Recent Clinical Techniques, Results, and Research in Wounds

Melvin A. Shiffman
Mervin Low *Editors*

# Pressure Injury, Diabetes and Negative Pressure Wound Therapy

Springer

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# Pressure Injury, Diabetes and Negative Pressure Wound Therapy

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# Foreword[1]

It is a great honour for me to be invited to provide a foreword for the series of six books edited by Dr. Shiffman and Dr. Low, which cover a broad expanse of subjects relevant to and important in the care of patients with wounds.

Wounds have existed since the beginning of time and, until recent years, have received scant attention unless major conflicts developed which necessitated innovation in the treatment of patients with wounds. However, in recent years there has been an increasing interest in this subject as evidenced by the explosion of journals, meetings, societies and associations and initiatives that have been developed in this field.

The need for an academic underpinning of the subject of wound healing is without question. Research papers published in recent years have undoubtedly enhanced the scientific basis for wound healing. This, coupled with demographic changes in many countries around the world, has led to increasing numbers of patients developing wounds or wound healing problems. It is recognised that in the vast majority of geographies globally the number of patients with wounds is increasing in everything other than major burns where better health and safety initiatives have been an effective preventive strategy.

This series of books not only attempts to deal with subjects that are normally seen in wound healing text but also provides a huge amount of space to the management of wounds seen in surgical practice, both general and specialist surgery. The sections on infection are an attempt to deal with a very common but poorly managed clinical problem and one that requires urgent attention in view of the global challenge of antimicrobial stewardship. The tradition chronic wounds are also included and provide a medical as well as a nursing and paramedical focus on these subjects.

It is particularly pleasing to see books and chapters focused on specialised surgical practice as these are areas that are rarely covered in other educational products in this area. The opportunity for new therapies, measuring the range of effective and appropriate outcomes and the use of new technologies are all included.

For those of us who work in the area of wound healing, these books will unquestionably be an important reference source. For those readers who want to get an insight into this common, expensive and complex problem they will without doubt find the content of these books an important source of informed opinion and refer to the rapidly expanding evidence base that is developing in this subject area.

I would urge you to immerse yourself in these books. Read, reflect and consider how information that you have had access to can and will change your clinical practice.

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[1]P. S.

We, Melvin A. Shiffman and Mervin Low, are greatly enthralled by Keith Harding's willingness to write the Foreword for the books on wounds. Keith Harding is the Director of TIME Institute (Translation, Innovation, Methodology and Engagement) and Head of the Wound Healing Research Unit in the School of Medicine at Cardiff University. He is Clinical Lead for Wound Healing in the Cardiff and Vale NHS Trust. In September 2013 Harding was appointed Dean of Clinical Innovation at Cardiff University. From 2002 to 2005 he was Head of the Department of Surgery at Cardiff University. He is Editor-in-Chief of the *International Wound Journal*. Harding is a Past President of the European Tissue Repair Society. He was the first President of the European Pressure Ulcer Advisory Panel and first Recorder of the European Wound Management Association. He was Chair of the International Working Group on Wound Healing in Diabetic Foot Disease in 2003. He was Chair of the Expert Working Group that produced a range of International Consensus Documents from 2004 to 2011. Professor Harding was appointed a Commander of the Order of the British Empire in the 2013 New Year Honours for services to medicine and healthcare.

# Preface

We are delighted to have the book on wounds extended into six volumes. There is so very much medical literature in journals and books that to cover the whole gamut of wounds would be virtually impossible. We tried to include as many of the experienced practitioners in wound care as possible, but many of them are too busy to spend the time committing to submitting a chapter.

The selection of topics in each of the volumes was decided by the number of authors responded to each of the subjects. As usual in editing a book, many authors who agreed to submit manuscripts finally were not available to complete the chapters. We contacted or tried to contact over 1500 authors and most of them did not respond or the responses were not as good as expected.

The volumes include:

1. Biofilm, Pilonidal Cysts and Sinuses
2. Burns, Infections and Wound Management
3. Pressure Injury, Diabetes and Negative Pressure Wound Therapy
4. Plastic and Thoracic Surgery, Orthopedics and Ophthalmology
5. Vascular Surgery, Neurosurgery, Lower Extremity Ulcers, Antimicrobials, Wound Assessment, Care, Measurement and Repair
6. Chronic Wounds, Wound Dressings and Wound Healing

There are many expert international contributors who have worked in various aspects of wound research as well as clinical practice. We have tried to have chapters that involved humans and in vivo results and avoided as much as possible animals and in vitro results. Chapter conclusions are those of the authors and may not be the same as those of the editors. At times the chapter may appear cumbersome, but the authors try to show some proof of their results. Language difficulties are common when translated into English so that grammar, spelling and sometimes words have to be corrected.

Hopefully, the reader will get information that adds to their care and treatment of patients. Researchers may gain knowledge of other researchers' progress and improve on the results or can continue their work in other directions. Controversy is many times a good thing since looking in other directions to prove or disprove a result can improve knowledge. We have a long way to go to be able to treat all wounds properly and successfully in as short a time as possible.

Tustin, CA, USA — Melvin A. Shiffman
Newport Beach, CA, USA — Mervin Low

# Contents

# Part I

# Pressure Injury

# Pressure Ulcers (Injury): Etiology, Prevention, Classification, Risk Assessment, and Treatment

Melvin A. Shiffman

## 1 Introduction

A pressure ulcer (PU) develops from continued pressure causing ischemia and necrosis of underlying structures. There are many methods to prevent pressure sores depending on the patient's disabilities. There are a number of classifications of pressure ulcers, and a number of risk assessments of pressure ulcers have been developed, and there are debates on which is the best risk assessment. Treatment of pressure ulcers is discussed, but this depends on the patient's ulcer cause and depth of the ulceration.

## 2 Etiology

A pressure ulcer develops from continued pressure causing ischemia and necrosis of underlying structures. The tissue over bony prominences is most often affected in patients with impaired mobility, decreased level of consciousness, and impaired sensation. Also at risk are patients who are dehydrated, elderly, malnourished, incontinent, and/or on steroid use. Often, there is friction, moisture, and shear besides the pressure.

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## 3 Preventive Measures

Preventive measures should be instituted by frequent position changes, repositioning to avoid stress on the skin, and body positions that minimize pressure on vulnerable areas. Other strategies include taking good care of your skin, maintaining good nutrition, quitting smoking, and exercising daily.

Clean the skin with mild soap and warm water daily at which time the skin is inspected for areas of redness, early ulceration, and areas that have pressure from clothing folds, buttons, etc. Use talcum powder to protect the skin vulnerable to excess moisture. Apply lotion to the dry skin. In nursing facilities and even at home, there may be a need to include frequent scheduled help with urinating, frequent diaper changes, protective lotions on the healthy skin, or urinary catheters or rectal tubes and changing bedding and clothing frequently.

Your care may include frequently scheduled help with urinating, frequent diaper changes, protective lotions on the healthy skin, or urinary catheters or rectal tubes.

There are specialty mattresses (Table 1) and specialty beds (Table 2) that could be utilized.

Diet is important and there may be a need to increase the amount of calories, protein, vitamins, and minerals. There may be a need to help in eating (watch for decreased urine, dark urine, dry or sticky mouth, dry skin, or constipation). Intake of liquid should be enough to keep the skin hydrated.

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**Table 1** Specialty mattresses (just some suggestions)

| |
|---|
| Pressure-relief mattresses and pads |
| Med Aire variable pressure pump and deluxe pad system |
| Spenco SILICORE pressure-relief mattress pad |
| Hill-Rom® 300 wound mattress |
| PressureGuard APM2 alternating pressure/lateral rotation mattress |

**Table 2** Specialty beds (just some suggestions)

| |
|---|
| Progressa® Bed System |
| Advanta™ 2 Med Surg Bed |
| Excel Care® ES Bariatric Hospital Bed |
| Hill-Rom® Resident® Long Term Care Bed |
| TotalCare SpO2RT® 2 ICU Bed |
| VersaCare® Med Surg Bed |

**Table 3** Shea stage of pressure ulcer [3]

| | |
|---|---|
| Stage I | Limited to the epidermis exposing the dermis |
| Stage II | Full-thickness skin loss exposing fat |
| Stage III | Full-thickness skin and fat defect exposing deep fascia |
| Stage IV | Full-thickness defect exposing the bone |

**Table 4** Torrance classification [5]

| | |
|---|---|
| Stage I | Non-blanching erythema of intact skin, heralding lesion of skin ulceration. In individuals with darker skin, discoloration of the skin, warmth, edema, induration, or hardness may be indicators |
| Stage II | Partial-thickness skin loss involving the epidermis, dermis, or both |
| Stage III | Full-thickness skin loss involving damage to or necrosis of subcutaneous tissue that may go down to, but not through, the fascia. The ulcer presents clinically as a deep crater with or without undermining adjacent tissue |
| Stage IV | Full-thickness skin loss with extensive destruction, tissue necrosis, or damage to the muscle, bone, or supporting tissues (e.g., tendon or joint capsule) |

## 4 Classifications of Pressure Ulcer

Pressure ulcer classifications are criticized for their low degree of inter-rater reliability [1].

### 4.1 Shea Classification of Pressure Ulcer

Shea reported his classification in 1975 [2]. Four grades of pressure can be recognized on the basis of pathophysiology of soft tissue breakdown overlying bony prominences (Table 3) [3].

### 4.2 Torrance Classification System

This pressure ulcer classification tool was devised in 1983 (Table 4) [4].

The major criticism of this tool is that it describes a grade 1 pressure ulcer as "blanching hyperemia." Critics have disputed that blanching hyperemia represents a warning sign and thus an underlying physiological reaction to pressure, rather than actual pressure damage. Furthermore, the term hyperemia is often used synonymously with erythema, but the terms mean different things [6–8].

### 4.3 Stirling Classification of Pressure Sore

This classification was developed in 1984 by Reid and Morrison [9].

The system complex has 0–4 grades with up to four subscales within some of the grades; thus, a deep necrotic infected ulcer would be labeled as 4.131.2. Grade 1 is described as "non-blanching erythema." This tool has several levels of descriptors within each grade; however, there is mixed opinion as to whether the descriptors assist or confuse practitioners when using this tool to assess the level of pressure damage [10]. Another major criticism is that the tool suggests a grade for an ulcer that is covered with eschar. This is certainly a contentious issue as many experts argue that eschar masks the depth of underlying damage, hence making it impossible to grade the level of harm [8].

Pressure ulcer grading scales are subjective measures of pressure damage. The major weakness of all classification systems is the lack of evidence to support their use, the most important factor being inter-rater reliability [11].

### 4.4 Yarkony-Kirk Scale (Table 5) [12]

**Table 5** Yarkony-Kirk classification of pressure ulcers

| | |
|---|---|
| Grade 1 | Red area |
| Grade 2 | Involvement of the epidermis and dermis. No subcutaneous fat seen |
| Grade 3 | Exposed subcutaneous fat with no muscle observed |
| Grade 4 | Exposed muscle without bone involvement |
| Grade 5 | Exposed bone |
| Grade 6 | Joint space involved |

### 4.5 National Pressure Ulcer Advisory Panel (NPUAP) (Table 6) [13]

**Table 6** National Pressure Ulcer Advisory Panel (NPUAP)

| | |
|---|---|
| Stage 1 pressure injury | Non-blanchable erythema of intact skin |
| Stage 2 pressure injury | Partial-thickness skin loss with exposed dermis |
| Stage 3 pressure injury | Full-thickness skin loss |
| Stage 4 pressure injury | Full-thickness skin and tissue loss |
| Unstageable pressure injury | Obscured full-thickness skin and tissue loss |
| Deep tissue pressure injury | Persistent non-blanchable deep red, maroon, or purple discoloration |

### 4.6 European Pressure Ulcer Advisory Panel (EPUAP) (Table 7) [14]

**Table 7** European Pressure Ulcer Advisory Panel (EPUAP) pressure ulcer classification system

| Grade | Definition |
|---|---|
| 1 | Non-blanchable erythema of intact skin. This may be difficult to identify in darkly pigmented skins |
| 2 | Partial-thickness skin loss involving the epidermis and/or dermis: the pressure ulcer is superficial and presents clinically as an abrasion, blister, or shallow crater |
| 3 | Full-thickness skin loss involving damage or necrosis of subcutaneous tissue that may extend down to, but not through, the underlying fascia: the pressure ulcer presents clinically as a deep crater with or without undermining of adjacent tissue |
| 4 | Extensive destruction tissue necrosis or damage to the muscle, bone, or supporting structures with or without full-thickness skin loss |

**Table 8** Norton scale for assessing risk of pressure ulcer

| | |
|---|---|
| 1. Physical condition | 1 = very bad, 2 = poor, 3 = fair, 4 = good |
| 2. Mental condition | 1 = stupor, 2 = confused, 3 = apathetic, 4 = alert |
| 3. Activity | 1 = bed bound, 2 = chairbound, 3 = walk with help, 4 = ambulant |
| 4. Mobility | 1 = immobile, 2 = very limited, 3 = slightly impaired, 4 = full |
| 5. Incontinent | 1 = urine and bowel, 2 = usually/urine, 3 = occasionally, 4 = not |

## 5 Scales for Assessing Risk of Pressure Ulcers

### 5.1 Norton Scale

Norton et al. [15] devised a scale for assessing the risk of pressure ulcers to help assess a patient's potential risk of pressure ulcer development and to determine the extent of pressure damage (Table 8). Low risk is over 18, medium risk is between 14 and 18, high risk is between 10 and 14, and very high risk is less than 10.

The Norton scale identified 38%, and the Braden scale identified 27% of patients as at risk [16].

### 5.2 Braden Scale Risk of Pressure Ulcer

The Braden scale was developed in 1984 (Table 9) [17]. The Norton scale overpredicted by 64%, whereas the Braden scale overpredicted by 36%.

### 5.3 The Waterlow Scale

Waterlow devised the scale in 1987 (Table 10) [18]. The tool identifies three "at-risk" categories:

1. A score of 10–14 indicates "at risk."
2. A score of 15–19 indicates "high risk."
3. A score of 20 and above indicates "very high risk."

The Waterlow scale has been criticized for its lack of research and its ability to overpredict and, consequently, result in the misuse of resources [19].

**Table 9** Braden scale risk of pressure ulcer [5]

| | |
|---|---|
| Sensory perception | |
| Completely limited | 1 |
| Very limited | 2 |
| Slightly limited | 3 |
| No impairment | 4 |
| Moisture | |
| Constantly moist | 1 |
| Very moist | 2 |
| Occasionally moist | 3 |
| Rarely moist | 4 |
| Activity | |
| Bedfast | 1 |
| Chair-fast | 2 |
| Walks occasionally | 3 |
| Walks frequently | 4 |
| Mobility | |
| Completely immobile | 1 |
| Very limited | 2 |
| Slightly limited | 3 |
| No limitation | 4 |
| Nutrition | |
| Very poor | 1 |
| Probably inadequate | 2 |
| Adequate | 3 |
| Excellent | 4 |
| Friction and shear | |
| Problem | 1 |
| Potential problem | 2 |
| No apparent problem | 3 |
| Score | Risk of developing pressure ulcer |
| 15–18 | Mild risk |
| 12–14 | Moderate risk |
| ≤11 | Severe risk |

**Table 10** Waterlow pressure ulcer risk assessment scale [20]

| Body mass index (BMI) | Score |
|---|---|
| Average (BMI = 20–24.9) | 0 |
| Above average (BMI = 25–29.9) | 1 |
| Obese (BMI ≥ 30) | 2 |
| Below average (BMI ≤ 20) | 3 |
| Skin type visual risk areas | Score |
| Healthy | 0 |
| Tissue paper (frail) | 1 |
| Dry | 1 |

**Table 10** (continued)

| Body mass index (BMI) | Score |
|---|---|
| Edematous | 1 |
| Clammy, pyrexia | 1 |
| Discolored grade 1 | 2 |
| Broken/spots grades 2–4 | 3 |
| Sex and age (years) | Score |
| Male | 1 |
| Female | 2 |
| 14–49 | 1 |
| 50–64 | 2 |
| 65–74 | 3 |
| 75–80 | 4 |
| 81+ | 5 |
| Continence | Score |
| Complete/catheterized | 0 |
| Fecal incontinence | 2 |
| Urinary + fecal incontinence | 3 |
| Mobility | Score |
| Fully | 0 |
| Restless/fidgety | 1 |
| Apathetic | 2 |
| Restricted | 3 |
| Bed bound, e.g., traction | 4 |
| Chairbound, e.g., wheelchair | 5 |
| Appetite | Score |
| Normal | 0 |
| Scarce/feeding tube | 1 |
| Liquid intravenously | 2 |
| Anorexia/absolute diet | 3 |
| Tissue malnutrition | Score |
| Terminal cachexia | 8 |
| Multiple organ failure | (Resp, renal 8, cardiac) |
| Peripheral vascular disease | 5 |
| Anemia <8 gm% | 2 |
| Smoking | 1 |
| Neurological deficit | Score |
| Diabetes, multiple sclerosis, CVA | 4–6 |
| Motor/sensory | 4–6 |
| Paraplegia | 4–6 |
| Major surgery or trauma | Score |
| Orthopedic/spinal | 5 |
| On table >2 h | 5 |
| On table >6 h | 8 |
| Score | Risk |
| 10+ | At risk |
| 15+ | High risk |
| 20+ | Very high risk |

The Braden scale has the best validity and reliability indicators across many studies and settings [3]. Both Braden and Norton scales predict PU development better than nurses' clinical judgment, while the Waterlow scale is highly sensitive but not very specific in predicting PU development [20]. The use of a PU risk assessment scale improves PU preventive interventions but is not, by itself, efficacious in decreasing PU incidence.

## 6 Treatment

### 6.1 Pain

Control pain with appropriate medications at appropriate doses on a regular basis. Ulcer pain can be reduced with nonadherent moist dressings. Reduce dressing changes to a minimum if possible. Ibuprofen-impregnated wound dressings can be helpful [21]. Cleanse the ulcer with saline or surfactants and/antimicrobials at the time of dressing change. Deep wounds can be irrigated.

### 6.2 Nutrition

There should be adequate nutrition and hydration [22].

Encourage protein, high-calorie foods, and fluids, unless contraindicated, and intravenous fluids when indicated. Monitor weight and skin turgor. Use dietary consultation when intake is inadequate.

### 6.3 Infection

Determine whether wound has bacteria imbalance (critical colonization and infection) [23].

If there is superficial increased bacterial burden, then use appropriate topical antimicrobial with low toxicity to cause allergy. If infection involves surrounding compartment, then obtain culture and sensitivity (C&S) and use topical and oral antimicrobial agents [24]. Deep wound infection requires intravenous antibiotics [25].

### 6.4 Debridement

Removal of nonviable tissues by debridement helps to control or prevent infection, removes growth medium and biofilm, defines the extent of the wound, and stimulates the healing process. Contraindications to debridement are dry stable eschar without infection, ischemic healthy tissue that is intact, and coagulation disorders (until controlled).

There are a variety of types of debridement including [26]:

1. Autolytic debridement in which the body's white blood cells and enzymes remove the necrotic tissue. This leaves healthy tissue behind.
2. Chemical debridement through the use of enzymes such as papain with urea (Accuzyme®, Ethezyme®, AllanEnzyme, Panafil, Gladase®, Papfyll), collagenase (Santyl®), castor oil/trypsin (Revina NLT, Granulderm, Xenaderm), and denaturing agents like sodium hypochlorite (Clorpactin®, Dakin's solution).
3. Mechanical debridement by pulsed lavage, whirlpool, and/or wet-to-dry dressings (may be painful).
4. Sharp debridement should be conservative. Excise only avascular tissue. General anesthesia may be necessary if too painful (surgical debridement).

### 6.5 Dressings

The physician must take into account the patient's pain and tolerance, the position of the ulcer, the amount of exudate, and the frequency of dressing change. The dressing should promote warm and moist environment for grade 2–4 pressure ulcers [27]. Hydrogel dressings were more effective than

other dressings for the proportion of pressure ulcers completely healed. However, there are different clinical indications for each dressing.

### 6.6 Surgery

With deep wounds it may be necessary to resect the ulcer, abnormal tissues, sinus tracts, bursa, and involved bone. If there is no infection, the wound may be closed primarily or with composite flaps. Flaps should be large so that the suture line is away from areas of direct pressure and has minimal tension at closure site [21].

## 7 National Pressure Ulcer Advisory Panel (NPUAP)

In 2016 the National Pressure Ulcer Advisory Panel (NPUAP) made a change in terminology from pressure ulcer to pressure injury and updated the stages of pressure injury in order to more accurately describe pressure injuries to both intact and ulcerated skin [28]. Arabic numbers are now used in the names of the stages instead of Roman numerals.

Pressure injury is defined as localized damage to the skin and/or underlying soft tissue usually over a bony prominence or related to a medical or other device. The injury can present as intact skin or an open ulcer and may be painful.

Stage 1 pressure injury: non-blanchable erythema of intact skin

Stage 2 pressure injury: partial-thickness skin loss with exposed dermis

Stage 3 pressure injury: full-thickness skin loss

Stage 4 pressure injury: full-thickness skin and tissue loss

Unstageable pressure injury: obscured full-thickness skin and tissue loss

Deep tissue pressure injury: persistent non-blanchable deep red, maroon, or purple discoloration

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# The Use of Oxygen in the Treatment of Pressure Ulcers

Jalil Azimian and Hossein Rafiei

## 1 Introduction

Wound healing consists of a series of physiological events that occur in response to tissue damages [1, 2]. Therefore, wound healing might progress with different pace and quality due to local and systemic factors and also because of the diversity among people [3, 4]. Nevertheless, it should be noted that many vital processes of wound healing are oxygen-dependent, and therefore oxygen-based therapeutic strategies for damaged tissues should be based on an understanding of the area with anoxia, hypoxia, and normoxia [5]. In this oxygen-based model, wound healing strategy should focus on the tissue in the hypoxia area in order to maintain the highest level of living tissue and stimulate the treatment process [5].

Throughout history, native healers (therapists) found that if a wounded patient was moved from thin air of mountainous environment to a more suitable climate (e.g., a very deep valley), the wounds were healed faster [6]. Treatment of a chronic wound requires the presence of enough oxygen. Actually, lack of enough oxygen is a challenge for chronic wounds' treatment [7, 8]. The amount of oxygen consumption in a cell depends on its type and biological status. Demand and consumption of oxygen significantly increase in case of tissue damage and ulcers. Since oxygen cannot be stored in the cells, the continuous supply of oxygen to the cells is necessary for wound healing [7]. Disruption in oxygen supply will make many problems for the wound healing process [7]. Therefore, oxygen therapy is a logical method to increase tissue oxygenation and accelerate wound healing [8].

Oxygenation of the skin may occur by two routes: delivery from the "inside out" route via the circulation and delivery from the "outside in" route via the atmosphere. In the first method (inside out), the oxygenated blood flows through the cardiovascular system, and the oxygen devolved is released in peripheral plasma cells such as the skin tissue. The oxygen is transferred from the capillaries into the interstitial space and eventually to the skin and cells because of concentration differences. In the second method (outside in), the oxygen penetrates the permeable surface of the skin into the tissue. The amount of oxygen diffusion into the cells and tissues depends on its pressure amount, which forms the basis for topical oxygen therapy (TOT) [5].

The history of using hyperbaric oxygen for tissue repair goes back to four decades ago [9]. The advantages of using hyperbaric oxygen are:

1. It increases oxygen in the wound area and prevents further damages.
2. It increases angiogenesis and improves microcirculation in the wound area.

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3. It improves wound healing process by reducing edema and inflammation of the area.
4. It sedates the wound area.
5. It reduces the risk of wound infections by destroying bacteria and increasing the power of the white blood cells.
6. It improves the microcirculation and detoxification.
7. It improves the performance of some antibiotics.
8. It reduces blood viscosity and its complications.
9. It improves the blood circulation in the lymphatic system.
10. It prepares the skin and bone before transplant surgery.
11. It improves recovery after surgery and increases the chances of successful transplantation [10, 11].

## 2 Hyperbaric Oxygen

Today, a new perspective has emerged in the use of topical oxygen for the treatment of wounds by new studies, and different everyday-developing technologies are used to deliver oxygen to the tissues [3, 12].

The ultimate goal of using the above methods is to deliver oxygen to the depth of the tissues and areas suffering from hypoxia where the cardiovascular system cannot deliver oxygen to the area due to different causes, or environmental barriers, such as an edema, prevent systemic methods to deliver oxygen to the wound [5].

In order to facilitate the understanding, the categories use oxygen for wound healing. The first category is related to the amount of pressure used in the system. Considering that, systems are divided into two categories of hyperbaric (a system with a pressure higher than atmospheric pressure) and normobaric (a pressure equal to atmospheric pressure). Hyperbaric oxygen (HBO) and topical hyperbaric oxygen (THBO) methods are in the hyperbaric category and transdermal continuous oxygen therapy (TCOT) is in the normobaric category. The systems can also be classified according to their systemic or topical application. In this case, the HBO method is in the systemic category (oxygen is systemically delivered to the patients), and TCOT and THBO are in the local category (oxygen is delivered to the wound topically).

Although the ultimate goal of all three methods of HBO, THBO, and TCOT is to increase the oxygen available to the damaged tissue and speed up wound healing, as noted, the three methods are different in their functional structure. In the HBO method, the patients are placed in a big chamber where they receive oxygen with high flow rate and pressure systemically. In this method, the patient receives 100% oxygen in a chamber at a pressure greater than atmospheric pressure for 90–120 min per day. In this method, treatment continues on a daily basis until the wound heals [13]. Since oxygen is not used topically, the chance of oxygen reaching the wound area is much reduced which is the main disadvantage of this method. The method has also some risks such as oxygen toxicity, barotrauma, and pneumothorax. There is a risk of explosion and fire threatening the patient, too. Due to the limitation of the patient inside the chamber and nonportability of the device, the method is used with less enthusiasm on the part of clinicians.

Many patients do not tolerate the side effects of systemic HBO. They do not have the access to the facilities necessary to carry out the procedure. In some cases, the cardiovascular system of the patient does not have enough power to deliver oxygen to the tissues of the patient's wound. In some cases, edema existed and oxygen cannot reach it through the circulation [3, 14]. In these cases, TOT as a preferred method is able to carry oxygen through the body surface to the wound, and since the oxygen reaches the wound directly and not through vascular capillaries, it minimizes the complications of oxygen therapy. Therefore, the topical hyperbaric oxygen therapy method is used. TOT can reach the outer surface of the body in the form of gas or dissolved in body fluids. However, biologically, the administered oxygen in the form of gas must be dissolved to be effective so that the target tissues can absorb it [5, 15].

## 3 Topical Oxygen

Topical oxygen gas system (oxygen boot) was proposed in 1932. In this method, the oxygen is delivered to the wound or affected limb in form of pure gas, or it is delivered to the wound through a machine that produces oxygen gas. In the THBO method, the 100% oxygen is directly delivered to the wound with a pressure more than atmospheric pressure [13]. In the THBO, the used pressure and time depend on factors such as the type of pathogen in the wound and the amount of angiogenesis around the wound.

A review study has suggested evidence-based recommendations for TOT. There are obstacles for carrying the oxygen in the form of gas bubbles to the target cells in the wound that must be overcome since these internal factors limit the performance of systemic TOT. Studies have shown encouraging results in the treatment of wounds using TOT with low pressure. In this method, the 100% oxygen is used at the atmospheric pressure or slightly higher with or without the use of high-pressure chambers. This method is more accessible than HBO and can be used by the patient at home.

Due to complications of HBO and THBO methods, the TCOT method was proposed which did not need the patient to remain still. In this method, the patient can receive other cares. This will reduce the costs, enable the patient to be active, and receive other cares. In the TCOT method, oxygen is directly delivered to the wound which is covered and moist at low flow rates (3–12 mL/h). In this method, the oxygen flow rate is 40 L per minute. TCOT might not be effective in wounds covered with scar tissue [7]. Few side effects are reported for using oxygen in chronic wound healing some of which are myopia, ear damage, and oxygen poisoning in rare cases [9]. The HBO method has contraindications in patients with deep vein thrombosis and severe heart failure. The method is not recommended for pregnant women [8].

Using this method has resulted in wound healing through angiogenesis in various studies on the wound healing of animals as well as human clinical studies. The use of a topical tool for administration of streams of 100% oxygen bubbles on the wound has healed the wound through epithelial healing.

The methods that deliver dissolved oxygen to the wound work in two ways. In the first way, these tools include portable dissolved oxygen to tissues and cells such as fluorocarbon. In the second way, these tools allow a gas tank of oxygen to deliver oxygen to the tissues [5]. Although there are reports of difficulty in stable fluorocarbon emulsion production, the results of some studies have been encouraging. The most important challenge in this method is delivering oxygen with high pressure gradient to the cells and tissues with hypoxia. If this challenge is resolved, TOT will be a complementary tool to systemic oxygen therapy report used by physicians in wound healing [5, 15].

Several previous studies have examined the effects of oxygen on the treatment of chronic wounds. In a study in 2016, Yu et al. [16] examined the effects of THBO in the treatment of chronic diabetic foot ulcers resistant to standard treatments. They divided 20 patients with diabetic foot ulcers into two groups of intervention and control. Oxygen therapy continued for 8 weeks in that study. Their results showed that the use of THBO significantly improved healing of chronic ulcers resistant to conventional treatments. In another study in this regard in 2017, Niederauer et al. [17] examined the effect of TCOT on wound healing in patients with diabetic foot ulcers. They divided 100 patients into two groups of intervention and control. The patients in the intervention group received TCOT for 12 weeks, and the patients in the control group were treated with standard methods. Their results showed that the rate of wound healing in patients treated with TCOT was significantly higher compared to the standard methods. They also showed that the rate of complete wound healing in patients treated with TCOT was higher compared to patients treated with standard methods.

The healing of pressure ulcers is very complex and costly. The use of THBO as an easy and cost-effective therapy in the treatment of pressure

ulcers is increasing [10]. Although there are few studies in this regard, they all suggest that the use of oxygen can enhance wound healing in pressure ulcers. One of the first reports on the application of HBO in the treatment of pressure ulcers is that of Fisher in 1990. Fisher [18] reported that the use of THBO increased granulation speed, reduced microbial growth, and enhanced wound healing. Fisher did not observe any side effects of the treatment. In another study published as a poster, Berlin et al. [19] examined the effects of TCOT on four patients with chronic ulcers resistant to conventional treatments. One patient in that study had a pressure ulcer in the heel.

In 2008, Bank and Ho [20] investigated the use of THBO on the healing of pressure ulcers in three patients with spinal cord injury with a grade 4 wound in the pelvic area. The results showed that in all three cases, the wound healing had improved significantly after treatment. In another study in 2015 in Japan, Sano [21] examined the effect of topical oxygen in the treatment of six patients with chronic wounds. One of the patients had a pressure ulcer in the sacral region. Their results showed that treatment with THBO has led to an increase in the partial pressure of oxygen in the area around the wound. A significant tissue granulation was also observed in the cells in that area. In another study in this regard in 2015 in Iran, Azimian et al. [3] examined the effect THBO on wound healing of pressure ulcers grades 2–4 in the sacral and ischium areas. They examined 100 patients in the two groups of intervention ($n = 50$) and control ($n = 50$). The patients in the investigation group received topical oxygen with a rate of 10 L per minute for 20 min, three times a day. The treatment continued for 12 consecutive days. They used the push criteria to study the wound healing process. At the end of the 12th day after the start of the study, the wounds of 16 patients in the intervention group healed completely, while the wound of only one patient healed completely in the control group. Their results also showed that the wound healing process in the intervention group had better conditions during the 12 days. Finally, they concluded that the use of THBO therapy is an effective and harmless method of pressure ulcer healing.

**Conclusions**

Generally, it can be said that the use of oxygen in the treatment of different chronic ulcers such as diabetic foot ulcers is very effective. Its application for patients with pressure ulcers requires further studies, although all the few studies on the effect of oxygen in the treatment of pressure ulcers indicated that this method will accelerate the healing process of pressure ulcers.

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# Less Invasive Surgical Technique for the Treatment of Unmanageable Pressure Ulcer with Pocket

Akitatsu Hayashi and Takumi Yamamoto

## 1 Introduction

Pressure ulcer is a typical disease among elderly and handicapped patients who cannot ambulate by themselves. Pocket, wound edge undermining, is one of the most demanding complications of deep pressure ulcers. Conventional therapies such as skin care/monitoring and frequent positional change play important roles in prevention/treatment of pressure ulcers, but further surgical procedures are generally required in cases with a pocket lesion, when refractory to negative pressure wound therapy (NPWT). Total resection of the lesion and reconstruction using a flap are recommended for an ulcer with a pocket [1–3]. However, since most elderly patients with pressure ulcer suffer from systemic complications, invasive surgeries cannot be applied in all cases.

Incision of the skin and the subcutaneous tissue to the wound edge undermining space is suggested to allow for the uncomplicated removal of the necrotic tissues around the deepest fold, and it facilitates voluntary wound healing [4, 5]. Traditional incisions cut through the whole area of the wound edge undermining space with several straight alignment incisions (Fig. 1). In spite of the fact that traditional incision facilitates voluntary wound healing with less invasiveness compared with flap reconstruction, the time required for thoroughgoing wound closure is long, because the whole area of the wound edge undermining space has to be cured through granulation and epithelialization. To reduce the time for wound closure and to minimize invasiveness, a new incision technique, parallel pocket incision (PPI), was developed in Japan [6]. "Pocket" is a term commonly used among Japanese wound-care professionals, and it means wound edge mining [7, 8].

## 2 Technique of Parallel Pocket Incision (PPI)

Different from traditional skin incision for a pocket lesion as shown in Fig. 1, parallel skin incision lines are designed in PPI technique. In this technique, one incision is designed including the ulcer and the other incision opposing site:

1. Incision lines are designed to allow easier irrigation of the whole wound area by cutting through the whole area of the deepest crease of the pocket.
2. Skin incisions are made as designed preoperatively after local infiltration anesthesia along

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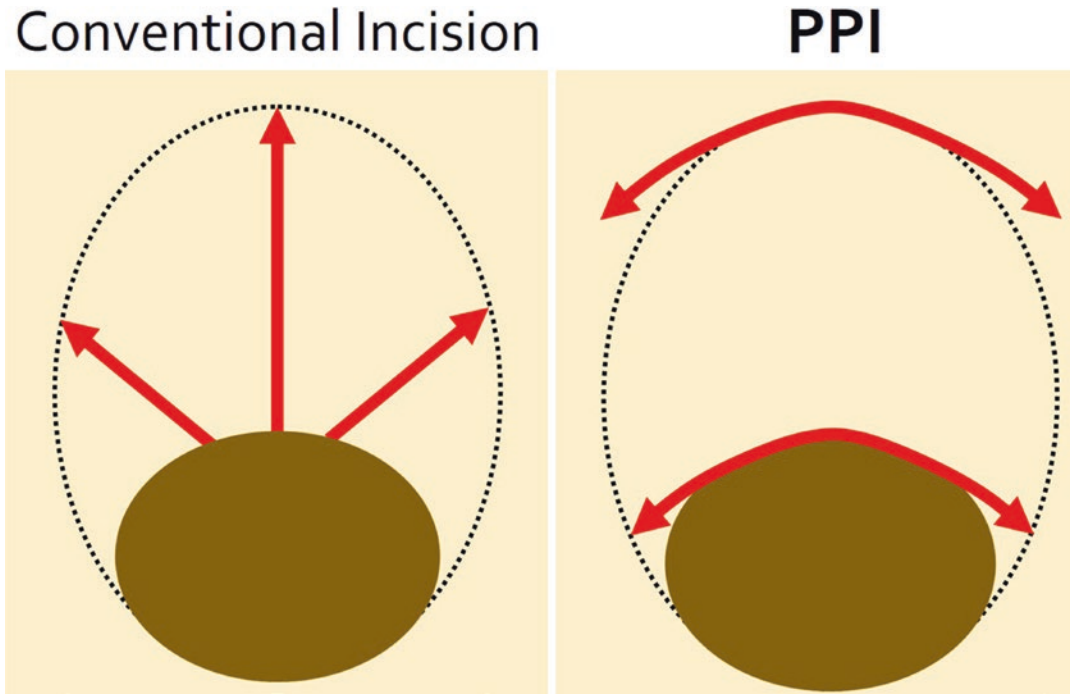


**Fig. 1** (*Left*) Traditional skin incision lines for a pressure ulcer with a wound edge undermining. The *dotted lines* indicate the area of undermining, and the *red lines* indicate incision lines. (*Right*) Parallel pocket incision lines for a pressure ulcer with a wound edge undermining. The *dotted lines* indicate the area of undermining, and the *red lines* indicate incision lines

the designed incision lines using 1% lidocaine with 1:100,000 epinephrine.

3. Careful attention is paid to cut through the deepest crease of the pocket to allow for the easier removal of the necrotic tissue onto the pocket edge.
4. After ascertaining that all the pocket edges can be easily irrigated through the incisions, gauzes are packed into the incisions to prevent postoperative hemorrhage. If bleeding is hard to control with gauze packing, then the bleeding wound is coagulated with electric cautery or bipolar coagulation.

The most important point in PPI procedure is to make the incisions long and deep enough to open up the deepest fold of a wound edge undermining. If incision is not long or deep enough to remove the necrotic tissue on the fold, the undermining would never be closed. However, as a skin flap created by primary PPI is a bipedicle flap, salvage re-PPI procedure (secondary PPI) can be safely performed with a low risk of skin flap necrosis. Therefore, a physician should confirm that the whole area of the deepest fold is completely opened, and it can be easily irrigated by nurses after PPI (Fig. 2).

## 3 Postoperative Care

Basically, postoperative care is not different from preoperative care, including wet-to-dry dressing, wound irrigation, and positional change under the use of air-loss beds by nurses.

The following are different from preoperative care: the packed gauzes are removed the day after PPI, and daily wound care is restarted as performed preoperatively. During the daily wound care, the pocket edge is thoroughly irrigated to remove the necrotic tissue.

As the necrotic tissue is removed and the granulation tissue begins to grow well, the skin overlying the pocket begins to be attached to

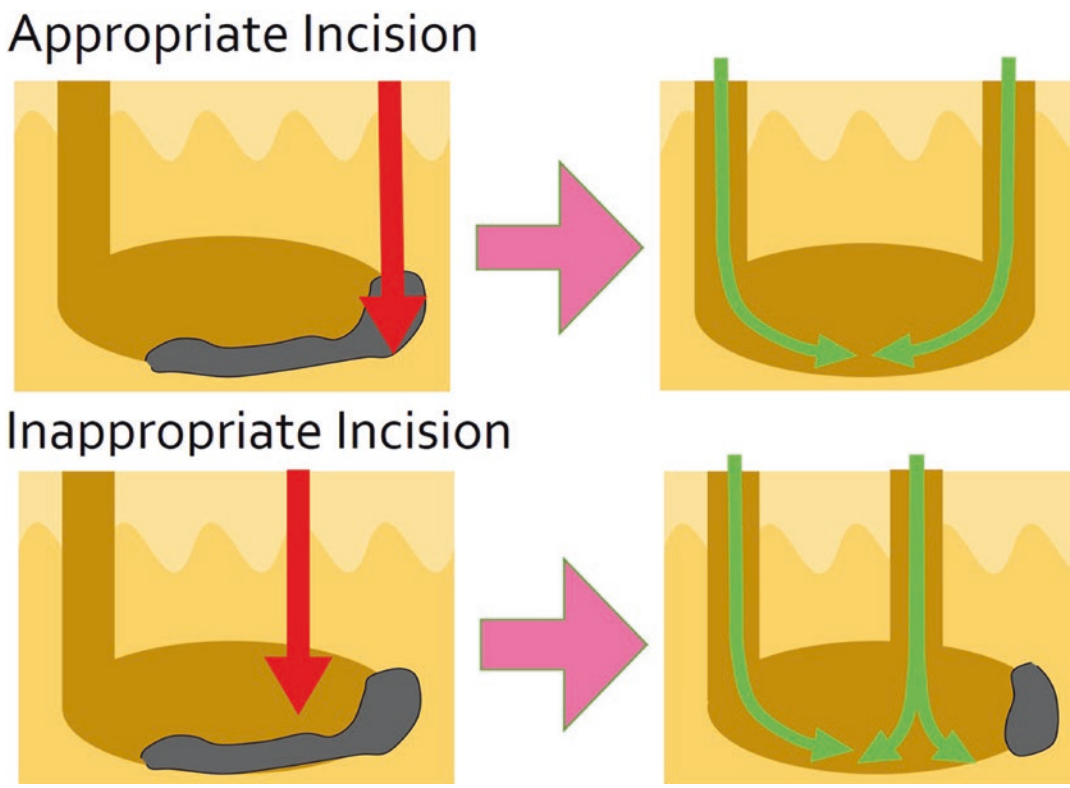


**Fig. 2** Appropriate and incision lines. (*Top*) After appropriate incision, the whole area of the deepest fold is completely opened, and it can be easily irrigated. (*Bottom*) After inappropriate incision, the deepest fold cannot be irrigated, and the necrotic tissue is not completely removed. The *red lines* indicate incision lines, the *green lines* the routes of irrigation, and the *black* regions the necrotic tissues

the pocket floor. When the overlying skin flap begins to adhere to the pocket floor, wound care is performed with caution not to dissect the adhesion. Two raw surface areas remain along the PPI sites after complete adhesion of the overlying skin.

Traditional treatments are continued until the raw surface areas are completely epithelialized. After complete wound closure, most wound area is covered with the skin flap, and only PPI sites are covered with skin after epithelialization.

## 4 Advantages

1. PPI can be safely performed under local anesthesia even on a patient with severe systemic comorbidities.
2. Intraoperative bleeding can be easily stopped using electric cautery or bipolar coagulation without any complication or blood transfusion. When electric cautery and bipolar coagulation are prepared, PPI can be safely performed at bedside. With bipolar and electric cautery prepared, PPI can be safely performed even on patients with antiplatelet and anticoagulation therapy.
3. As a skin flap created after PPI is a bipedicle flap that has good vascularity, the partial necrosis of the skin overlying a wound bed is less likely.
4. Resection and flap coverage are considered an optimal treatment for a pressure ulcer with a pocket, but they cannot be performed on patients with severe comorbidities due to its invasiveness. PPI is indicated for ulcers complicated with eccentric pocket

formation, when flap transfer cannot be applied. Taking this into consideration that an intractable pressure ulcer requires lifelong wound care, potentially curative PPI is considered to be cost-effective.

## 5 Disadvantages

1. When a pocket is not eccentric or the opening of the pockets is large, PPI is difficult to apply, and conventional incision seems better indicated.
2. PPI can be performed for the treatment of the pocket with its opening smaller than half of the pocket.

### Conclusions

PPI procedure is a less invasive surgical intervention to an intractable pressure ulcer with a wound edge undermining, which can be performed under local infiltration anesthesia safely even on a patient with severe systemic comorbidities. The incision facilitates spontaneous wound healing by allowing for the easier removal of the necrotic tissue in the deepest fold of an undermining.

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# The Potential Role of Zinc Supplementation on Pressure Ulcer Healing in Older Adults

Melissa Heintschel and Roschelle Heuberger

## 1 Introduction

The development of pressure injuries (PI) is a significant problem in healthcare settings. Pressure injury prevalence varies from 8.52 to 32.2% in long-term care and 2.9 to 19.1% in home care patients, and stage 1 and 2 PIs make up the majority of skin lesions [1, 2]. Approximately 2.5 million patients are treated annually for PIs in US healthcare facilities, and an estimated $11 billion is spent annually for PI treatment [3, 4]. There are 88.3% US long-term care residents ≥65 years and 45.2% aged ≥85 years old [5]. This population is prone to compromised skin integrity [2]. Additionally, they are susceptible to nutrition-related risk factors, including decreased appetite and altered thirst, dysphagia, self-feeding deficits or other eating problems, and unintentional weight loss [6]. These risk factors contribute to malnutrition and nutritional deficiencies including zinc (Zn) deficits. A cross-sectional study found that Zn deficiency in older adults was associated with risk for malnutrition [7]. Consequently, nutritional deficiencies and malnutrition associated with aging further increase the chances of developing a skin injury or delay in wound healing [6, 8].

Pressure injuries are defined as "localized damage to the skin and/or underlying soft tissue usually over a bony prominence or related to a medical or other device" [2]. There are three stages of wound development and healing. The first stage, the inflammatory phase, increases fluid and cell influx to the wound location to provide needed oxygen and nutrients to enhance regenerative processes and, consequently, results in decreased immunity and increased susceptibility to infections [8, 9]. The second stage is the proliferative phase, also considered the building phase [8]. This phase consists of the synthesis of collagen, reticulin, and elastin from fibroblasts for new tissue growth [8]. The final stage is the remodeling phase of healing where cellular activity and number of blood vessels in the area return to normalcy [8, 10]. In order to support the immune response during these first two phases, nutritional needs increase from 25–30 kcal/kg energy and 0.8–1.0 g/kg protein daily to 25–35 kcal/kg and 1.2–1.5 g/kg for PI wounds [8].

Repositioning programs for pressure relief, selection of mattresses, and wound care are important considerations in PI treatment [11]. Currently, there are more than 3000 nonnutritional products on the market for adjuvant therapeutic use [12]. Nutritional interventions can include oral vitamins/minerals, therapeutic diets,

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and oral nutritional supplementation (ONS). Considering the connections between nutritional deficiencies, risk of malnutrition, and PI development, close nutrition monitoring along with appropriate interventions should be considered to improve wound healing outcomes.

Zinc plays a vital role in protein metabolism, regulation of gene expression, immunity, and inflammatory response, all of which play an important role in wound healing. Zinc deficits may delay processes in all phases of wound healing [13]. The purpose of this review is to determine whether or not there is a body of evidence to support the hypothesis that poor pressure injury healing (as measured by both surrogate measures and biomarkers) in older adults is a result of suboptimal zinc status.

## 1.1 Zinc

Zinc plays a significant role in several biochemical and physiologic functions [14]. These functions include but are not limited to control of gene transcription and translational regulation vital for DNA repair, transcriptional regulation, and protein metabolism, as well as catalytic enzyme activity for many biologic processes in intermediary metabolism and insulin signaling [15–17]. Zinc also plays a role in immune competence. Without adequate Zn availability, T- and B-helper cells fail to reach maturation, subsequently leading to lymphopenia along with impaired natural killer cell and phagocytic cell function [15]. Suboptimal Zn levels impair secretion of cytokines, regulation of interferon-$\gamma$, tumor necrosis factor, and production of interleukin-2 [15]. These are vital for immune response [15]. Most relevant to this literature review is the need for Zn for normal functioning of anabolic processes for growth, tissue maintenance, and wound healing [18].

The recommended dietary allowance (DRI) for Zn in adults (age $\geq$ 19) is 8–11 mg/d, and the American Society for Parenteral and Enteral Nutrition (ASPEN) has suggested that nutritional needs are further increased for critically ill patients [18, 19]. Dietary sources of Zn can be found in meat products, including oysters, organ meats, fish, and beef [16]. In contrast, some plant-based foods may be good sources of Zn but are less bioavailable due to the presence of phytate, iron, and fiber, which inhibits Zn absorption [18]. The Third National Health and Nutrition Examination Survey (NHANES III) found that 57.5% of the advanced age population ($\geq$71 years old) consumed inadequate levels of Zn from the diet and that advanced age contributes to decreased dietary Zn intake below recommended guidelines ($\leq$77% RDA) [5]. Additionally, Zn is primarily transported from the small intestine, and intestinal absorption decreases with aging [14]. Consequently, it is difficult to assess the adequacy of dietary Zn levels in older adults due to reduced intakes and absorption. Surrogate measures, such as dietary assessment of a patient's overall nutritional status, should be evaluated when considering a possible Zn deficiency. These assessments include inadequate intake, reduced absorption, increased losses, or increased nutrient needs [18].

Zinc homeostasis, with respect to intracellular concentrations, is maintained via multiple feedback controls to preserve tight regulation and adequate levels. There is no site for storage; Zn is recycled through an internal reservoir or "pool" [18, 20]. A further obstacle in evaluating Zn status is the lack of a reliable biomarker [13]. Although plasma Zn concentrations are the most widely used biomarker to assess Zn status, it is an insensitive marker for deficiency.[21] Many factors can result in false-positive hypozincemia, such as hypoalbuminemia, that have an impact on the validity of these test results [18, 21]. The normal range for plasma Zn level is 12–18 μmol/L (78–118 μg/dL) [13]. Zinc can also be measured by urinary Zn but has little validity due to several factors affecting its loss in urine aside from Zn status [21]. The pancreas, prostate, and mammary glands have unique Zn requirements for metabolic processes, but measuring Zn from tissue biopsies is not used in practice [22]. Several other biochemical markers have been considered but have not been found to be useful indicators of Zn status [18]. A potential biomarker that may be useful in future research is the copper to zinc ratio (CZr), which has been a suggested biomarker for physical

and functional decline associated with aging. Though the role of this marker is to predict mortality rather than evaluate Zn status, it may be useful in determining associations with immobility and risk of development in PUs in the aged population [23]. Future research is needed to validate the use of this measure. As a result, it is difficult to assess Zn status from biochemical markers. Despite being unable to measure the efficiency of Zn absorption and imprecise measures of Zn status using plasma Zn, the International Zinc Nutrition Consultative Group currently recommends the monitoring of Zn status by assessing plasma zinc concentration and dietary Zn intakes, though alternative indicators are under investigation [24].

Due to risk of poor dietary intakes, and decreased efficiency in zinc absorption with aging, monitoring zinc status for older patients with pressure injuries should be a part of clinical practice for optimal wound healing outcomes. In the event of deficit, nutritional interventions should be instituted. These nutritional interventions include standard oral nutrition supplements (ONS), specialty ONS formulas, Zn sulfate, and potentially L-carnosine (CAR) and its Zn complex, polaprezinc (PLZ).

## 2 Methods

Search strategies included the use of the following MESH terms in PubMed: "zinc status," "pressure ulcer," "pressure ulcers in older adults," "wound healing," and "zinc sulfate." PubMed was chosen for its comprehensive database of peer-reviewed journals in all aspects of medical and allied health, often encompassing the contents of several other databases used for queries of medical, nursing, and dietetics research. A total of 41 full-text journal articles, books, and credible web-based content were evaluated. The inclusion criteria were adequate sample size, sound methodology, generalizability of findings, and adherence to Medical Subject Heading categorization. Articles between 2006 and 2016 were selected. One article from 2001 was selected that investigated adverse effects of supplementation, and one article from 1968 was reviewed in order to incorporate contrasting information on oral zinc supplementation effects alone. Inclusion criteria consisted of long-term care and home care populations and articles investigating chronic wounds, including pressure injuries, and healing progression. A large number of papers reporting on intensive care units and home health settings were excluded. Exclusion was due to the duration of the study being too short, or having insufficient follow-up, with no ability to consider course of wound healing. For comparative purposes, one study from intensive care, home health, and dietary zinc alone was evaluated [25, 26]. There was a lack of research that measured nutrient loss from PI wound exudate in the elderly, but one study was included that investigated select vitamin and trace element loss from wound exudates in adults [27]. The use of topical Zn as an intervention was also excluded, as this is not ingested and therefore not considered a nutritional intervention. Search results were narrowed by English language, time frame, and peer review.

## 3 Results

In a total of 10 studies, 41 were analyzed and evaluated. These findings include four observational studies and six clinical trials, which are examined in Tables 1 and 2, respectively. Outcome assessments were analyzed, including nutritional status, Zn biomarkers, micronutrient measurement in wound exudate, and PI healing. These findings included Braden Pressure Ulcer Risk Assessment Score (BPURAS), Pressure Ulcer Scale for Healing (PUSH) tool and other skin assessments, subjective global assessment (SGA), Patient-Generated-Subjective Global Assessment (PG-SGA), and micronutrient biomarkers in plasma and wound exudate.

Three observational studies found relationships between inadequate oral intakes, including Zn, and pressure injuries, suggesting that there is a connection between malnutrition and both PI development and healing. One of these studies determined that micronutrients are also lost from wound exudate. Of the clinical trials, three concluded that wound-specific ONS formulas in

combination with standard PI care are superior to standard ONS formulas for wound healing outcomes. However, one clinical trial did not find one formula to be preferable over the other.

No studies were found using Zn tissue measurements or the copper to zinc (CZr) ratio per exclusion criteria. Also, studies that use oral Zn sulfate as the intervention have not been conducted within the last 10 years. All intervention trials of oral nutrition supplements (ONS) followed wound care protocols in all groups. ONS intervention studies that met the criteria contained several nutrients, in addition to Zn, also known to be beneficial for wound healing. These studies demonstrate that dietary inadequacies are likely in older adults with pressure injuries, and nutritional interventions are beneficial in improving wound healing.

**Table 1** Review of observational literature (2005–2015)

| Study | Subjects/age | Measurements | Findings | Statistical analysis |
|---|---|---|---|---|
| Raffoul et al. 2005 | Nine patients with ulcerations, 71 ± 10 SD y/o[a] | Food intake assessed by standardized meals, Plasma Zn[b] measured | Micronutrient status alterations & malnutrition observed. Energy & protein meal intakes inadequate to meet estimated nutritional needs. ONS[c] compensated for these deficits. | Energy consumption: 76 ± 21% meals, ONS additional 35 ± 12% of energy target. Baseline plasma Zn levels: (sub-optimal) 9.4 μmol/L[d], slight improvement with ONS ($P = 0.07$). |
| Banks et al. 2010 | 2208 acute (Average 66.5 y/o) & 839 aged (78.9 y/o) care facilities | SGA[e] measured Ntr status, Pls[f] categorized by the Australian Wound Management Association | Malnutrition associated with increased risk & odds ratio of developing a Pl of >2 times. Increased severity associated with increased odds ratio of having a Pl, Pl stage, and number of Pls. | Subjects with malnutrition, as measured by SGA, have an adjusted odds ratio of 2.6 (95% CI 1.8–3.5, $P < 0.001$) & 2.0 (95% CI 1.5–2.7, $P < 0.001$) acute & aged care facilities, respectively for having a Pl. |
| Wojcik et al. 2011 | 31 home care clients with PUs (21) or venous stasis ulcers (10), Mean 68.05 y/o | BPURA[g] score, 3-day food records analyzed by electronic database comparing EAR[h]. | Clients with chronic wounds are at risk for nutrient deficiencies, which could delay wound healing and increase wound severity. | 59% subjects adequate energy intakes without ONS. 26% clients: >1 one PI. 36% subjects: Stage 3/4 Pls. 18 used ONS, 77.8% adequate zinc intakes. 11 subjects without supplement, of those 45.5% had adequate Zn intake. Higher BPURA in higher protein intakes ($P < 0.05$). |
| Hourigan et al. 2015 | 17 patients with wounds (Mean 45 y/o) | Wound exudate samples collected to analyze specific micronutrients | Significant amounts of micronutrients can be lost wound exudates. Nutrition support should be considered with these losses. | Mean 24-h loss Vitamin A, C, E: 0.3, 2.8, & 11 mg, respectively, & Zn, Fe[i], & Cu[j] were 0.5, 0.4, & 0.25 mg[k], respectively. Greater micronutrient loss from open wounds. |

[a]Year old
[b]Zinc
[c]Oral nutrition supplement
[d]Micromole per liter
[e]Subjective global assessment
[f]Pressure injury
[g]Braden pressure ulcer risk assessment score
[h]Estimated average requirement
[i]Iron
[j]Copper
[k]Milligram

**Table 2** Review of clinical trial literature (2005–2015)

| Study | Subjects/age | Measurements | Findings | Statistical analysis |
|---|---|---|---|---|
| Houston et al. 2001 | Older adults with stage 2–4 Pls[a], Mean age not noted. | Healing rate of pressure sores, Pl volume | Zn[b] supplementation should be used with caution with consideration to toxicity. | 26 patients received large dose 440 mg[c] (100 mg elemental Zn/d) zinc sulfate & 44 patients controlled. Increased incidence of adverse events; infection & nausea/vomiting in treatment group. |
| Heyman et al. 2008 | 245 patients with; stage 2–4 Pls at 61 LTC[d] facilities, Average 82.2 y/o[e] | Pl area, calculated by measuring the lesion's width and length | High protein ONS[f] with fortifications significantly reduces the mean Pl area of LTC residents when used with standard Pl care. | Treatment group received ONS fortified 200 mL[g] ONS, 2.3 serving&####; daily providing 46 g protein, 6.9 g arginine, 87 mg Vitamin E, 575 mg Vitamin C, and 21 mg Zn for 9 weeks. Reduction of Pl area; from 1580 ± 3743 mm square to 743 ± 1809 mm square; a 53% reduction ($P < 0.001$) |
| Cereda et al. 2009 | 28 elderly participants with stage 2–4 Pls of recent onset, mean 81.8 y/o | PUSH[h] tool and are measured | Use of a ONS enriched with protein arginine, Zn, and vitamin C is preferable to a standardized ONS, which accelerates the rate of Pl healing. | Treatment group received additional 400 mL ONS enriched with 34 g protein, 6 g arginine, 18 mg Zn, & 500 mg vitamin C. PUSH scores revealed a higher mean reduction in Pl area in Treatment 72% from Control group 45%. Treatment group had higher Zn serum levels ($P < 0.01$). |
| Bauer, Ienring and Waterhouse 2013 | 24 patients with Pls Mean 67.8 y/o | PUSH, & PG-SGA and 24 hour food recall. | Standard ONS may be more effective at wound healing than a specialized wound ONS | Wound specific ONS with arginine, vitamin C, and Zn compared to standard ONS. PUSH scores improved 33.4% in standard ONS group and 4.3% improvement in wound specific ONS. |
| Sakae et al. 2013 | 42 patients with Pls, Mean 64.9 y/o | Rate of Pl healing as measured by the PUSH tool and serum Zn biomarker measured | CAR11 and PLZ12 groups were not significantly different from each other. CAR[i] and PLZ[j] may improve Pl healing during 4 weeks. Future studies with larger sample sizes are needed to confirm these results. | (1) Control group: untreated. (2) PLZ group: 150 mg PLZ (116 mg CAR & 34 mg Zn). (3) CAR group: 116 mg CAR.PUSH scores >in group 2 & 3 ($P = 0.02$) & ($P = 0.009$). Serum Zn significantly increased ($P < 0.001$). Mean. PUSH scale improvement: (1) 0.8 ± 0.2; (2) 1.6 ± 0.2; (3) 1.8 ± 0.2 |
| Cereda et al. 2015 | 200 malnourishe; patients with Pl, Mean 81.4 y/o | Percentage of change in Pl area at 8 weeks | Use of a ONS enriched with arginine, Zn, and antioxidants along with wound care management is an additional benefit to wound healing. | Treatment group: ONS enhanced with arginine, Zn, and antioxidants (400 mL). Controlled group received standard ONS. Treatment group had greater reduction in PU area; mean reduction&###; of 60.9% compared to controlled group 45.2% |

[a]Pressure injury
[b]Zinc
[c]Milligram
[d]Long-term care
[e]Years old
[f]Oral nutrition supplement
[g]Milliliter
[h]Pressure ulcer scale for healing
[i]L-Carnosine
[j]Polaprezinc

## 4 Discussion

The elderly adult population is at risk for compromised skin integrity, inadequate dietary intakes, reduced Zn absorption, and malnutrition. As a result, development of PIs is a common yet detrimental complication that can lead to poor clinical outcomes. Though Zn deficiency is difficult to diagnose due to lack of sensitive Zn biomarkers, studies indicate that Zn status is likely to be compromised in older adults with PUs. Recognizing these conditions in this target population is vital in initiating nutritional interventions as Zn plays an important role in wound healing for collagen synthesis and improved immune response. The use of both standard and specialty ONS as nutritional interventions should be considered for PI wound healing.

Identifying nutrient inadequacies. Both macro- and micronutrient oral intakes were found to be suboptimal in the majority of older patients with PIs. Two observational studies measuring oral intakes demonstrated that food intake alone is inadequate in meeting sufficient nutrient intakes for both caloric targets and Zn intake in older adults with PIs. Raffoul et al. [25] found that target energy consumption was variable through food intake 76 ± 21%, and the addition of ONS provided 35 ± 12% of energy requirements [25]. Prior to the onset of offering ONS, plasma Zn levels measured a median of 9.4 μmol/L, below the normal range, and improved slightly ($P = 0.07$) by day 10 after ONS consumption. These findings suggest that ONS acceptance reduces these energy and mineral intake deficits. Similarly, the Wojcik et al. study found that only 59% of subjects met their energy requirements and 41% met estimated protein needs for wound healing without supplementation [26]. Additionally, intakes of Zn were least likely to meet Estimated Average Requirement compared to other mineral biomarkers measured; 45.5% of subjects had inadequate Zn intakes with food alone. Alternatively, only 22.2% of subjects accepting the ONS consumed inadequate Zn levels indicating a measurable improvement. These improvements in nutrient consumption, including higher protein intakes, were associated with higher BPURAS scores. These findings suggest that older adults with PUs are at risk for a number of nutrient deficiencies, including Zn, which may impair the wound healing process.

An observational study investigated vitamin and trace element losses from wound exudates and found 0.5 mg Zn loss from open abdominal wounds and 0.3 mg Zn loss from soft tissue wounds over 24 h [27]. This loss accounts for 5% and 3% of the RDA for Zn, respectively. While this study did not focus on the older adult population with PUs, it is important to consider as nutrient loss may also occur from wound exudate in PIs. These studies support the previously stated premise that older adults with PIs are prone to inadequate dietary intakes, as well as nutrient losses from wound exudate, which increases risk for malnutrition, a condition that is associated with increased severity in PIs [28]. Though identifying Zn deficiency may be difficult, interventions for PI healing should consider this possible insufficiency, in addition to the other known factors.

Effects of zinc in ONS for treatment of wounds. Nutritional interventions for PIs are beneficial in order to decrease wound healing time [28–32]. Heyman et al.'s [29] clinical trial of ONS containing fortification of several nutrients providing additional protein, arginine, vitamins C and E, and Zn, in addition to standard wound care in the aged, is supported in use for the reduction in PI area and optimal for wound healing. The use of specialized ONS designed to improve wound healing versus standardized ONS remains controversial. One investigative study concluded that specialized ONS formula, enriched with arginine, Zn, and antioxidants, was superior to standard ONS resulting in a greater reduction in PI area with a mean reduction of 60.9% compared to 45.2%, respectively [30]. Another clinical trial found that both high protein-, arginine-, Zn-, and vitamin C-rich ONS and standard nutrition formula improve PU healing, but the enriched ONS had higher rates of PI healing as measured by PUSH score and ulcer area ($P < 0.05$) concluding that it is a preferable formula for wound healing [31]. Serum Zn levels were also monitored. There was a 107.5 ± 106.6 μg/dL Zn increase from

baseline to week 12 in the treatment group, whereas serum zinc dropped to 32.5 ± 87.1 μg/dL in the control group suggesting that nutritional inadequacies are more efficiently repleted with the treatment formula.

In contrast, a study investigating a wound-specific ONS, enriched with immune-enhancing nutrients including arginine, zinc, and vitamin C, versus standard high protein ONS found that the standard ONS formula was of more benefit in improving PUSH scores and healing ($P = 0.044$) [32]. At the same time, nutritional status and quality of life remained similar between both groups. These variable findings in the literature should prompt healthcare clinicians to implement an appropriate plan of care based on individual needs to most effectively treat each patient.

The use of Zn as polaprezinc. Another avenue for possible nutritional interventions in wound healing is L-carnosine (CAR), a dipeptide composed of β-alanine and L-histidine, and its zinc complex, polaprezinc (PLZ) [33]. Because CAR has many biological functions linked to antiaging activity, it is presumed to have an effect on age-related diseases, including wound healing [34]. The first controlled clinical study to investigate these ONS for PU treatment in their respective groups, CAR and PLZ, along with a control group, receiving no supplementation, resulted in significant improvements in PUSH scores in both treated groups ($P = 0.02$ vs. control) and ($P = 0.009$ vs. control), respectively [33]. The PLZ treatment group experienced increases in serum Zn levels ($P < 0.001$), though serum copper had decreased ($P < 0.001$). These findings suggest that both CAR and PLZ may be potential treatments for PUs. Future research should involve larger sample sizes with randomized trial methods and special consideration to appropriate length of PLZ treatment in order to ensure safe dosing regimens.

Use of Zn alone for wound healing. Due to Zn's notable role in immunity and wound healing, clinical trials testing the effects of Zn sulfate as an oral supplement date back to the 1960s [35]. However, recent studies with oral Zn as the sole intervention are much less frequent, likely as a result of disappointing findings. Moreover, older patients receiving high doses of Zn sulfate (440 mg) experienced adverse effects attributed to the supplementation, including infection, which required more treatment for antibiotic therapy, and nausea/vomiting [36]. Recent studies have shown more promising results with wound healing by incorporating a variety of nutrients through ONS.

Cost-effectiveness in treatment. Another consideration to clinical care is cost-effective treatments. A clinical trial completed a cost analysis on the difference in medical costs of PI care and cost-effectiveness of a disease-specific nutritional formula, enriched with arginine zinc and antioxidants, versus a standard formula. Both groups had improved wound healing, though the experimental group had greater improvements in the reduction in PI area ($P = 0.012$). The specialty formula costs significantly more ($P < 0.001$), but the patients receiving this formula had reduced costs in nonnutritional wound care management of PIs ($P = 0.001$). This care included dressing materials, pressure relieving mattresses, nursing expenses, and antibiotics [37]. As a result, the use of a specialized formula should not be discounted based on initial higher cost.

## Conclusion

This descriptive review had the following limitations: Zn as the primary nutrient under consideration, smaller sample sizes, and studies short in duration. Further research is needed to investigate variations in trace element supplementation, including the combination of minerals versus the addition of single minerals as supplements. Dosing regimens should also be considered when gauging efficacy. To further strengthen conclusions regarding trace element mixture administration, careful consideration of methodology and study design should be undertaken. This review has raised the question of whether or not the RDA for older adults should be reevaluated in order to adjust for impaired absorption in Zn. Optimal Zn nutriture would improve the quality of life in older adults with wounds.

Monitoring patient risk and implementing appropriate nutritional interventions based on individual needs are vital for maintaining nutritional status, thereby optimizing patient outcomes. In conclusion, for older adults, the clinical application of supplementation of zinc, along with calories, protein, and other nutrients in PIs, improves outcomes, shortens healing time, and decreases comorbidities.

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# Universal Pressure Ulcer Prevention Bundle with WOC Nurse Support: A Pressure Injury Prevention Journey

Megan Anderson

## 1 Introduction

The occurrence of pressure injuries in the United States continues to be a significant issue for patients in the acute care setting. Pressure injuries are painful and debilitating as well as costly. Incidence rates range from 14 to 42% in critical care units [1, 2]. This is particularly true in critical care units. Critical care units in 196 hospitals reported an average rate of 7.79–13.89% for stage II or greater [1]. Changes in reimbursement from the Center for Medicare and Medicaid Services (CMS) were made in 2008 related to the growing pressure injury issue. Nurses often think of the extremely critically ill patients as those that develop pressure injuries. Such as those with hemodynamic instability, unstable spinal cord injuries, or patients that require prone therapy related to pressure injuries, and the frail elderly. Those with a moderate risk as defined by the Braden scale may be overlooked or not as closely scrutinized by nursing staff until an issue has already developed.

The National Pressure Ulcer Advisory Panel (NPUAP) now defines a pressure ulcer as an injury, "A pressure injury is a localized damage to the skin and/or underlying soft tissue usually over a bony prominence or related to a medical or other device. The injury can present as intact skin or an open ulcer and may be painful. The injury occurs as a result of intense and/or prolonged pressure or pressure in combination with shear. The tolerance of soft tissue for pressure and shear may also be affected by microclimate, nutrition, perfusion, co-morbidities and the condition of soft tissue" [3]. Pressure injuries may affect patient's quality of life for a limited to infinitive time as well as the financial burden of treating the injury. The long-term impact that a pressure injury may have on a patient is often not apparent while the patient is still hospitalized.

Often the patient who comes to the hospital as a healthy adult is overlooked based on the assumption that they are "alert and oriented" or "were up and moving" prior to the hospitalization. However, they are immobilized and often have many medical devices. It takes an interdisciplinary effort to support nursing in the care of complex patients with multiple medical devices that are being managed and assessed by other disciplines than nursing alone. This often includes the bedside nurse, skin champion, WOC nurse, physician/provider, respiratory therapists, dieticians, nursing leadership/advanced practice registered nurses (APRNs), and specialty clinicians (orthopedics and trauma, etc.).

Education for nursing staff alone is not an effective intervention that will sustain over time, whether due to staff turnover or competing priorities. Nurses' lack of knowledge may be

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another contributing factor to the pressure injury problem, indicating that alternative methods to applying a protocol may increase the ability for nursing staff to adhere to it. A study by Padula et al. [4] taken from pressure injury prevention experts indicates that internal factors that influence evidence-based practice are hospital prevention campaigns, the availability of nursing specialists, and the level of preventative knowledge among hospital staff. External factors that influence best practice are financial concerns, application for Magnet recognition, data sharing among peer institutions, and regulatory issues.

## 2 Background

North Memorial Health Hospital is a level one Trauma Center in Minneapolis, Minnesota (MN). There were three intensive care units (ICUs) and occasionally a fourth overflow unit in 2012. Focused efforts on decreasing pressure injuries had been in place for as long as 10 years. Initially a skin champion role was created by the quality improvement team on the Trauma-Neuro ICU. The role of the skin champion was to round on all of the patients in the unit once to twice weekly, assess patients, and make recommendations for pressure injury prevention. Eventually the skin champion role was adopted by the other ICUs and most recently the medical-surgical general floors. Together this group formed the hospital-wide skin champion team led by the Wound, Ostomy, and Continence (WOC) Nurse. Transformations started to happen with skin champions focusing on assessments and educating nursing at the bedside during assessments. If any new issues or challenges were noted, the WOC nurse was notified for an assessment and pressure injury prevention plan.

Eventually, the effectiveness of this intervention depended on the ability to have a skin champion available to round with nursing. Frequently, interventions were not taken on by nursing independent of the skin champion or WOC nurse. There was also a lengthy pressure injury prevention protocol in place (4 pages and approximately 37 interventions). The protocol was accessed by computer or printed to be placed at the bedside. Printed copies at the bedside eventually became discouraged due to version control once updates were made.

North Memorial participated in a quarterly prevalence and incidence audit. A 1-day snapshot audit was used for comparison to the National Data Nursing Quality Indicator (NDNQI) and designated Magnet institutions. From 2011 to 2012, the incidence of critical care hospital-acquired pressure injuries ranged from 3 to 15% on any one of those quarterly audits. At that time, the national median was 0% with a national high of 10% and national low of 0% according the NDNQI.

North Memorial Health Hospital applied for a grant from the Wound, Ostomy, and Continence Nursing Society to research a better delivery for pressure injury prevention. Reasoning behind the action was due to higher rates of pressure injuries during the 1-day NDNQI audits, the need for additional information about the true rate of critical care hospital-acquired pressure injuries, the need to change the current outcome to benefit patients, and the need to understand practice changes that have potential to improve outcomes. The grant was awarded and our focus shifted toward simplifying and standardizing our pressure injury prevention practices in the ICUs.

In order to simplify the bedside protocol, the desire was to review the current evidence and gather a core set of interventions that when applied consistently would have the highest level of impact [5]. The bundle as a protocol is designed to be applied to all patients regardless of their risk. The SAFER bundle that was developed was structured with the idea that all critical care patients are at risk for pressure injuries. The actual number of patients that are considered unstable or complex is actually minimal at any given time within a unit. The SAFER bundle offers the first layer of pressure injury prevention until the WOC nurse or other consultants or members of the interdisciplinary team are able to determine what additional interventions may be necessary in addition to the bundle.

The idea of using a bundle for care was not new at this time. It had been applied to patients

with central lines for prevention of central line infections as well as intubated patients for the prevention of ventilator associated pneumonia. Using a bundle approach also requires a champion(s). In this case, it was the proactive support of the WOC nurse. Bundles are currently being utilized for care of several best practice initiatives including catheter care, fall prevention, prevention of delirium, and pressure injury prevention. The Minnesota Hospital Association (MHA) has adopted the bundle idea based on the Agency for Healthcare Research and Quality (AHRQ) Injury bundle. The Minnesota Hospital Association also went one step further and developed a medical device-related pressure injury (MDPRI) bundle in order to address the growing issue of medical device-related pressure injuries [6].

The current protocol was to consult the WOC nurse for a patient with a Braden risk score of 12 or less. Other patients considered to be at highest risk and may benefit from a WOC consult included continuous renal replacement therapy (CRRT), diagnosis of sepsis, induced hypothermia, impaired perfusion, cardiac or hemodynamic instability, persistent malnutrition and protein loss, decreased level of consciousness (LOC) and inability to participate in own care, receiving mechanical ventilation therefore the presence of an endotracheal tube (ETT) or other devices that may create pressure on skin or mucous membranes, and those undergoing surgery or procedures lasting more than 3 h or multiple procedures.

The WOC nurse consult needed to be placed into the computer, which was a completely separate step and often missed until a skin champion rounded or was assigned to care for the patient. The WOC nurse would then consult with nursing and assess the patient head to toe and initiate a pressure injury prevention plan of care (POC) and document findings. The plan of care at that time reflected a "check in the box" on a paper form that the pressure ulcer/injury prevention protocol was in place. The WOC nurse would initiate intervention that had not been applied previously (specialty mattress selection, device-related care, incontinence skin care protocols, heel suspension boots, etc.) This was especially helpful at that moment in time when the skin champion or WOC would assess the patient from head to toe to determine a prevention plan. More often than not, the plan was similar or the same for several patients on a unit. However, the WOC nurse consult was found to be insignificant in long-term success without proactive early basic interventions. The ongoing assessment and monitoring of the patient's skin and adherence to the pressure injury prevention plan was not consistent and pressure injuries still developed. This consult was often delayed as well due to the unavailability of the WOC nurse.

While protocols and standard of care are effective in providing best practice care, the bundle is general enough to apply to all patients and nursing to recognize that each individual patient has a specific set of circumstance, body, and comorbidities contributing to a unique pressure injury risk. Not every trauma patient is going to fit in the same cast or splint, not every cervical collar is going to fit every patient the same nor will they have the same level of mobility or limitations. Patients have different anatomy; this can be as simple as the shape of the nare. Some patients have pointed nares that will be troublesome when trying to reposition a nasogastric tube or feeding tube. One patient may have gastric leakage around their percutaneous enteric gastric (PEG) making them more susceptible to pressure under the device, while another patient may not. Pressure injury prevention needs an overall frame work for nursing staff to apply with each patient. From there, it will depend on the patient's particular situation, and this may often change from day to day or hour to hour, especially in critical care patients. One of the many complexities of pressure injury prevention is that each patient interaction has the potential to prevent or cause additional pressure, friction, and shear. An example of this is when repositioning a patient, once the HOB is elevated, the heels may slide off the pillows. If this is not feasible, heel suspension boots should be applied for consistent elevation. Another example is when repositioning the patient with a tracheostomy, the ventilator tubing needs to be supported and repositioned with the

patient so that there is not additional tension causing friction at the site of the trach flange. This will be especially troublesome if the patient has unusual anatomy, tighter than normal sutures, or a significant amount of secretions. Is the tubing supported so that the weight of it is not applying additional pressure against the trach flange? While head-to-toe assessments are an essential part of nursing practice to catch early warning signs of pressure injuries, it is just as crucial to consider positioning of pressure sources at all times throughout the care of the patient.

## 3 The Pre-intervention Phase

The first phase of the study was to simply study our current protocol and continue with twice weekly skin champion rounds, quarterly NDNQI audits, and WOC nurse consults based on specific triggers or a Braden score of 12 or less. It was estimated by historical data that the current rate or incidence would be 9% and the goal for the study was to decrease the incidence to less than 3%. The first phase of the study lasted approximately 6 months and demonstrated an incidence rate of 12%. In order to be involved in the study, patients were assessed by a trained skin champion or the WOC nurse upon admission to the critical care unit. This was to determine that they did not have a pre-existing pressure injury. It was felt that the overall prevalence of community acquired pressure injuries noted on admission to the hospital was increased due to the fact that patients were thoroughly assessed by a skin champion or WOC nurse at the time of their admission. These pre-existing pressure injuries also received more timely assessments and interventions due to early discovery.

During the initial phase and data collection, the SAFER bundle (Table 1) was developed. It was based on the pressure injury interventions that had the highest level of evidence at the time of the initiation of the study. The Institute for Clinical Systems Improvement (ICSI) and NPUAP guidelines were reviewed as well as literature. It should be noted that a dietician consult and early intervention was not included as the facility had a well-established and proactive process for dietary consults in the critical care units. The use of prophylactic foam dressings was also widely established. Silicone foam dressings for pressure injury prevention and management are frequently utilized. They are stocked on each unit with the theory that the staff will use what is first available to them and while it may not be the best long-term solution or dressing choice, it is perfect for prevention or management of moisture and friction, microclimate, and especially under devices. We did not include this in our bundle because we were not able to adapt a protocol that would support application of silicone foam to every patient or even necessarily a specific group due to body habitus differences and in large, incontinence. We have a consistent practice in using silicone foam dressings prophylactically when appropriate.

### 3.1 Skin Emollients

It was routine practice for the WOC nurse to write a nursing order for a dimethicone-based skin barrier to be applied to the sacral-coccygeal area preventatively for skin health and to prevent friction and shear as well as treatment of microclimate.

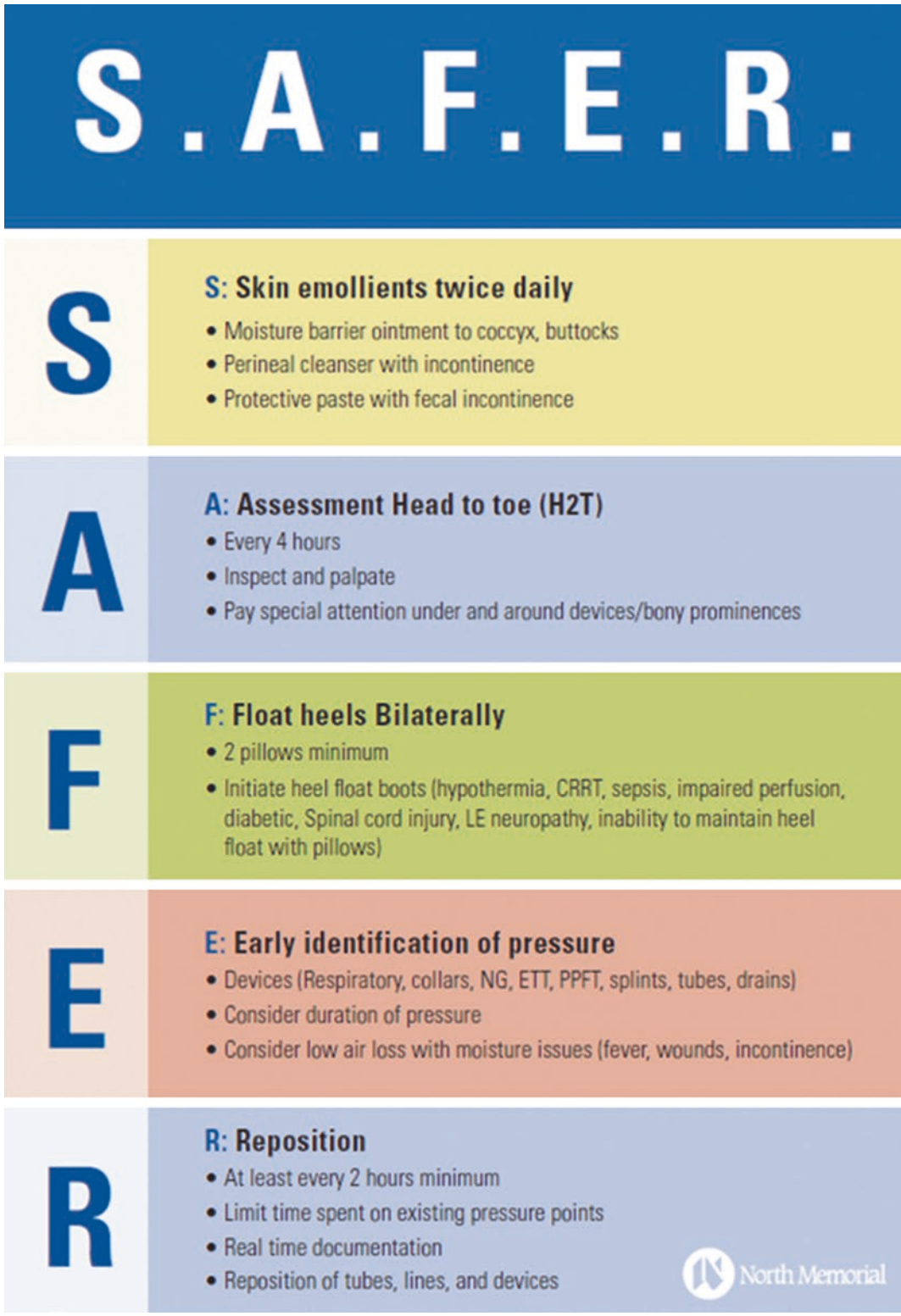
### 3.2 Assessment

Critical care units at North Memorial perform head-to-toe assessments every 4 h. While this is an aggressive time line, the thought was that catching early warning signs and removing sources of pressure early would be beneficial. It also places an emphasis on the fact that devices need to be reevaluated frequently, especially when the patient is repositioned.

### 3.3 Floating of Heels

Routine practice was to place one pillow between the patient's knees and ankles. This was enough to sometimes off-load or reduce pressure on one heel, it did not elevate or protect the medial

**Table 1** SAFER bundle



surface, and often when the head of bed was elevated greater to 30° after the patient was positioned, the heel(s) would again come in contact with the bed surface. Specific patients were triggered to automatically have heel suspension boots based on previous results from the facility as well as NPUAP guidelines. This applied to patients with spinal cord injuries (especially new spinal cord injured patients), those with decreased or lack of sensation to the lower legs or neuropathy, patients receiving multiple vasopressors or continuous renal replacement therapy (CRRT), and induced hypothermic states. The second focus of this intervention was to ensure that if pillows were the practice of choice, that there was a two pillow minimum to off-load bilateral heels rather than just one at a time.

### 3.4 Early Identification of Pressure Sources and Need for a Specialty Bed

Our practice was varied in how to secure and manage or reposition nasogastric and feeding tubes. There were multiple devices or ways of securing them, and repositioning was infrequent and sporadic. Due to each individual set of circumstances, we were unable to specify one specific way to secure tubes for all patients. In some instances, it depended on the position the patient was in at the time, and this was changed at least every 2 h. Medical devices were not necessarily viewed as sources of pressure. It was not clear what devices should or should not be removed for head-to-toe assessments, and this practice was varied as well, often due to comfort level or past experience. There was a confusing practice about who would reposition endotracheal tubes (ETT) between nursing and respiratory therapists and how best to reposition based on best practice recommendations to reposition every 2 h. Nursing was not comfortable with the specialty bed ordering process, and it was often assumed that the skin champion or the WOC nurse would order the specialty bed when they rounded. Early placement of specialty beds, specifically low air loss, was missed or often delayed for this reason.

### 3.5 Repositioning of Patient and Devices

The hospital standard was to reposition patients that were at risk as defined by the Braden score at least every 2 h. The fine print in the lengthy protocol discussed repositioning more often for a HOB greater than 30° or weight-shifts/micro-turns for patients deemed too unstable to turn. This piece was also infrequent and not commonly understood or practiced. The second point of repositioning is to point out that devices are properly fitted or repositioned with the patient to ensure that there are not devices, cords, tubes, etc. left under the patient or applying more friction against the skin when in a certain position. A specific example of this would be adopting the practice that ETTs are recommended to be repositioned every 2 h while using a commercial tube stabilizer. The patient is turned every 2 h, if the rotation of the ETT is off sync with the rotation of the patient, there will be additional pressure and friction against the oral mucosa because the tubing is positioned opposite from the direction the patient is turned. Simple reminders such as turning the patient and repositioning tubes together make sense as does assigning the rotation of the ETT to nursing because both interventions may be completed together. When the patient is turned left, the ETT will be rotated and left and when turned right, the ETT will be positioned to the right. Another similar practice to adopt is assessing the Foley catheter securement and positioning each time the patient is repositioned to ensure that it is not under the patient or between the legs.

**Table 2** Frequencies of UPUPB focus recorded in rounding logs

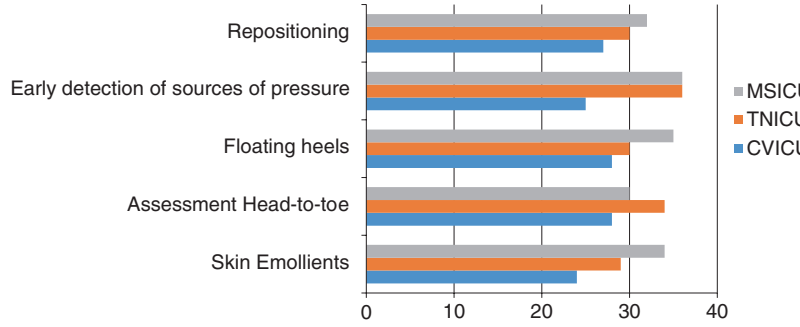
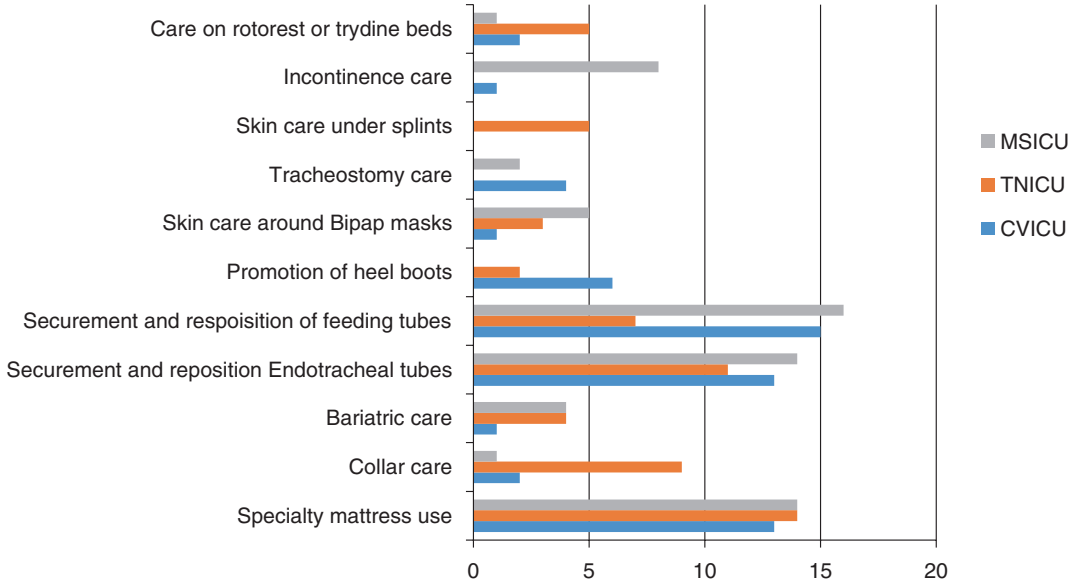



**Table 3** Frequencies of additional WOC nurse interventions recorded in rounding logs




## 4 The Intervention Phase

During the pre-intervention phase, it was noted that nursing was not comfortable with injury terminology, staging, and documentation. Once the intervention phase was initiated, nursing was assigned to complete the first two modules of the NDNQI pressure ulcer/injury online learning modules. Their knowledge was tested at the end of the module, and they were required to pass. Approximately 90% of the critical care staff completed this module.

The intervention phase of the study was approximately 2 months in duration. Two WOC nurses with a total FTE of 1.8 began rounding proactively in each of the ICUs twice weekly (Tables 2 and 3). During these biweekly rounds, WOC nursing introduced the SAFER bundle to staff. Consults specifically placed for a Braden score of 12 or less were still completed; however, often the WOC nurse was already aware of the patient and had discussed appropriate interventions and assisted nursing with application during biweekly rounds.

The staff was a little unsure how to view their new bundle approach. There were several comments about how "we already do those things" or "that's nothing new." One nurse actually commented that it didn't seem like "rocket science." The WOC nurses reinforced that the interventions were not in fact new and were evidence based. The emphasis was on the early intervention by bedside nurses and applying the bundle to all critical care patients without delay in a consistent manner. Care was taken during rounds to explain the reasoning behind the interventions and demonstrate proper technique or troubleshooting how to secure certain devices. Orders would be placed in the chart for staff to follow if there was a recommended intervention that needed to be more specific than the SAFER bundle. The WOC nurse would often complete more frequent consults and reconsults due to the frequency of rounding and often did not need to perform an entire head-to-toe assessment. The skin champions still rounded twice weekly on the units as well and stayed in communication with the WOC nurse dividing the work or working together on complex scenarios.

The SAFER bundle was printed on large posters to be placed at a central location on each unit and easy for viewing. There were also smaller versions posted in each critical care room. This made for more frequent review and easier access than printing a lengthy protocol addressing the availability and ease of use of a new delivery approach.

The WOC nurse rounds were well received by nursing. Rounds were performed on all three shifts between days, early and late evening, and early mornings with the night shift. This allowed the WOC nurse not only to touch base with each patient but to gain a greater audience regarding at the elbow education and troubleshooting by meeting with the off-shifts that did not historically have access to a WOC nurse and were dependent on placing a consult.

## 5 The Post-Intervention Phase

During the third phase of the study, information was again collected on pressure injury incidence with the SAFER bundle in place and proactive twice weekly WOC nurse rounds. Staff would remark that they remembered they had a question or issue to consult when the WOC nurse would round on the unit. Staff would also share recent experiences where they did not have access to the WOC nurse and would ask for confirmation about the intervention they put in place, or ask, "What should I do next time?" The rapport between WOC nursing and staff nurses strengthened while practice began to shift toward the WOC nurse validating the care that was being put in place by nursing rather than looking over every patient head to toe at one moment in time. The bundle would be reviewed and the patient was assessed for any further needs that were not met by the bundle.

## 6 Results of Study

At the conclusion of the study, North Memorial had a decrease in ICU-related hospital-acquired pressure injuries from 15.5 to 2.2%. There were no reportable pressure injuries as defined by the Minnesota Hospital Association (MHA) (stage 3, 4, or unstageable pressure injuries) that developed during this post-intervention phase.

The success of the bundle and WOC proactive nurse rounding was adopted into practice; however, the ability to maintain twice weekly proactive WOC rounds was difficult to sustain due to changes in WOC staff for a lengthy period of time. Several turnovers in staff in the ICUs also presented a challenge as the knowledge base that was reinforced during the intervention and post-intervention phases as well as the rapport with the WOC nurse deteriorated. Eventually, it was determined that the WOC nurse would have a "shadow" day with each new hire for critical care

that consisted of 2–4 h and preventative rounds on the ICUs. This provided the nurse with valuable one-to-one experience and training with one of the WOC nurses specifically related to pressure injury prevention in the ICU.

A process was developed where this was built into orientation and a head-to-toe assessment competency was also completed with the WOC nurse on an ICU patient during this shadow experience. The WOC nurse would also seek out opportunities to show the new nurse hire some complex situations for repositioning as well as common and not so common devices that could be expected in the ICU. A focus was placed on ownership of devices, and if the care of a device was unknown, nursing would know who to notify or consult with regarding orders and routine care. The idea behind the shadow experience is not to show the nurse complex wounds and how to provide wound care, but rather to focus on the tools and resources available for prevention as well as how to troubleshoot and escalate potential pressure injury risks and situations when necessary.

A critical care clinical nurse specialist was hired and began to work closely with the WOC nurses on pressure injury prevention in the ICUs. Standards were developed further and practices better defined for bedside nursing staff. Compliance with the SAFER bundle was audited by skin champions and leadership. Gradually the role of championing pressure injury prevention and the SAFER bundle was becoming a team effort with WOC nursing, clinical nurse specialists (CNS), and nursing leadership.

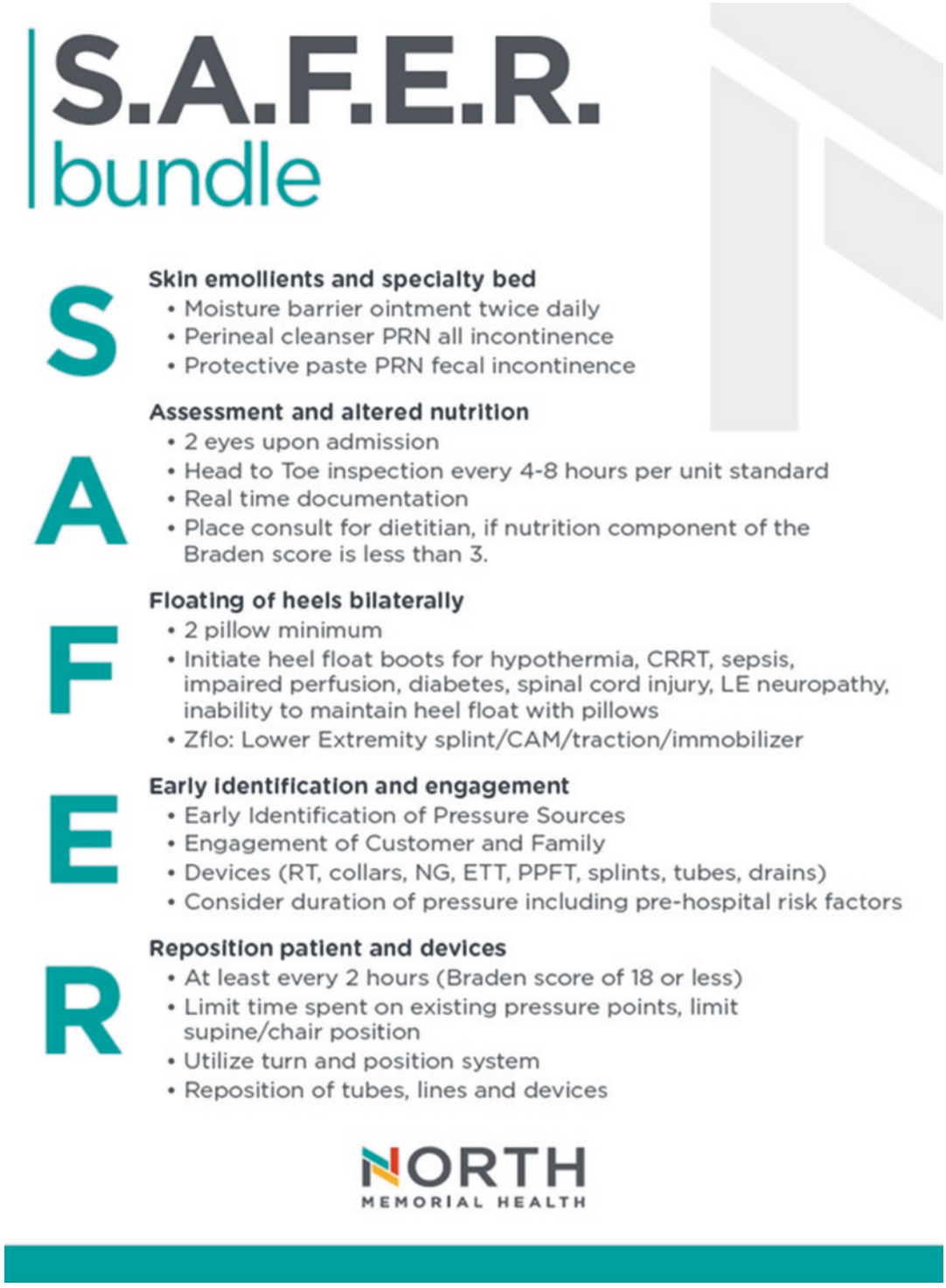
While the ICUs were focusing on putting a standard bundle in place, there were still several pressure injuries developing on the medical-surgical floors that lacked early intervention of basic pressure injury prevention interventions. The skin champions felt strongly that SAFER bundle would be beneficial for all patients throughout the facility. There were some gaps noted upon transfer from the ICU that the SAFER bundle interventions were not consistent or known well enough to the nursing staff on the medical-surgical presenting delay in care or reconsult to the WOC nurse. The bundle interventions were not placed in the order section of the chart unless the WOC nurse had specifically written them. This was particularly the case with specialty beds, the transfer process did not include transferring the bed when a patient left critical care, and often a new bed was ordered a few days later by someone on the receiving unit. New practices and refinements to the original SAFER bundle were needed as it had been in place for 3 years.

## 7 The SAFER Bundle 2.0 All North Memorial Health Hospital Patients

A small work group of CNSs and WOC nurses met and began updating the SAFER bundle 2.0 version to be applicable to the entire hospital (Table 4). Best practice interventions were reviewed; it was determined at that time to put the dietician consult in place. The importance of educating our patents and families had become apparent over the past few years, and this was also added to the bundle.

The limited supine position campaign had been gaining steam throughout the facility. The basis of this intervention was that when the entire length of the hospitalization was reviewed, patients were often spending one half to two thirds of their hospitalization supine due to frequent procedures, surgeries, and the long-established practice of turning our patients left, center, right, center, left, etc. Best practice guidelines pertaining to positioning patients off their surgical position post-op were being noted, and it was becoming more and more clear that patients did not need to be positioned supine unless it was for meals or procedures. This practice also refers to having the patient sit up in the chair for a limited amount of time and then returning to bed

**Table 4** SAFER bundle 2.0



positioned on their side. Limited supine position was added to the repositioning portion of the bundle with the intention to place emphasis on the fact that many patients spend significant amounts of time (6–12 h) at a time in the supine position while technically repositioning from bed to chair.

A house-wide rollout of the SAFER bundle 2.0 bundle was carefully planned. Tips and discussion points were prepared for nursing leadership as they introduced the bundle their staff reinforced by the CNSs and WOC nurses. It was again posted on every unit and is now championed or reinforced by not only the WOC nurse in consultation but also the CNSs and nursing leadership. Adherence is monitored with feedback directly from the manager to nursing staff regarding opportunities for improvement.

### Conclusions

The SAFER bundle is a simple, routine set of interventions that when consistently applied early in a patient's hospitalization have been shown to reduce the incidence of hospital-acquired pressure injuries [1]. Recommended interventions need to be readily available for nursing staff to utilize, and routine monitoring of adherence to the proactive utilization of the interventions is recommended. A bundle of interventions will not replace consultation with the WOC nurse, however, will free time spent on basic interventions to focus on complex scenarios or education.

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# Part II

# Diabetes

# Cellular and Molecular Mechanisms of Impaired Angiogenesis and Delayed Wound Healing in Type 2 Diabetes: Amelioration Using siRNA-Pluronic Acid-Based Technology

Milad S. Bitar

## 1 Introduction

Successful wound healing in response to an incision, trauma, and tissue death caused, for example, by myocardial infarction necessitates an overlapping, highly coordinated sequence of cellular and biochemical events including the arrest of hemorrhage, followed by inflammatory response characterized by infiltration of neutrophils and macrophages, reepithelization, formation of granulation tissue rich in immature collagen bundles and newly formed blood vessels, and finally remodeling. Understanding the cellular and molecular events regulating tissue repair mechanisms will help to optimize and maximize the design of effective therapy for non-healing chronic wounds (e.g., diabetic foot ulcers (DFUs), pressure ulcers (PUs), and chronic venous leg ulcers). The latter phenomenon represents a major healthcare burden that is reaching epidemic proportions in the United States and throughout the world. For example, among the 27 million Americans diagnosed with type 2 diabetes (T2D), more than 6 million exhibit chronic non-healing skin wounds, leading to secondary bacterial infection and costing the health-care system more than $25 billion [1, 2]. An approximately 71,000 patients with DFU undergo limb or digit amputations each year. Further data showed that advanced stage of PUs (stages III and VI) have a mortality rate of 68% [3] and can incur costs for the hospital as high as $124,000 per episode [4]. To this end, it seems imperative to recognize that chronic wounds repair is a mortal disease analogous to cancer [5]. Indeed, recent studies revealed that the 5-year mortality rate for patients suffering from DFU or ischemic ulcers is much higher than that of prostate or breast cancer [5–7].

Despite the recent advances in understanding the science of wound healing, the pathogenetic mechanisms underpinning chronic wounds are elusive, and efficient treatment options are currently missing. In view of this dilemma, a number of hypotheses have been proposed to explain chronic wound biology; these include persistent inflammation, epidermal hyper-proliferation, interruption of keratinocyte migration, and dysregulated signaling and/or expression of aberrant or specific microRNAs [8–10]. Similarly, heightened state of oxidative stress and fibroblast senescence in connection with decreased fibroblast migration and responsiveness to growth factors including IGF-1, TGF-β, and PDGF have also been suggested to contribute to delayed wound healing [9, 11–15]. Last but not

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least, we and others have advanced the notion that angiogenesis is a key controlling point for normal and delayed wound healing. In this context, a defect in angiogenesis appears to be a common feature of all chronic non-healing wounds including DFU, PU, arterial ulcers, and chronic venous leg ulcers [16, 17]. Indeed, one of the challenges facing clinician when devising strategies to promote healing of chronic wounds is the initiation of angiogenesis and the formation of a stable vasculature to support tissue regeneration.

This chapter is intended to provide clinicians with a better understanding of the molecular mechanisms and clinical applications of angiogenesis. Surgeons and wound-care specialists including dermatologists can use their knowledge regarding angiogenesis to identify defects and select evidence-based therapeutic regimen to enhance wound angiogenesis and, henceforth, speed up healing. To this end, we will describe the "angiogenesis model of wound healing" including the regulation of angiogenesis by endogenous pro-angiogenic (e.g., VEGF, PDGF, and IGF-1) and anti-angiogenic (e.g., thrombospondins) mediators. The cellular and molecular cascades that coordinate angiogenesis in healthy and diabetic wounds will be addressed. Finally, rather than providing an encyclopedia survey, we will focus on a recent discovery by our laboratory confirming a new molecular target (e.g., CREM/ICER-HIF-1-VEGF axis) that may have translational potential in providing therapeutic avenues aimed at advancing the treatment of angiogenesis-dependent disorders including delayed wound healing.

## 2 Discussion

Blood vessels enable hematopoietic cells to patrol the organism for immune surveillance and provide oxygen and a variety of nutrients, inflammatory cells, cytokine, chemokines, and growth factors, and they are also capable of disposing of waste products. In a healthy adult, blood vessels exist in a quiescent state, and their wall is composed of an endothelial cell lining known as phalanx cells, a basement membrane made up mainly of collagen IV and laminin and a layer of cells called pericytes. Quiescent endothelial cells have long half-lives and are protected against insults by the autocrine action of maintenance signals exemplified by VEGF, fibroblast growth factor (FGF), angiopoietin-1 (ANG-1), and NOTCH. Tight cell-cell adhesion, occurring through inter-endothelial junctions (IEJs)/integrin receptors, provides a barrier that helps maintaining blood flow. IEJs encompass tight junctions, gap junctions, and adherence junctions. Although occludins and claudins are the keystones of tight junctions, connexins constitute gap junctions, and VE-cadherin is necessary for formation of adherence junctions. Similarly, linking endothelial cell to ECM is achieved via the connection between integrin receptors and matrix proteins including fibronectin and vitronectin. Finally, endothelial cells are usually sheathed by pericytes, which suppress endothelial cell proliferation and release cell-survival mediators including VEGF, FGF, and ANG-1.

### 2.1 Mechanisms of Neovascularization

Restoration of a functional vascular network in response to traumatic injury or surgical wounds represents one of the most important constituents of successful tissue repair mechanisms. Angiogenesis—the outgrowth and proliferation of capillaries from preexisting blood vessels—is one of the most important components of successful wound healing. Clinically, the new capillaries first become visible in the hypoxic wound bed, 3–5 days post-injury, and peak around day 10 when blood vessel density is more than double that of non-wounded tissue [18]. The final reestablishment of a functional vascular network is crucial in providing damaged tissue with oxygen and nutrients required to support the growth and function of reparative cells.

This process, the so-called neovascularization or the formation of new blood vessels, is accomplished via two major mechanisms including vasculogenesis and angiogenesis (sometimes referred to as "sprouting angiogenesis" Fig. 1). Vasculogenesis is the de novo formation of new blood vessels by bone marrow-derived endothelial

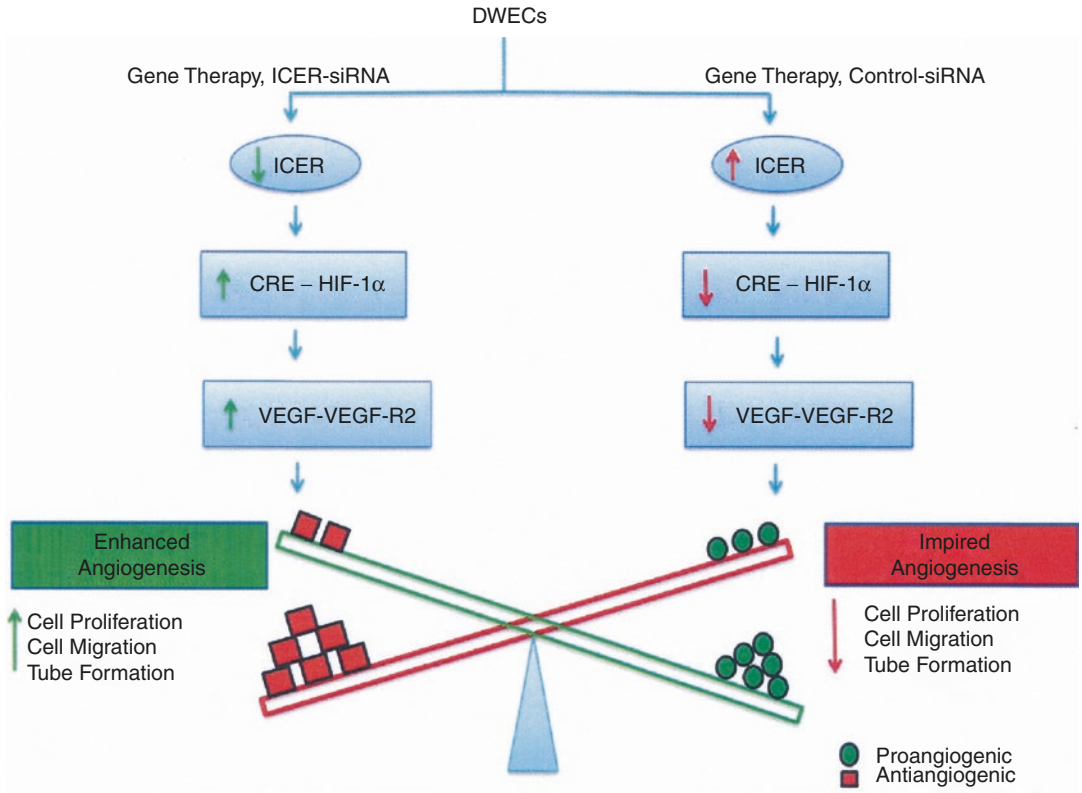


**Fig. 1** How CREM/ICER siRNA-based strategy restored angiogenic balance and promoted angiogenesis in diabetic WECs. Diabetes stabilizes and elicits persistent elevation in CREM/ICER level in diabetic WECs. This in turn suppresses the CRE-HIF-1-VEGF signaling network and induces a shift in angiogenic balance in favor of antireparative response. Consequently, impaired angiogenesis and delayed wound healing may ensue. A gene-based therapy exemplified by the CREM/ICER siRNA restored diabetic WEC VEGF content, enhanced angiogenic response, and ameliorated wound healing impairment during the course of type 2 diabetes. *DWECs* diabetic wound endothelial cells, *CREM/ICER* cAMP-responsive modulatory inducible cAMP early repressor, *CRE* cyclic AMP response element, *VEGF* vascular endothelial growth factor, *HIF-1* hypoxia-inducible factor

progenitor cells. These precursor endothelial cells are recruited to sites of active angiogenesis, where they proliferate and differentiate into mature endothelial cells. Another important component of neovascularization is sprouting angiogenesis, the formation of new blood vessels from preexisting vascular network, and this phenomenon will be discussed in the context of acute and chronic wound healing. We will focus on key molecular targets in angiogenesis and highlight our preclinical experience of treating non-healing diabetic wounds with a functional gene therapy model that is based on altering the dynamics of key intracellular mediators, which regulate the formation of pro-angiogenic molecules.

## 2.2 Angiogenesis Dynamics

Sprouting angiogenesis is a complex process occurring in an orderly cascade of molecular and cellular events within injured tissues or wound bed. When a quiescent vessel senses an angiogenic signal elicited by the release of VEGF, FGF, ANG-2, or chemokines from hypoxic or inflammatory cells within injured tissue or wound bed, a sequence of molecular and cellular events becomes activated culminating in the initiation of sprouted angiogenesis. This process can be conveniently divided into stages of quiescence, activation and resolution. During the activation stage, pericytes

detach from the vessel wall and liberate themselves from the basement membrane via matrix metalloproteinases (MMP)-mediated proteolytic degradation. In the meantime, endothelial cells become activated and start loosening their cell-cell contacts and the nascent vessels dilate [19]. Two types of cells characterize the "activated endothelium," the tip cells and the stalk cells. The tip cells form the migrating front of the vascular buds, and they direct vascular growth by sensing a gradient of pro-angiogenic mediators like VEGF. The neighbors of the tip cells assume subsidiary positions as stalk cells, which proliferate and migrate (stimulated by NOTCH, WNTs, FGF) in the direction of the tip cell, resulting in elongation of the sprouting vessel and the establishment of the lumen (mediated by VE-cadherin, CD34, VEGF, [19]). During the resolution phase of angiogenesis, vascular sprouts fuse with neighboring sprouts to establish blood flow. For a vessel to become functional, it must become mature and stable. Accordingly, endothelial cells resume their quiescent phalanx state and become covered with pericytes via mediators such as PDGF-B, ANG-1, mTGF-β, ephrin-B2, and NOTCH. Junctions are reestablished, and a new basement membrane is formed via tissue inhibitors metalloproteinases (TIMPs) and plasminogen activator inhibitor-1 (PAI-1) to ensure optimal flow distribution. This eventuates in the completion of the process of sprouting angiogenesis.

Aberration in the mechanisms regulating physiological angiogenesis may contribute to the pathogenesis of numerous diseases. To name just a few, insufficient angiogenesis and abnormal vessel regression can lead to stroke, myocardial infarction, neurodegeneration, and non-healing chronic wounds. In contrast, overproliferation of blood vessels or abnormal remodeling fuels psoriasis, cancerous tumors, liver fibrosis, inflammatory disorders, and diabetic retinopathy; in these conditions, it is advisable to halt angiogenesis [20, 21]. Accordingly, pro-angiogenic and anti-angiogenic signals must operate in balance to assure optimal physiological health.

## 2.3 Impaired Angiogenesis in Non-healing Chronic Wounds

Insufficient angiogenesis is a common phenomenon in virtually all chronic wounds and is often predictive of poor healing outcomes in diabetic foot ulcers [22]. An aberrant endothelial activation with reduced proliferation and migration has been demonstrated in wound angiogenesis during the course of diabetes, ischemic ulcers, and venous insufficiency ulcers [17, 23]. Similarly, reduced mobilization of bone marrow-derived EPCs to the wound milieu was also evident in these disease states [24, 25]. A number of studies including those derived from our laboratory have documented significant decrease in the expression of growth factors and their receptors in human and experimental models of type 2 diabetes [12, 15, 16, 26]. For example, the levels of pro-angiogenic molecules such as PDGF, IGF, VEGF, and FGF in diabetic wounds are decreased during the various phases of healing [27]. In contrast, anti-angiogenic mediators including ANG-2 and thrombospondin appear to be increased as a function of diabetes [16]. In addition to the aforementioned functional abnormalities accompanying the angiogenic signal, diabetic blood vessels suffer from a variety of structural changes including a reduction of capillary size, thickening of the basement membrane, and hyalinosis of arterioles [17, 23]. As a result, impaired physiological fluid exchange, cellular migration, and capacity to confront infection may ensue [17, 23]. Collectively, these structural and functional defects in diabetic endothelial cells lead to impaired angiogenesis, and this is, perhaps, among the most important parameter contributing to aberrant wound healing during the course of diabetes.

Although the biochemical basis of impaired angiogenesis in diabetic wounds is not well understood, multiple mechanisms have been proposed, and we will highlight each of them in terms of evidences supporting or refuting their involvements. In this context, oxidative stress, which is defined as an imbalance between prooxidant and antioxidant systems, has been

regarded as an independent risk factor and may be a biomarker for many vascular complications including impaired angiogenesis [28]. However, the failure to demonstrate clinical benefit from ROS scavenger necessitates further studies regarding the role of this process in diabetic vascular pathology. Endothelial dysfunction and loss of endothelial-derived nitric oxide bioavailability has been shown to be important; however, the cause and effect in relation to angiogenic insufficiency during the course of diabetic wounds remain to be illustrated [29]. MicroRNAs may play a role in delayed angiogenesis, but the regulatory mechanism regarding these molecules is currently unknown [30]. Finally, growth factors deficiency has long been thought to contribute to impaired angiogenesis. However, treatment with these growth promoting polypeptide, especially in non-healing wounds resulted in low to moderate efficacy [17], suggesting a defect in the receptor signaling.

## 2.4 VEGF-Based Novel Angiogenic Signaling Network

A number of growth factors generated in response to injury can stimulate angiogenesis. One of the most important pro-angiogenic mediators is the well-characterized VEGF (also known VEGF-A), a matricellular protein produced by a variety of cells including keratinocytes, activated fibroblasts, mast cells, macrophages, and endothelial cells during the early phases of the healing process, peaking at about 5-day post-injury [31, 32]. Given the complexity of wound angiogenesis, it is remarkable that a single growth factor, VEGF, regulates this process so predominately.

VEGF exerts its effects on endothelial cells by binding to and activating multiple membrane receptor-linked tyrosine kinase including VEGF-R1 and VEGF-R2 (also known FLK1). Neuropilins such as NRP1 and NRP2 are VEGF co-receptors, which enhance the activity of VEGFR-R2 in a context-specific manner [33]. The two receptors differ in their ligand binding properties and tyrosine kinase activity with VEGF-R1 having higher affinity for VEGF, whereas VEGFR-2 exhibits stronger inherent tyrosine kinase activity [34]. It is believed that VGFR-2 is more important than VEGFR-1 in regulating endothelial cell function and angiogenesis during the course of wound healing. Upon binding to VEGF, phosphorylation of tyrosine residues within the VEGF-R2 enhances key intracellular mediators including protein kinase B (Akt), which inhibits cell apoptosis; ERK1/ERK2, which promotes cell proliferation; and Src kinase/focal adhesion kinase, which mediates cell migration [35]. Emerging evidence indicates that the aforementioned functional effects of VEGFR2 signaling depend on its subcellular localization—for example, for VEGF to induce arterial morphogenesis, VEGFR2 must signal from intracellular compartments [36]. Activating VEGFR2 mutations causes vascular tumors, and genetic polymorphisms in VEGF and/or its receptor co-determine pathological angiogenesis, whereas the blockage of VEGF signaling can target angiogenic vessels in malignant and ocular disease in humans [37, 38]. VEGF protein or gene transfer stimulates vessel growth in ischemic tissues and the loss of, even a single copy of the VEGF gene, results in embryonic lethality at early stages of development [39, 40].

Recent studies indicate that paracrine VEGF, released by myeloid cell (e.g., monocytes and macrophages) and keratinocyte, affects not only angiogenesis and the increase in vessel branching but also other components of the healing process including wound closure and epidermal repair, granulation tissue formation, and the quality of repair—both in terms of wound tensile strength and tissue scar formation [41–43]. In this connection, delayed wound closure, reduced vessel density, and decreased granulation tissue formation have been reported in mice lacking VEGF in myeloid cells and keratinocytes [41–43]. Similarly, reducing VEGF activity by treating with neutralizing antibody or small inhibitors of VEGFR-2 or conditional genetic deletion of VEGF leads to fewer blood vessels beneath the epidermis, reduced reepithelization rate, and delayed wound healing [41, 43–45]. Finally, additional support for the important of VEGF in wound closure comes

from models of impaired healing or severe injury. We have found that wounds of type 2 diabetic animals showed less VEGF with concomitant decrease in tissue repair efficiency [16]. Clinical studies also support the concept that sufficient levels of VEGF are required for effective healing, since poor vascularization and impaired angiogenesis represent the hallmark of non-healing diabetic wounds [22].

## 2.5 Novel Signaling Network

Impaired angiogenesis and delayed wound healing are common features of both clinical and experimental diabetes. Current therapeutics for the aforementioned pathologies include the topical applications of growth factors such as PDGF, tissue-engineered dressing, hyperbaric oxygen, and negative pressure [46]. These strategies are inadequate, and a new treatment is deemed necessary in lieu of the demographical fact that the number of patients suffering from chronic wounds and impaired healing is reaching epidemic proportions. Accordingly, in the below discussion, we will focus on our recent data elucidating a novel signaling control mechanism of VEGF expression, production, and angiogenic function that can be exploited therapeutically.

It is now well established that angiogenesis is regulated by a dynamic balance between endogenous pro-angiogenic (e.g., VEGF) and anti-angiogenic (e.g., TSPs, PEDF) molecules. Bitar and colleagues using a polyvinyl alcohol (PVA) sponge model of wound angiogenesis have shown that in type 2 diabetes, this angiogenic balance is shifted in a manner, which is consistent with an overproduction of the TSP and PEDF angiostatic factors and a downregulation of VEGF expression [16]. This duality of effect of type 2 diabetes as a suppressor of VEGF and an inducer of TSPs/PEDF could represent one of the mechanisms underlying impaired angiogenesis and delayed wound healing during the course of the disease. From a basic science and preclinical perspective, VEGF represents an ideal option for stimulating the formation of new blood vessels in response to injury and for the amelioration of non-healing wounds. Unfortunately, in a phase II clinical trial, topical VEGF failed to improve diabetic foot ulcer healing. This may very well be due to a defect in VEGFR2 signaling pathway, a premise that deserves to be explored, especially when viewed in the context of our recent data documenting that wound VEGF content is diminished as a function of diabetes [16]. Similarly, others have shown that endothelial cell homeostasis is regulated primarily by the autocrine action of VEGF [47, 48]. Indeed, a VEGF-deficient endothelium appears to associate with reduced level of VEGF-R2 and a decrease in cell proliferation, migration, and tube formation [47, 48]. Advancing those previous findings prompted Bitar and his colleagues to firstly identify in diabetic wound endothelial cells (WECs) a novel therapeutic target exemplified by the cAMP response element modulator (CREM/ICER) that was shown to negatively regulate cAMP response element (CRE)-HIF1-VEGF-VEGFR-2-dependent pathway and secondly to use on these cells a gene-based therapy (e.g., CREM siRNA) to promote reparative angiogenesis and ameliorate delayed wound healing during the course of diabetes.

Our study examined the protein kinase A (PKA)-cyclic AMP response element binding protein (CREB) and HIF-1α dynamics in diabetic WECs. This strategy harmonizes with the wealth of evidence indicating that in a variety of cell lines, including cancer cells [49] and human umbilical vein endothelial cells [50, 51], activation of PKA-CREB- or HIF-1α-dependent pathways increases both VEGF transcriptional activity and the formation of new blood vessels. We recognize that most of these previous studies used endothelial cells that were not exposed to an elaborate set of microenvironmental cues ex vivo. For example, the fluid bathing the wound tissue reflects the wound microenvironment and shapes the functional response of wound-related cells, such as endothelial cells [52]. Bitar and his colleagues addressed this gap by isolating intact endothelial cells from the actual wound milieu of subcutaneous sponge implants, a well-established in vivo model of angiogenesis. They found that

diabetic WECs exhibited a significant reduction in VEGF expression both at the mRNA and protein levels in connection with a defect in cAMP-PKA-CREB-dependent signaling. Intriguingly, however, CREB-DNA binding and CRE transcriptional activity, essential parameters for HIF-1α and VEGF formation, were similarly diminished during the course of diabetes.

Panoply of evidence suggests that the outcome of CRE-mediated gene expression is dictated by CREB activation and the competitive binding of several dimerized transcription factors, including activators and repressors of gene transcription [53]. Among the members of the CREB, CREM/ICER serves as an endogenous repressor of genes containing a CRE sequence within their promoters [54]. Under physiological conditions, CREM/ICER induction is a transient phenomenon that allows cAMP signaling to return to the basal state [55]. By contrast, prolonged or inappropriate induction of ICER can elicit pathological consequences [55]. In this context, we have shown that CREM/ICER mRNA and protein expression were elevated in diabetic WECs in connection with a reduction in a number of CRE target genes, such as NURR1, IRS2, and VEGF. These findings are not unique to the diabetic WECs as a persistent elevation of CREM/ICER, and the concomitant suppression of gene transcription was also evident in other pathological conditions and cell types, such as hypercortisolemia [56], hypercatecholemia [57], and hyperglycemia [58], in addition to angiotensin-II-treated cardiomyocytes [59] or oxidized LDL-treated insulin-secreting cells [60].

VEGF gene expression can also be modulated by the total and nuclear levels of HIF-1α, a transcription factor that plays a central role in tumor progression and angiogenesis [61]. HIF-1α protein content and activity are controlled not only by hypoxia but also by growth factors (e.g., IGF1) and cAMP- or PKA-inducing agents [62, 63]. In this context, it has been shown in a variety of cell lines (e.g., INS-1, PC-3, SK-Hep1, MDA-MB-231) that forskolin, norepinephrine, or isoproterenol upregulate HIF-1α protein expression, in part through PKA-mediated activation of insulin receptor substrate 2 (IRS2)-protein kinase B (AKT)-mTOR-dependent signaling [49, 63]. Ensuing studies in diabetic WECs unveiled a diminution in total and nuclear protein contents of HIF-1α both under basal conditions and in response to the PKA activator MB-cAMP. Consistent with these data, we also found that the ability of MB-cAMP to induce the activity of HRE-luc and the binding of HIF-1α to the HRE within the promoter region of VEGF were attenuated as a function of diabetes. A pressing question that follows these observations is how does diabetes inhibit HIF-1α expression and its translocation to the nucleus? PKA activation enhances IRS2 accumulation through CREB-CRE-dependent mechanisms [63]. IRS2 in turn increases HIF-1α accumulation and activity by stimulating Akt-mTOR signaling [63]. Contrastingly, cells exposed to inhibitors of PKA (e.g., H89), CREB, or mTOR activity (e.g., rapamycin) showed marked suppression of HIF-1α expression [63]. Analogous data were obtained using siRNA-mediated knockdown of IRS2 [63]. These findings harmonize with the data obtained from Bitar laboratory documenting that the overexpression of CREM/ICER in diabetic WECs inhibits CREB-CRE transcriptional activity with concomitant reduction in cellular contents of IRS. This diabetes-induced IRS2 downregulation could, by means of inhibiting the Akt-mTOR axis, contribute to the observed decrease in HIF-1α total protein levels. To this end, the dysregulation in CREM/ICER dynamics in diabetes can lead to a decreased in IRS2 cellular content with concomitant suppression of the Akt-mTOR-mediated increase in HIF-1α expression. Consequently, a decrease in VEGF production and henceforth impairment of angiogenesis may ensue.

An adequate level of HIF-1α and its target gene VEGF might necessitate reciprocal and flexible signaling between ICER and the CREB-IRS2-mTOR pathway. In diabetic WECs harboring siRNA against ICER, MB-cAMP-induced activation of the IRS2-Akt-mTOR axis was significantly higher than that in corresponding cells that had been transfected with control siRNA. Consistent with these findings, we also

demonstrated in these cells a marked increase in HIF-1α and VEGF levels. The impact of this strategy on the downstream signaling of VEGF, as well as its functional relevance to in vitro and in vivo angiogenesis, during the course of type 2 diabetes is currently under consideration in our laboratory. A case in point in this regard is our initial unpublished findings, which characterized the expression, production, and function of CREM/ICER in the context of dermal wound repair. We found as in WECs that this negative regulator of pro-angiogenic signal (e.g., CREB-CRE-IRS2-pAkt-mTOR-HIF-1α) was elevated in dermal wound of type 2 diabetes. More intriguingly, the topical application of ICER siRNA in a biocompatible controlled-released gel, commonly used in drug delivery system, to an 8-mm diabetic wound signified relative to control siRNA higher blood vessel density at 10-day post-injury.

## Conclusions

Designing effective therapies for impaired angiogenesis and delayed wound healing during the course of diabetes continues to be a demanding challenge in current medical and pharmaceutical sciences. Despite this, preclinical and clinical managements of wound disorders have emerged with a notion favoring the concept that insufficient angiogenesis stemming from prolonged imbalance of pro- and anti-angiogenic mediators contributes in large part to non-healing diabetic wounds. The advent of using pro-angiogenic agents such as VEGF and PDGF in the treatment of chronic non-healing wounds is an excellent example of moving research from bench to bedside; such strategy, however, failed to improve DFUs in phase II clinical trial. This omission might be due to the potential defect in the growth factor receptor and/or post-receptor events. Recent evidence confirmed that endothelial homeostasis, a major player in the formation of new blood vessels, is maintained via the autocrine action of VEGF on VEGFR2. Accordingly, this chapter highlights attempts to promote endogenous VEGF formation and to stimulate dysfunctional angiogenesis in diabetes by targeting intracellular signal transduction pathways exemplified by the CRE-HIF-1α-VEGF-dependent cascade. This signaling event is negatively regulated by the CREM/ICER feedback loop, which appears to be over-expressed in diabetic WECs. A gene-based therapy involving diabetic WECs bearing siRNA for CREM/ICER confirmed higher levels of intracellular VEGF together with improvement in angiogenic functions when compared to their corresponding control siRNA values. More intriguingly, topical applications of CREM siRNA in pluronic acid gel to diabetic wounds markedly improved the healing process and its responsiveness to VEGF therapy. Overall, our recent data of identifying a new molecular target with a therapeutic potential may open avenues for maximizing the efficacy of VEGF in the treatment of non-healing diabetic wounds. The reviewed information in connection with our current data may shed light on innovative approaches to in vivo directed modulation of angiogenesis using upstream and downstream VEGF signaling dynamics. If this is taken further and validated in human diabetic subjects, it could have a major impact on clinical wound care in the future. Indeed, a tight interaction between preclinical and clinical research is crucial to achieve these goals. Furthermore, it may enable surgeons and wound-care specialists to use such knowledge regarding angiogenesis to identify defects and select interventions that promote wound granulation and healing.

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# FOXO1 has a Dual Function to Promote Normal but Inhibit Diabetic Wound Healing

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## 1 Diabetic Wound Healing

Diabetes mellitus is characterized by deficient insulin production and hyperglycemia. By 2025 it is estimated that there will be 300 diabetics worldwide [1]. Wound healing is a significant complication of diabetes along with blindness, heart disease, stroke, kidney failure, neural impairment, and periodontal disease [2]. Deficient healing in diabetics is caused by a number of factors including hyperglycemia, formation of advanced glycation end products (AGEs), increased inflammation, reduced insulin signaling, and higher levels of oxidative stress [3]. Impaired wound healing in diabetic patients is accompanied by increases in inflammatory cytokines and chemokines and decreases in growth factors [4]. Increased expression of IL-1α, IL-2, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), CCL3, and CCL4 is observed in wounds of type 1 diabetics [5]. In diabetic animal models, TNF-α and IL-6 are increased, and the anti-inflammatory cytokine, IL-10, is decreased [6, 7]. In contrast diabetic wounds have reduced levels of critical growth factors such as TGFβ1.

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Stem cells are important during wound healing. Epithelial stem cells are largely derived from cells in the hair follicle, whereas mesenchymal cells may be recruited from the peripheral blood or adjacent to the wounded tissue. Mesenchymal stem cells are important in generating fibroblasts and myofibroblasts and also produce growth factors such as VEGF-α, EGF, and KGF [8, 9] as well as anti-inflammatory mediators that reduce the level of inflammation [10]. Diabetic animals have reduced numbers of MSC, and the addition of exogenous MSCs improves healing of diabetic ulcers [11]. They increase reepithelialization, angiogenesis, and generation of growth factors and reduce inflammation [8, 12]. Reepithelialization involves migration of keratinocytes from the wound edges and epithelial stem cells from hair follicles or sweat glands [13, 14]. Keratinocytes produce TGF-β, VEGF, EGF, KGF, and TGF-α [15–17]. Migration of keratinocytes is impaired by diabetes in vivo [18] and in vitro by high-glucose conditions [19].

Macrophages play a key role in wound inflammation. Depletion of macrophages in the early wound healing interferes with the healing process [20]. Macrophages in diabetic wounds have impaired efferocytosis with deficient clearance of apoptotic cells that increases inflammation [6]. Macrophages can form different phenotypes that are functionally distinct: M1 macrophages are inflammatory and M2 macrophages are anti-inflammatory. The latter produce growth factors such as TGF-β and VEGF [21]. Diabetic wounds

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have increased M1 macrophage polarization and decreased M2, which is thought to increase inflammation and reduce the healing response [22]. Hyperglycemia induces macrophages to increase production of IL-6, IL-1β, TNF-α, and IFN-γ in vivo and in vitro [23].

In normal wound healing, the highest levels of TNF-α are seen 12–24 h after wounding [24]. After completion of the proliferative phase of wound healing, TNF-α returns to basal levels. During the early phase of wound repair, TNF is predominantly expressed in polymorphonuclear leukocytes and later by macrophages. It is also expressed in the hyperproliferative epithelium at the wound edge. TNF-α stimulates upregulation of antimicrobial defenses [25]. TNF-α levels are elevated in diabetic wounds in part through increased oxidative stress that prolongs inflammation [26, 27], and TNF levels are threefold higher found in wound fluid from nonhealing venous leg ulcers than in healing ulcers [28].

## 2 FOXO Transcription Factors

Impaired diabetic wound healing has been linked to increased TNF-α [29]. TNF-specific inhibitors increase proliferation of fibroblasts in vivo and reduce fibroblast apoptosis in diabetic wounds [27, 30, 31]. Inhibiting TNF also improves angiogenesis, wound closure, and the overproduction of small noncoding RNAs such as miR-200b [32]. TNF may also reduce insulin signaling in keratinocytes to interfere with reepithelialization [33]. To investigate mechanisms through which TNF could inhibit diabetic healing, we performed a transcription factor array and identified FOXO1 as a transcription factor that was significantly upregulated by TNF in vitro and modulated by TNF in vivo [27]. This may be particularly significant since FOXO1 activity is increased in several diabetic conditions and FOXO1 has the potential to increase cell cycle arrest, increase apoptosis, and enhance inflammation, although in some cases it can have the opposite effect [26]. For example, TNF-α-induced endothelial and pericyte cell death is mediated by FOXO1 in vivo and in vitro [34, 35]. FOXO1 DNA-binding activity is increased in diabetic dermal and mucosal wounds and in diabetic fractures [27, 36, 37]. Advanced glycation end products induce activation of FOXO1, and FOXO1 mediates AGE-stimulated apoptosis [38]. The latter activate FOXO1 through intermediate steps that include enhanced generation of reactive oxygen species and ceramide that in turn stimulate p38 and JNK MAP kinase activity [38]. In contrast, the phosphatidylinositol 3-kinase/Akt pathway inhibits FOXO1 activation.

Forkhead box (FOX) transcription factors were originally identified in mammalian cells by homology with the forkhead gene found in *Drosophila* [39]. This large family of transcription factors is divided into subfamilies and is the largest family of transcription factors in humans [40]. The forkhead box "O" subfamily has three members (FOXO1, FOXO3, FOXO4) which have a high degree of homology [41] and another member, FOXO6, that is less homologous [42]. FOXO transcription factors modulate gene expression to regulate cell survival, cell cycle, and embryonic pattern formation [43, 44] and have a significant effect on formation of cancer [45]. FOXO1, FOXO3, and FOXO4 bind to similar consensus sequences and may induce similar target genes. However, they interact with coactivator and corepressors in a distinct fashion and have lineage-specific expression patterns so that their function does not necessarily overlap. Thus, the biological function of FOXOs may overlap under some and be divergent in other conditions. Thus, it is difficult to predict in the absence of experimental evidence some of the FOXO1 effects.

FOXOs induce expression of target genes by interacting with similar FOXO response elements. Activation of FOXO transcription factors typically involves nuclear translocation, interaction with other proteins, binding to DNA, and export from the nucleus. Because FOXOs are important in maintaining homeostasis, their activation and deactivation are tightly regulated [46]. FOXO proteins can be modified, particularly by acetylation or phosphorylation to enhance or impede each step. Each of these aspects of FOXO activation is regulated by a number of different

processes including posttranscriptional and post-translational mechanisms, including miRNA-mediated repression [47], acetylation, phosphorylation, ubiquitination, methylation, and glycosylation [48]. FOXOs have four distinct domains that include a forkhead DNA-binding domain, nuclear localization, nuclear export, and transactivation domains. There are two different consensus of FOXO DNA-binding sequences: a Daf-16 binding element (5′-GTAAA(T/C)AA) and an insulin-response element (5′-(C/A)(A/C)AAA(C/T)AA). The core DNA sequence 5′-(A/C)AA(C/T)A is recognized by all FOXO family members. The nuclear localization domain is rich in phosphorylation and acetylation sites and is regulated by kinases and acetylases that modify them. The chaperone protein 14-3-3 binds to the FOXO nuclear export domain and removes FOXOs from the nucleus, an important step in FOXO deactivation [49]. FOXOs are phosphorylated by several kinases to modulate FOXO subcellular location, DNA binding, and transcriptional activity [50, 51]. A major negative regulatory pathway of FOXOs is the phosphoinositide 3-kinase (PI3K) pathway that is stimulated by insulin. Thus, a major effect of insulin is the inactivation of FOXO1 by phosphorylation of the nuclear localization domain which prevents its nuclear translocation and reduces binding to DNA consensus elements.

FOXOs maintain homeostasis and facilitate adaptation to changes in environment [52]. FOXO1 plays a diverse role in the innate and adaptive immune response, such as dendritic cell activity [53, 54], epithelial cell responses to Gram-negative bacteria [55], CD8 T-cell response to chronic viral infections [56], macrophage activation in parasitic and bacterial infections [54], and antibody class switching by B cells [57]. FOXO1 plays a role in the onset of diabetes by increasing gluconeogenesis and has complex effects on beta cells in the pancreas [58]. As an example of its diverse array of functions, FOXO1 can protect cells from oxidative stress but under certain conditions promote cell death induced by reactive oxygen species formed as a result of oxidative stress [26]. Thus, it can be difficult to predict how FOXO transcription factors will function in different circumstances. It is our hypothesis that the environment regulates the specific promoter regions of the FOXO1 downstream gene targets and this modulation determines its impact [59].

## 3 FOXO1 and Diabetic Reepithelialization of Dermal Wounds

Wound healing is initiated by inflammatory events which are followed by migration and proliferation of cells that participate in the healing process such as endothelial cells, stem cells, myofibroblasts, neural cells, and others as well as the formation of an extracellular matrix and remodeling [60]. Diabetes dysregulates these processes and can lead to significant morbidity and limb amputation if a biofilm forms on a slowly healing wound [61]. The underlying mechanisms for dysregulated healing in diabetics are not well understood [62], although it has been understood for several years that diabetic wounds are characterized by increased levels of advanced glycation end products (AGEs) and inflammation, particularly TNF-α. As noted above, diabetic wound healing is improved when AGEs or TNF is blocked [27, 63], and both TNF and AGEs stimulate FOXO1 activation [26].

Reepithelialization plays a critical role in covering a wound, particularly in humans where there is less contraction than in other mammals such as rodents [64]. Migration of keratinocytes is essential for reepithelialization. Deficient wound closure can lead to colonization and formation of a nonhealing ulcer by promoting bacterial colonization. Keratinocyte migration is regulated by growth factors, integrins, extracellular matrix molecules, and metalloproteinases (MMPs) [65]. FOXO1 has been shown to regulate genes that participate in each of these events. Factors that are increased by diabetes such as a high-glucose environment and high levels of TNF-α and AGEs induce FOXO1 activation [26, 35, 66, 67]. FOXO1 is upregulated in wounds of diabetic animals and in osseous fracture [27, 68, 69].

We examined the role of FOXO1 reepithelialization of dermal wounds by studying experimental mice with keratinocyte-specific *FOXO1* deletion in which Cre recombinase was driven by a promoter element from the *keratin-14* gene and littermate control mice. One of the reasons for focusing on FOXO1 in keratinocytes was due to findings that FOXO1 expression and activity were considerably higher in the epithelium compared to the connective tissue healing wounds and that FOXO1 was important in keratinocyte behavior in vitro [70–72]. Lineage-specific deletion of Foxo1 in diabetic mice enhanced reepithelialization establishing that the overall effect of FOXO1 activation in keratinocytes in diabetic wounds was to interfere with reepithelialization in both mucosal and dermal wounds [59, 72]. On a quantitative level, deletion of FOXO1 in keratinocytes reduced the rate of closure by half. The inhibitory effect of FOXO1 on reepithelialization was also investigated in vitro using high glucose and insulin deficiency as an in vitro equivalent to the "diabetic condition" in vivo. FOXO1 silencing enhanced closure of a scratch wound created in keratinocyte cultures in high glucose [59, 72]. Interestingly siRNA-specific knockdown of FOXO3 had little effect [71]. Taking a gain of function approach, overexpression of FOXO1 in keratinocytes in high glucose in vitro reduced reepithelialization of a scratch wound supporting the role of FOXO1 as an inhibitor of reepithelialization in hyperglycemic environments [59].

The impact of FOXO1 on reepithelialization by its effect on keratinocyte migration was investigated in vivo and in vitro. In diabetic animals, keratinocyte migration was significantly reduced in both mucosal and skin wounds [59, 72]. Keratinocyte-specific Foxo1 deletion in diabetic mice reversed the impact of diabetes by increasing the migration of keratinocytes twofold in vivo. In vitro keratinocyte migration in high glucose was reduced more than half compared to standard media. FOXO1 knockdown reversed the negative effect of high glucose on keratinocyte migration. To understand how FOXO1 affected migration of keratinocytes in high glucose, we examined a number of potential factors. Because TGFβ1 was previously shown to be critical in keratinocyte migration in healing wounds, we examined the ability of FOXO1 to regulate TGFβ1 [73]. Chromatin immunoprecipitation (ChIP) assays showed that in high-glucose conditions, FOXO1 did not bind to the TGFβ1 promoter [59]. Interestingly, FOXO1 deletion had no effect on TGFβ1 expression in high glucose, and overexpression of FOXO1 in keratinocytes in high glucose had no effect on TGFβ1 promoter activity. Thus, under high-glucose conditions, FOXO1 is unable to bind to the TGFβ1 promoter and unable to increase TGFβ1 transcription. Similarly, in high-glucose FOXO1 is also unable to regulate TGFβ1 expression in mucosal keratinocytes [72]. This result is striking since in low-glucose conditions, FOXO1 is a potent regulator of TGFβ1 expression [59]. Moreover, we found that treatment of diabetic wounds with TGFβ1 in vivo or incubation of keratinocytes in high-glucose media with TGFβ1 in vitro could rescue the deficit in keratinocyte migration. Thus, keratinocytes in hyperglycemic conditions have inadequate TGFβ1 production caused by a failure of FOXO1 to induce TGFβ1 expression because FOXO1 is unable to bind to the promoter region of TGFβ1 when cells are exposed to hyperglycemic conditions [74].

The results indicate that diabetes impairs healing due to the fact that hyperglycemia prevents FOXO1 from inducing TGFβ1. However, our results also suggested that FOXO1 had an inhibitory effect on healing that could be explained by inducing expression of a negative healing factor. To search for such a factor, a microarray was performed, and serpin peptidase inhibitor, clade B (ovalbumin), member 2 (SERPINB2), and chemokine (C-C motif) ligand 20 (CCL20) were potential inhibitors of reepithelialization that were regulated by FOXO1 expression and increased by high glucose [59]. In vivo and in vitro experiments confirmed that both were enhanced by high glucose at the protein and mRNA levels in a FOXO1-dependent manner. Moreover, high glucose

drove FOXO1 binding to the promoters of both genes. Their negative impact on keratinocyte migration was examined by in vitro transwell assays. Knockdown or inhibition of SerpinB2 or CCl20 in keratinocytes in high glucose significantly improved migration [59].

The results indicate that high glucose is problematic to keratinocytes because of its effect on FOXO1. We carried out additional experiments to assess whether an AGE, carboxymethyl-lysine-modified bovine serum albumin (CML-BSA) also had a negative effect on keratinocyte migration through FOXO1. AGEs are elevated in diabetic skin [75, 76]. AGEs blocked FOXO1 from inducing TGFβ1, similar to high-glucose conditions [59]. Furthermore, it increased SERPINB2 and CCL20 expression in a FOXO1-dependent manner and interfered with keratinocyte migration. Foxo1 ablation reversed the inhibitory effect of AGE on keratinocyte migration.

## 4 FOXO1 and Diabetic Reepithelialization of Mucosal Wounds

Like dermal wounds, healing of mucosal wounds is delayed by diabetes which is primarily related to a deficit in keratinocyte epithelial migration and, to a lesser extent, a moderate decrease in proliferation. Although not compared directly, the contribution of proliferation to mucosal reepithelialization appears to be greater than on dermal reepithelialization [72]. High glucose significantly reduced migration of mucosal epithelial cells. Like dermal wounds, Foxo1 elevation in diabetic mucosal wounds is significant since FOXO1 nuclear localization closely parallels functional activity of FOXO1 [48]. Lineage-specific deletion of Foxo1 in keratinocytes in vivo rescued deficient reepithelialization in diabetic animals by improving migration and proliferation [72]. Furthermore, high glucose in vitro caused the same effect on keratinocyte migration and proliferation which was rescued by FOXO1 knockdown. Interestingly, insulin treatment in vitro had the same effect. This is likely due to the impact of insulin on decreasing Foxo1 nuclear localization. Thus, the effect of diabetes on reepithelialization could be twofold, partly due to the effect of hyperglycemia and partly due to the reduced insulin signaling which prevents FOXO1 hyperactivity [72].

To investigate mechanisms through which Foxo1 in diabetic conditions negatively impacts keratinocyte migration, we examined CCL20 and IL-36γ, which are upregulated by wounding and linked to inflammation [77, 78]. CCL20 and IL-36γ expression in vivo was increased in diabetic mucosal epithelial wounds in a FOXO1-dependent manner [72]. Moreover, high expression of CCL20 and IL-36γ interfered with mucosal keratinocyte migration. When FOXO1 was knocked down or deleted, the high levels of CCL20 or IL-36γ were reduced demonstrating FOXO1 dependence on expression. Antibodies that inhibit CCD20 or IL-36γ rescued the negative effect of high glucose on keratinocyte migration showing that these mediators are inhibitory [72]. This was confirmed by reduced mucosal keratinocyte migration with the exogenous addition of CCL20 and IL-36γ in vitro. Thus, Foxo1 regulated CCL20, and IL-36γ expression in diabetic mice inhibits keratinocyte cell migration.

## 5 FOXO1 and Normal Reepithelialization

Experiments were performed to test the role of FOXO1 in reepithelialization of normal wounds using the lineage-specific deletion of FOXO1 in keratinocytes by Cre recombinase driven by a *keratin-14* promoter element. This deletion impaired wound closure and reepithelialization in vivo and in an in vitro "scratch assay" of both dermal keratinocytes [71]. The delay in dermal reepithelialization caused by the loss of FOXO1 was primarily due to reduced keratinocyte migration. One of the principal ways that FOXO1 enhances keratinocyte migration is

through induced TGFβ1 expression. The importance of FOXO1-regulated TGFβ1 expression was conclusively established by rescue of deficient wound healing in FOXO1-deficient mice by treatment with exogenous TGFβ1. There were several lines of evidence to support the FOXO1-TGFβ1 axis [71] as follows: (a) Cre recombinase deletion of FOXO1 in keratinocytes in vivo or in keratinocytes explanted from experimental mice had substantially diminished TGFβ1 expression; (b) FOXO1 deletion in keratinocytes caused a reduction in downstream TGFβ1 signaling as demonstrated by reduced SMAD2/SMAD3 phosphorylation; (c) FOXO1 knockdown by siRNA in vitro substantially reduced TGFβ1 expression in keratinocytes and reduced TGFβ1 promoter activity; and (d) overexpression of FOXO1 significantly increased TGFβ1 promoter activity. These results suggest a mechanism through which FOXO1 regulates wound healing via induced transcription of TGFβ1 agreeing well with previous reports that TGFβ1 regulates keratinocyte migration and wound reepithelialization [79, 80]. Thus, FOXO1 is needed for adequate TGFβ1 expression, normal keratinocyte migration, and dermal wound healing. Similar results were found in mucosal wounds where FOXO1 activation in mucosal keratinocytes was needed for TGFβ1 expression in mucosal wounds and subsequent migration and proliferation of mucosal keratinocytes [72]. Both activities involved FOXO1-driven TGFβ1 expression.

Because keratinocyte migration is complex and requires the expression of integrins and MMPs [73, 79], we examined these parameters for dependence on FOXO1. Lineage-specific deletion of FOXO1 in keratinocytes significantly reduced expression of integrins-β6 and integrins-α3 and decreased matrix metalloproteinase-3 (MMP-3) and MMP-9 expression. Silencing FOXO1 also caused a decrease in collagen IV by keratinocytes, a basement membrane protein that contributes to keratinocyte migration. FOXO1 regulation of these proteins provides further insight as to how it contributes to overall keratinocyte migration beyond regulating TGFβ1 expression.

FOXO1 can also affect normal wound healing by protecting keratinocytes from oxidative stress, which at high levels reduces migration and increases apoptosis [81]. Reduced FOXO1 levels in keratinocytes increase ROS levels and enhance oxidative damage in healing wounds. Increased oxidative stress caused by FOXO1 deletion impairs keratinocyte migration, which can be rescued by application of an antioxidant. Interestingly application of TGFβ1 can rescue impaired keratinocyte migration when oxidative stress is mild but cannot with high levels of oxidative stress [71]. FOXO1 protects keratinocytes against oxidative stress by induced expression of glutathione peroxidase 2 and cytoglobulin. In addition FOXO1 is protective by stimulating expression GADD45α, which repairs DNA damaged by oxidative stress. Thus, FOXO1 protects keratinocytes from oxidative damage which facilitates keratinocyte wound healing behavior including migration under stressful conditions such as wound healing.

## 6 FOXO1 Expression and Keratinocyte Regulation of Connective Tissue Healing

Dermal keratinocytes play an important role in connective tissue repair, and FOXO1 is essential in this process [82]. *FOXO1* deletion specifically in dermal keratinocytes reduces by ~40–50% the amount of granulation tissue formed and the amount of extracellular matrix produced. This is similar to the degree of connective tissue healing when macrophages are deleted [83–85] and indicates that keratinocytes have an equivalent role in connective tissue repair. The in vivo data support previous in vitro studies that factors produced by keratinocytes can promote connective tissue formation during wound healing [86, 87]. Foxo1 is needed to activate fibroblasts to stimulate fibroblast proliferation, myofibroblast differentiation, and production of extracellular matrix [82]. As discussed above regarding keratinocyte migration, FOXO1 regulation of TGFβ1 in keratinocytes is a key factor through which keratinocytes

regulate connective tissue healing. TGFβ1 may directly stimulate fibroblasts but also stimulate the expression of connective tissue growth factor (CTGF), which mediates many of the pro-fibrotic effects of TGFβ [88, 89]. Thus, in healing wounds, TGFβ1 expression is followed by CTGF production.

We found that keratinocytes were a primary source of TGFβ1, whereas CTGF expression occurred predominantly in the connective tissues [82]. Interestingly keratinocyte-specific deletion of *FOXO1* reduced CTGF expression in connective tissue, linking it to keratinocyte-produced TGFβ1. Antibody blocking studies in vitro revealed that keratinocyte-produced TGFβ1 upregulates CTGF in fibroblasts. Moreover, keratinocytes are able to induce MSC differentiation to myofibroblasts in vitro, which was blocked by FOXO1 knockdown or by inhibition of TGFβ1 and/or CTGF. Keratinocyte-produced CTGF may also promote connective tissue healing. In addition, keratinocytes produce vascular endothelial growth factor (VEGF) expression in a FOXO1-dependent manner, and FOXO1 activation in keratinocytes significantly contributes to angiogenesis in the underlying connective tissue [90]. The role of FOXO1 in contributing to normal healing is shown in Fig. 1.

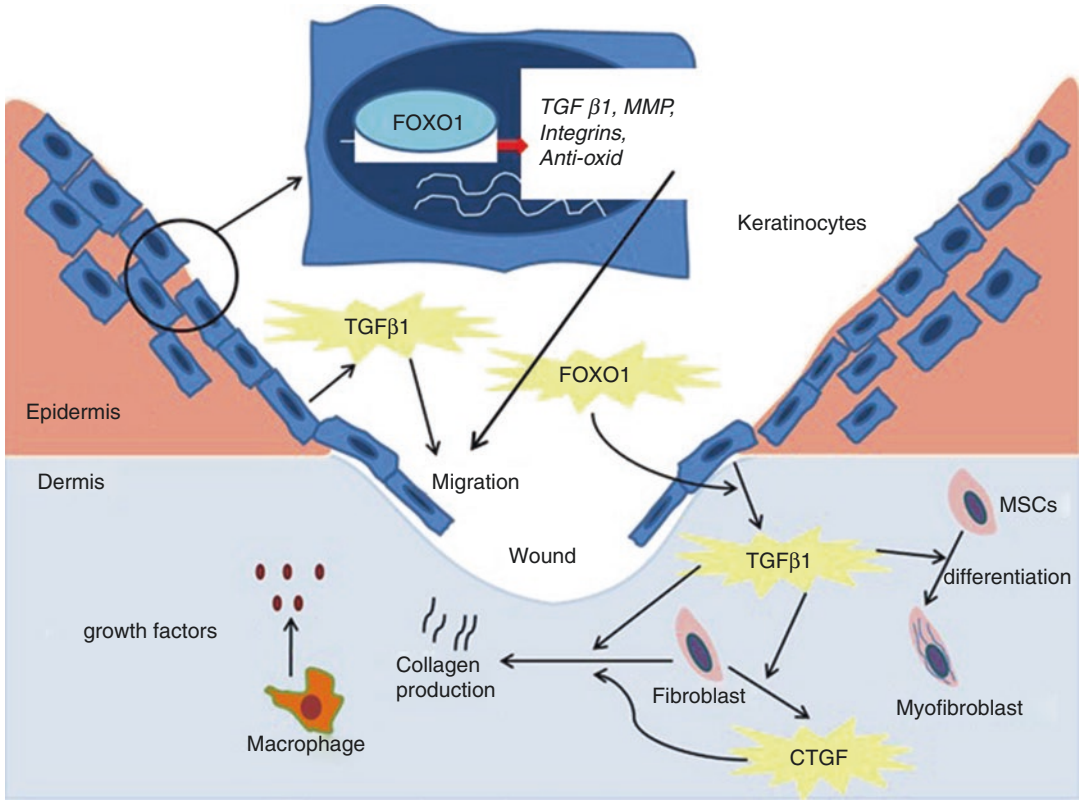


**Fig. 1** FOXO1 organizes keratinocyte activity to promote healing. During normal wound healing, FOXO1 binds to the TGFβ1 promoter in keratinocytes to upregulate TGFβ1 expression, integrins, MMPs, and antioxidants that facilitate keratinocyte migration to close wounds. TGFβ1 promotes connective tissue wound healing directly and also induces CTGF production. TGFβ1 and CTGF stimulate differentiation of myofibroblasts and connective tissue formation. In addition, keratinocytes produce VEGF that enhances angiogenesis. Leukocytes, particularly macrophages, also produce growth factors to enhance healing. In diabetic healing, FOXO1 does not bind well to the TGFβ1 promoter and fails to induce sufficient levels of TGFβ1 expression causing the loss of an important growth factor. Instead, FOXO1 in diabetic conditions (high glucose, AGE, and TNF levels) stimulates production of higher amounts of CCL-20, SerpinB2, and IFN-36γ which interfere with keratinocyte migration

## Conclusions

One of the primary mechanisms through which FOXO1 was shown to enhance healing was through upregulation of TGFβ1. Since its functional role in diabetic healing has not been investigated, we carried out experiments to address this issue. Surprisingly, lineage-specific deletion of *Foxo1* led to enhanced wound healing behavior of keratinocytes in diabetic wounds, and in wounds of normal mice, the opposite was observed. Thus, in diabetic mice, FOXO1 has the opposite effect on keratinocyte wound healing behavior as it does under normal conditions. The differential effect of FOXO1 on normal and diabetic healing was due to changes in its regulation of downstream targets, which was modulated by factors that are elevated in diabetes. In normal conditions, FOXO1 binds to the *TGFβ1* promoter and upregulates TGFβ1 expression, which promotes keratinocyte migration. When stimulated in vitro with high glucose, AGEs, or TNF-α, FOXO1 fails to bind to the *TGFβ1* promoter and does not upregulate TGFβ1 expression. Instead, FOXO1 enhances the expression of factors that lead to reduced keratinocyte migration. This provides a mechanistic explanation for impaired reepithelialization in situations where the levels of glucose, AGEs, and TNF-α are elevated such as diabetes. Additional studies demonstrate that FOXO1 upregulates expression of TGFb1 and VEGF in keratinocytes and that FOXO1 expression in keratinocytes plays a significant and important role in enhancing connective tissue healing and angiogenesis.

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# Nanohybrid Scaffolds for the Treatment of Diabetic Wounds

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## 1 Introduction

Diabetic wounds are the main cause of mortality in patients with diabetes. Patient education, blood sugar control, wound debridement, off-loading, surgery, and advanced therapies are still the standard care of therapies for treating diabetic wounds. Currently, diabetic wound treatment is focused on early diagnosis, prevention, and patient education [1, 2]. Although the pathogenesis of diabetic wound healing is multifactorial, the long-term inflammation accompanied by infections with improper tissue management is the principal factor that impairs wound healing [3, 4]. To date the active dressings which have emerged are targeted to either control the infection by delivering antimicrobials [5–8] or modify the MMP levels in the wound site [3, 9, 10], apart from tissue management.

In tissue engineering, especially in diabetic wound healing, collagen-based products have become popular over the years because of easy manufacturing methods and potential applications. They are available in various forms such as films, gels, fibers, and sponges [11]. Among these various types, porous 3D collagen scaffolds have received greater attention as they promote cell-biomaterial interactions, cell adhesion, and ECM deposition [12]. The collagen molecule in the scaffolds is degraded by collagenase, which leads to the formation of gelatinized fragments that are cleaved by several nonspecific proteases. This results in cellular infiltration of fibroblasts which synthesize new extracellular matrix (ECM) components for tissue regeneration. In general, a balance exists between these two processes [13]. However, in diabetic conditions this balance is disturbed resulting in wounds with higher levels of inflammatory mediators and oxidative stress conditions causing collagen degradation alone [14]. Various collagen scaffolds have been used to deliver antibiotics, growth factors, cytokines, or mediators of genetic engineering as a regenerative medicine in diabetic wound healing. However, persistent inflammatory conditions of chronic wounds cause degradation of these growth factors as well as collagen resulting in diabetic wounds falling short of the optimal goal

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[15–17]. Hence, the therapeutic intervention to reduce inflammation and initiate tissue regeneration is considered vital in achieving faster healing of diabetic wounds. Another main disadvantage of collagen to be utilized as a scaffold is its biological instability. In an effort to reduce the easy degradation of collagen and enhance its weak mechanical property, chemical cross-linking [18–20] or combined hybridization with synthetic polymers [21] or natural polysaccharides [22–24] is actually regarded as an efficient methodology to fabricate collagen-based scaffolds with superior properties. Natural polymers are preferred over synthetic polymers because of their excellent biodegradability and biocompatibility [25]. Although the cross-linking process may improve the biodegradability and mechanical properties of collagen scaffolds, the mechanical properties still need to be enhanced to implant the scaffolds for in vivo testing. For this, various co- and synthetic polymers were used in combination with collagen. Accordingly, in this study, alginate was blended with collagen and then cross-linked to improve its physical stability and also to provide a moist wound environment.

CUR is a widely known anti-inflammatory and antioxidant agent [3]. The wound healing activity of CUR can be attributed to its biochemical effects that include its anti-infectious, anti-inflammatory, and antioxidant activities [26]. Hence, the use of CUR can be a significant approach in enhancing the impaired healing of diabetic wounds. However, regardless of such distinct biological activities of CUR, it suffers from poor bioavailability and stability [27]. Moreover, the mode of polyphenol application is a matter of concern, due to toxic responses at higher concentrations of CUR [28]. Therefore, a water-soluble formulation with sustained release property is desired for clinical application of CUR. In this work, CUR was incorporated into chitosan (CS), a naturally derived polymer possessing wound healing activity [29] nanoparticles (CSNPs) for better stability and controlled release. CSNPs prevent the rapid clearance of drug from the site of inflammation to the systemic circulation due to the vascularity and tissue permeability associated with inflammation. Additionally, CSNPs safeguard CUR from the severe chronic wound environment [30].

Doxycycline hyclate (DOX), an antibiotic of the tetracycline family of drugs, is an inhibitor of matrix metalloproteinases (MMPs). It inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit. DOX has bacteriostatic activity against a broad range of gram-positive and gram-negative bacteria both *in vitro* and in clinical infections. DOX also provides an effective means of treating MRSA infections [31]. Several animal studies reported that treatment with DOX or other tetracycline analogues improved healing parameters. In the current era of increasing antibiotic resistance when systemic antibiotic treatment of wound infections is increasingly likely to fail, the ability to deliver high concentrations of antimicrobial agents locally to prevent wound infections is highly desirable in case of infections associated with diabetic wounds. Additionally, topical application greatly reduces the risk of antibiotic-associated systemic toxicities. Thus, a wound dressing with antimicrobial properties that decreases the risk of infection may have significant clinical benefit particularly in settings (e.g., contaminated diabetic wounds) where there is a high likelihood of wound infection developing. DOX is a broad-spectrum drug and is effective against various microorganisms which are present in DFIs.

The constantly flourishing field of biomaterials and tissue regeneration research has contributed to the evolution of novel materials to be incorporated for the treatment of various morbid circumstances. To the best of our knowledge, we have yet to come across a research paper detailing the wound healing capabilities of CUR-CSNP-incorporated collagen/alginate scaffolds, let alone diabetic wound healing in particular [32].

Contemplating the above literature, in this current research, CUR-loaded CSNPs were fabricated and incorporated to COL-ALG scaffolds along with DOX that aids in reducing the inflammation as well as tissue regeneration in infected diabetic wounds. Further CUR-CSNPs in COL-ALG scaffolds (nanohybrid scaffolds) also help to improve physical attributes, corrosion rate, and biodegradation of composite scaffolds. In turn, the scaffolds act as drug depot for enhanced sustained release of CUR and DOX. This nanohybrid scaffold not only minimizes the inflam-

mation and infections but also aids in enhancing cell proliferation and tissue regeneration in diabetic wounds.

## 2 Materials and Methods

### 2.1 Materials

Doxycycline hyclate, collagen (porcine type I), and chitosan (MW 100–300 kDa) were purchased from MP Biomedicals (India) Pvt. Ltd., Mumbai, India. Polyvinylpyrrolidone (PVP) K30, sodium tripolyphosphate (TPP), 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, MW = 191.7), N-hydroxysuccinimide (NHS), 2-morpholinoethane sulfonic acid (MES), collagenase, streptozotocin, acetonitrile (HPLC grade), methanol HPLC grade, and triethylamine were procured from Sigma Aldrich, USA. Trypsin, Dulbecco's modified essential medium (DMEM), and fetal bovine serum (FBS) were purchased from HiMedia, India. Glutathione/ GSH and glutathione reductase were purchased from Fluka Chemicals, India.

### 2.2 Preformulation Studies

#### 2.2.1 Compatibility Study Between CUR and DOX Using Differential Scanning Calorimetry (DSC)

Compatibility between the selected drugs (CUR and DOX) was studied using DSC Q200 (TA Instruments, USA). Under nitrogen flow of 40 mL/min, the samples were sealed in aluminum pans and heated (10 °C per min, 30–300 °C temperature). An empty pan was used as a reference. Thermograms were obtained for CUR, DOX, and their physical mixture.

#### 2.2.2 Compatibility Study Between CUR and DOX Using Fourier Transform Infrared Spectroscopy (FTIR)

Compatibility between the selected drugs was further studied using FTIR. A physical mixture of both the drugs (alone or in combination) was prepared and mixed with anhydrous potassium bromide (KBr) in a ratio of 1:4. About 100 mg of this mixture was ground into fine powder using mortar and pestle followed by compression to form a transparent KBr pellet using a hydraulic press at 15 tons pressure. Each KBr pellet was scanned at 4 mm/s at a resolution of 2 cm over a wave number region from 4000 to 400 $cm^{-1}$ in a FTIR spectrophotometer (Shimadzu 8400-S, Japan). Spectra of the physical mixture and API (1:1 ratio) were compared and their IR peaks matched to detect any appearance or disappearance of peaks.

#### 2.2.3 Saturation Solubility Studies

The saturation solubility of CUR in simulated wound fluid (SWF) was determined at pH 7.4 with or without sodium lauryl sulfate (SLS) at 37 ± 0.5 °C in isothermal shaker (IKA KS400 I, Germany) for 72 h. SWF consisted of sodium chloride (7.996 g), sodium bicarbonate (0.350 g), potassium chloride (0.224 g), dipotassium phosphate (0.228 g), magnesium chloride (0.305 g), calcium chloride (0.278 g), sodium sulfate (0.071 g), tris(hydroxymethyl) aminomethane (6.057 g), and hydrochloric acid (to adjust pH 7.4) dissolved and made up to 1000 mL with distilled water having final pH of 7.4. CUR was added in increments of 1 mg till the saturation was achieved. Drug solubility was determined by UFLC at the end of 72 h ($n$ = 3).

### 2.3 Simultaneous Analytical Method Development for the Estimation of CUR and DOX Using Ultrafast Liquid Chromatography (UFLC)

A UFLC (Shimadzu LC2010A HT, Japan) instrument possessing two LC20AD solvent delivery modules, SPD-M 20A PDA detector and a Rheodyne injector (model 7125, USA) valve fitted with a 20 µL loop, was employed for chromatographic determinations. A system controller (SCL-10A) was used for operation through a personal computer with the Shimadzu chromatographic software (LC Solution, Release 1.11SP1) installed in it. During validation procedure, dual-wavelength mode was used, with CUR monitored at 424 nm, and DOX at 353 nm.

Chromatographic separation was achieved on a Hibar C18 (250 × 4.6 mm i.d., 5 µm) column. The mobile phase was a mixture of acetonitrile (ACN):5 mM Pot. dihydrogen orthophosphate (pH 3.0) (65:35 v/v).

## 2.4 Preparation and Optimization of CUR-CS Nanoparticles (NPs) Using Ionic Gelation Method

CUR-loaded CSNPs have been prepared using ionic gelation method. 8 mg of CUR was taken and dissolved in 10 mL of absolute ethanol to prepare 800 µg/mL of CUR solution. A CS solution was prepared using 2% acetic acid. Subsequently, the pH of resulting CS was adjusted to 5 using 2 M NaOH. PVP K30 solution was added to CS solution to achieve a concentration of 0.1%. Spontaneous nanoparticle formation resulted from the dropwise addition of CUR into the CS solution and subsequent addition of sodium triphosphate (TPP) solution (0.125% *w*/*v* in deionized water) under constant magnetic stirring (1000 rpm). To ensure CS:TPP weight ratios of 2:1, 3:1, 4:1, and 5:1 (w/w), appropriate volumes of CS and TPP solutions were incorporated. This suspension was subjected to specific time-based stirring (45 min) to produce CSNP suspensions. The appearance of opalescence was used as an indicator for nanoparticle formation [33].

## 2.5 Characterization of CUR-CS Nanoparticles

### 2.5.1 Determination of Encapsulation Efficiency

The encapsulation efficiency (EE) of CUR within the drug-loaded CSNPs was determined by pelletizing the NPs. In brief, the CUR-CSNPs were separated from the nanosuspension by ultracentrifugation using Remi laboratory centrifuge (REMI R-8C, India) at 10,000 rpm for 30 min. The resulting pellet was redispersed in deionized water and was lyophilized. A known quantity (2 mg) of lyophilized sample was taken in 10 mL of ethanol; the solution was sonicated thoroughly using a probe sonicator (Bandelin RK 100 H, Germany) for 15 min. The final solution was again centrifuged (10,000 rpm, 15 min), the supernatant was collected, and drug concentration was quantified in it upon analyzing the peak area obtained by using corresponding chromatogram which corresponds to the peak area of CUR. The amount of CUR encapsulated in nanoparticles was expressed as EE% and calculated as follows:

$$\mathrm{EE}\,(\%) = \frac{\text{Mass of the drug in nanoparticle}}{\text{Mass of the drug used in the formulation}} \times 100$$

### 2.5.2 Determination of Particle Size, Zeta Potential, and Polydispersity Index

The prepared nanoparticles were washed with double-distilled water (filtered through 0.22 µm) several times before particle size analysis. The average particle size and zeta potential of the CUR-CSNPs were determined by particle size analyzer (Malvern ZS 90, UK) which allows sample measurement in the range of 0.020–2000 µm.

### 2.5.3 Morphological Characterization Using Scanning Electron Microscopy (SEM)

The morphology of the CUR-CSNPs (size and shape) was verified using SEM. Lyophilized NPs were resuspended in distilled water; subsequently they were placed on a silicon grid and left to dry at room temperature. The NP suspension was then vacuum coated with gold for 3 min before SEM analysis. Surface characteristics of the samples were observed under a SEM (Carl Zeiss, Germany) operated at 15 keV pulse under different resolutions.

### 2.5.4 Fourier Transform Infrared Analysis

FTIR spectra of CUR, CS, TPP, and CUR-CSNPs were assessed using FT-IR spectrophotometer (Shimadzu 8400-S, Japan).

#### 2.5.5 Differential Scanning Calorimetry

DSC analysis of individual samples (CUR, CS, and TPP) and CUR-CSNPs was performed using DSC Q200 (TA Instruments, USA).

#### 2.5.6 X-ray Diffraction Studies (XRD)

An XRD peak mainly depends on the crystal size as they indicate the crystalline nature at particular value at $2\theta$ range. An X-ray diffractometer was employed to determine the molecular arrangements of CUR alone and in nanoparticulate formulations (PANalytical X'pert PRO, The Netherlands) using CuKα radiation.

### 2.6 Preparation of COL/ALG Scaffolds Impregnated with CUR-CSNPs and DOX (Nanohybrid Scaffolds)

Freeze-drying method was used to prepare scaffolds. COL solution (4 wt.%) was made by dissolving COL in 0.5 M acetic acid and ALG solution (4 wt.%) was prepared by dissolving ALG powder in distilled water. Subsequently, the COL–ALG blends were prepared; initially the pH of acidic COL was adjusted to 7 by adding 2 M NaOH at 4 °C, then the ALG solution was added dropwise to COL solution, and the final pH was adjusted to 6–7. Finally, a clear homogenous blend was obtained by continuous stirring for 2 h, which was then centrifuged at 4000 rpm for 15 min to remove entrapped air bubbles. Later, the resulting blend was poured into molds to form hydrogels. The hydrogels were then washed with deionized water and then placed into polystyrene culture flasks at −80 °C for 72 h and lyophilized, ensuing in porous COL matrices. COL–ALG composite scaffolds with COL–ALG ratio of 50/50 (*w*/*w*) were prepared and thereafter expressed as COL–ALG scaffolds (placebo scaffolds). The obtained scaffold discs were chemically cross-linked with EDC/NHS (EDC-cross-linked) in MES buffer solution (pH 5.5, 0.05 M) for 24 h at 4 °C [34]. To prepare drug-loaded scaffolds, CUR-CSNPs and DOX were added to ALG solution under magnetic stirring and it was added dropwise to COL solution to obtain a final concentration of 1% (w/v) CUR and 1% (w/v) DOX. The resultant drug-loaded COL–ALG composite mixture was then stirred overnight to ensure uniform mixing of the CUR-CSNPs and DOX and then immediately poured into a culture flask, frozen, and lyophilized. The freeze-dried scaffolds were preserved in a desiccator for further evaluation. The CUR-CSNP- and DOX-loaded COL–ALG scaffolds would be thereafter referred as nanohybrid scaffolds.

### 2.7 Scaffold Characterization

#### 2.7.1 Matrix Morphology

The cross-sectional morphologies of COL–ALG scaffolds and nanohybrid scaffolds were observed using field emission scanning electron microscopy (Carl Zeiss, Germany). In general, pore size is expressed as the distance between the struts and assessed by using SEM images. In this study, 40 pores were randomly selected and then measured from scaffolds.

#### 2.7.2 Differential Scanning Calorimeter

DSC (DSCQ-200, TA Instruments, USA) was employed to assess the thermal stability of the nanohybrid scaffolds.

#### 2.7.3 Tensile Strength Measurement

For the assessment of the scaffold's mechanical properties, the scaffolds were cut into small strips ($10 \times 20$ mm$^2$). The measurement was performed on the tensile machine (Chemilab Top-tech 2000, South Korea). A stretching speed of 0.5 mm s$^{-1}$ was employed for the measurement of the scaffolds' stress–strain curves. All values are mean ± SD ($n = 3$).

#### 2.7.4 Swelling Behavior

The cross-linked and non-cross-linked nanohybrid scaffold discs [8 mm (diameter) and 1.5 mm (thickness)] were weighed as $W_{dry}$. The scaffold samples were then soaked in a closed tube

comprising 5 mL SWF, pH 7.4, maintained at 37 °C. The swollen samples were removed from the tube after72 h and the excess liquid was removed by blotting with a filter paper, and weighed as $W_{wet}$. The swelling ratio ($S$) was calculated as follows:

$$S = \frac{\left(W_{wet} - W_{dry}\right)}{W_{dry}} \times 100$$

### 2.7.5 Matrix Degradation Studies

The EDC-cross-linked nanohybrid scaffold was subjected to *in vitro* biodegradation tests by collagenase digestion; non-cross-linked scaffolds were also studied for comparison. A measure of the extent of COL degradation from the subjected scaffolds was obtained through the analysis of hydroxyproline content [35, 36]. Briefly, the scaffolds of each group were immersed in SWF (pH 7.4, 0.01% sodium azide) containing 265 U/mg (56 units) of collagenase at 37 °C. At a given time point, instant incubation of the assay mixture in an ice bath halted the degradation process. Post-centrifugation (10,000 rpm, 10 min), the obtained supernatant was subsequently hydrolyzed with 6 M HCl at 110 °C for 28 h. The hydroxyproline content in the test scaffolds was measured at a wavelength of 570 nm on a spectrometer (Bio-Rad 550, Bio-Rad Laboratories, India) while employing a standard curve. The definition for degree of biodegradation is "the percentage ratio of the hydroxyproline released from the scaffolds at different times to the completely degraded one with known composition and known weight." The values were expressed as the mean ± standard deviation ($n$ = 3).

### 2.7.6 In Vitro Drug Release Studies

In vitro release of CUR from CSNPs was carried out using dialysis bag membrane method. CUR-CSNPs (100 mg) suspended in 2 mL of phosphate buffer saline, pH 7.4, maintained at 37 °C were added to a dialysis tube (MW CO 12,000 D, 16 mm diameter, HiMedia, India) with both ends tightly tied. Further it was immersed in 200 mL of SWF (pH 7.4, 37 °C, constant stirring). The release of CUR and DOX from nanohybrid scaffold was carried out using 20 mL of SWF maintained (pH 7.4 at 37 °C) in which composite scaffold (3 $cm^2$ [1.5 × 2 cm]) was dispersed. At regular time intervals, the supernatant was pipetted out and replaced with equivalent volumes of fresh phosphate buffer solution. The extent of drug release was subsequently evaluated by UFLC analysis at 424 nm using a standard curve of CUR in ethanol [37].

### 2.7.7 In Vitro Drug Release Kinetics

To examine the drug release kinetics, the release data were fitted to models representing zero order, first order, and Higuchi square root of time kinetics.

## 2.8 In Vitro Cytotoxicity Studies on Fibroblast-3T3 Cells [38]

Cell viability of the nanohybrid scaffolds was performed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Scaffolds of standard dimensions (5 mm × 5 mm × 3 mm) were sterilized in 75% ethanol for 30 min, rinsed 5 times in sterile water for 5 min, and then placed in 24-well plates with appropriate DMEM (2 mL) added to each well. Following this, the NIH-3T3 cells ($2 \times 10^4$ cells/well) were seeded in the 24-well plate with or without the scaffolds. In addition to this, a media control was also maintained to facilitate better comparison and the test was performed for 72 h. Photographs of the controls and tested scaffolds were acquired using a light microscope (Motic AE31E, Motic®, China). The experiment was performed in triplicate.

## 2.9 In Vitro Antibacterial Studies [39]

The antimicrobial activity of the drug-loaded and placebo scaffolds was tested against *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli* (*E. coli*). Suspensions of *S. aureus*, *P. aeruginosa,* MRSA, and *E. coli* were prepared from

fresh colonies after overnight incubation and the turbidity was adjusted to 0.5 McFarland standards ($\sim 10^5$ c.f.u/mL). Scaffolds' antibacterial activity in these microbes was estimated by the Kirby-Bauer disc diffusion method. Scaffold samples (discs) of constant weight and 5 mm diameter were sterilized and incorporated in the study. A nutrient agar plate with evenly spread bacterial suspension had the samples placed upon its surface and subjected to overnight incubation (37 °C) followed by zones of inhibition measurement.

## 2.10 Skin Irritation Studies [40]

A modified Draize method was employed for skin irritation assessment of the test scaffolds. Albino Wistar rats (130–160 g) were allowed for acclimatization in the animal facility for a period of 7 days prior to study commencement. The fur on the dorsal side of the animals was epilated while ensuring the protection of the skin 4 h prior to the experiment. Left dorsal surface of the animal was treated with 0.9% (w/v) NaCl solution as a control and right dorsal surface of the animal was treated with COL–ALG (placebo) and nanohybrid scaffolds. The scaffolds were applied to approximately 25 mm diameter of the skin followed by application of secondary application as support. The animals were then kept in their cages and were examined at 24, 48, and 72 h. At the end of the contact time, the dressings were removed and the sites were inspected for dermal reactions such as erythema and edema.

## 2.11 In Vivo Diabetic Wound Healing Studies

### 2.11.1 Diabetes Induction and Wound Creation

Healthy, adult male Wistar rats (170–200 g) were utilized for the in vivo wound healing studies. They were housed in standard polycarbonate cages with ad lib access to standard chow and water and maintained on a 12:12-h light:dark cycle in a climate-controlled room. Following 10 days of acclimatization, a single injection of STZ (60 mg $kg^{-1}$) in citrate buffer solution (0.1 M, pH 4.5) was intraperitoneally administered to initiate the induction of diabetes. After 2 days of STZ injection, animals with blood glucose levels ≥300 mg/dL (Glucometer, Accu-Chek, USA) were kept under observation for 4 more days and only those that consistently demonstrated elevated blood glucose levels were selected for the subsequent procedures. A wound measuring 2 × 2 $cm^2$ (≈400 $mm^2$) was created on the dorsal thoracolumbar region of the diabetic rats under ketamine and xylazine anesthesia (100 mg/kg and 10 mg/kg, respectively). After anesthesia recovery, the animals were housed individually and closely monitored. The animals were grouped into five groups. Group 1 was treated with sterile gauze (control), group 2 was treated with COL–ALG scaffold (placebo group), group 3 was treated with CUR-CSNP-loaded scaffold (test group 1), group 3 was treated with DOX-loaded scaffold (test group 2), and group 4 was treated with nanohybrid scaffold (test group 3). All animal experiments were approved by Institutional Animal Ethical Committee, JSS College of Pharmacy, Ooty (Proposal no. JSSCP/IAEC/PhD/PH-CEUTICS/01/2015–2016).

### 2.11.2 Wound Area Measurement

Percentage wound contraction was measured using the formula

$$\%\ \text{wound contraction} = \frac{\text{day 0 wound area} - \text{wound area on a particular day}}{\text{day 0 wound area}} \times 100$$

### 2.11.3 Collection of Tissue

On days 3, 7, 14, and 21, two animals from each group were sacrificed with an overdose of anesthesia and the wound tissue excised and halved. One half was stored in 10% formalin for histopathology, while the other half was processed for biochemical evaluation. Post-homogenization [ice-cold lysis buffer, 1% Triton × 100, 10 mM phenylmethylsulfonyl fluoride, 1 mg/mL aprotinin, and 1 mg/mL leupeptin in phosphate buffer saline (pH 7.4)] and centrifugation (12,000 rpm, 10 min, 4 °C), the supernatants were aliquoted and refrigerated at −80 °C for use in further assays.

#### 2.11.4 ELISA Assay for TNF-α and IL-10

The protein levels of tumor necrosis factor- α (TNF-α) and IL-10 in wound lysate were determined using ELISA kits (Koma Biotech Inc., Seoul, Korea) according to the manufacturer's protocol using a multimode plate reader (Tecan M200, Austria). Samples of cytokine and protease were used in duplicate and OD measurements were then verified against a standard curve. The results are expressed as pg of TNF- α and IL-10 per mg of total protein.

#### 2.11.5 Western Blot Analysis of MMP-9

The wound tissue homogenate was centrifuged at 3000 rpm for 10 min to obtain a pellet. The pellet was resuspended in three volumes of lysis buffer (100 mM/L KCl, 10 mM/L HEPES, 1 mM/L EDTA, 1% Triton ×-100, and protease inhibitor). Post-centrifugation (2000 rpm, 10 min) the supernatant was again centrifuged (10,000 rpm, 30 min, 4 °C); the eventual pellets were resuspended in lysis buffer (100 μL) and mixed with the supernatant of the second centrifugation. Bradford's method was used to determine the total protein content. Fifty micrograms of protein from each sample was subjected to separation by SDS-PAGE using 8% polyacrylamide gels followed by transfer of proteins to a nitrocellulose membrane. The membranes were then incubated overnight with mouse MMP-9 (1:1000) at 4 °C. Anti-mouse IgG-HRP (1:2000) was added at room temperature and incubated for 60 min as a secondary antibody. Quantification was performed by taking GAPDH band intensities as loading control. The images were developed using an image scanner connected to a computer.

#### 2.11.6 Histopathological Analysis

Hematoxylin and eosin (H&E) staining was used to determine the gross morphological changes at the wound site. The granulation/healing tissues fixed in 10% formalin were embedded in paraffin. Rotary microtome (Leica Biosystems, India) was used to obtain 5 μm tissue slices and subjected to H&E staining followed by visualization (Olympus C×31, Japan) at 40× magnification.

### 2.12 Statistical Analysis

The numerical data was expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was performed to obtain the statistical significance. GraphPad Prism® v5.01 (San Diego, CA, USA) was used to generate the statistical significance and the values having $p < 0.05$ or lower were considered significant.

## 3 Results

### 3.1 Preformulation Studies

#### 3.1.1 Compatibility Study Between CUR and DOX Using DSC and FTIR

Since in this research two drugs, i.e., CUR and DOX, have been selected to synergize the diabetic wound healing, the compatibility studies between these drugs have been performed using DSC and FTIR studies. Figure 1 shows the DSC thermograms of CUR and DOX. CUR has shown a sharp endothermic peak at 187.49 °C while DOX has a melting point of 184.70 °C. The physical mixture of both the drugs has a melting point of 186.20 °C. It indicates that CUR and DOX may be compatible with each other and also the absence of new peaks further indicates that both drugs are compatible.

These results were further confirmed to FTIR studies. Figure 2 shows the FTIR spectra of CUR, DOX, and their physical mixture. FTIR spectra of CUR have shown the characteristic peaks at 1161.91 (C—O—C) and 3499.90 (OH) while DOX has shown the characteristic peaks at 1627.01 (C=O), 3296.46 ($NH_2$) and 3411.08 (OH). The FTIR spectra of CUR and DOX physical mixture have shown the same characteristic peaks which are present in individual compounds without any presence of new peaks and absence of characteristic peaks. These results further support and conform the compatibility between CUR and DOX.

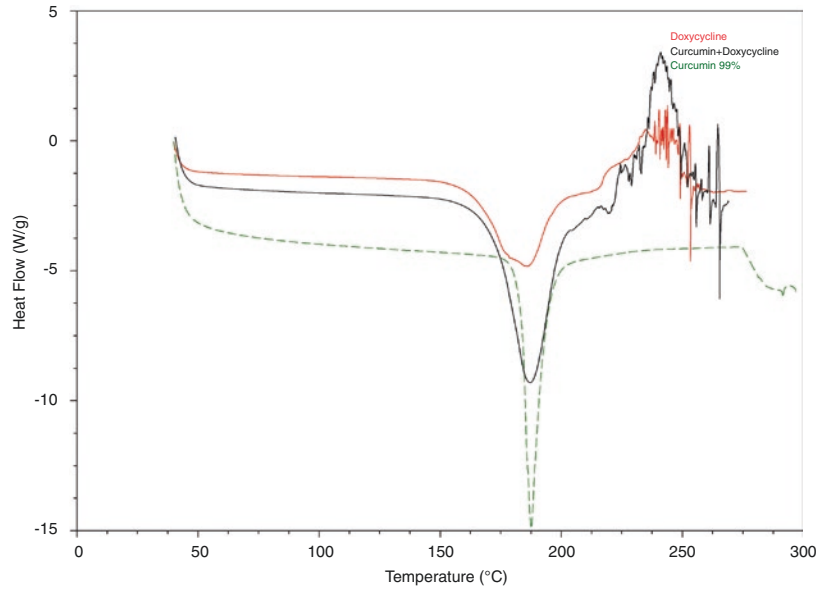



**Fig. 1** DSC thermograms of CUR, DOX, and their physical mixture

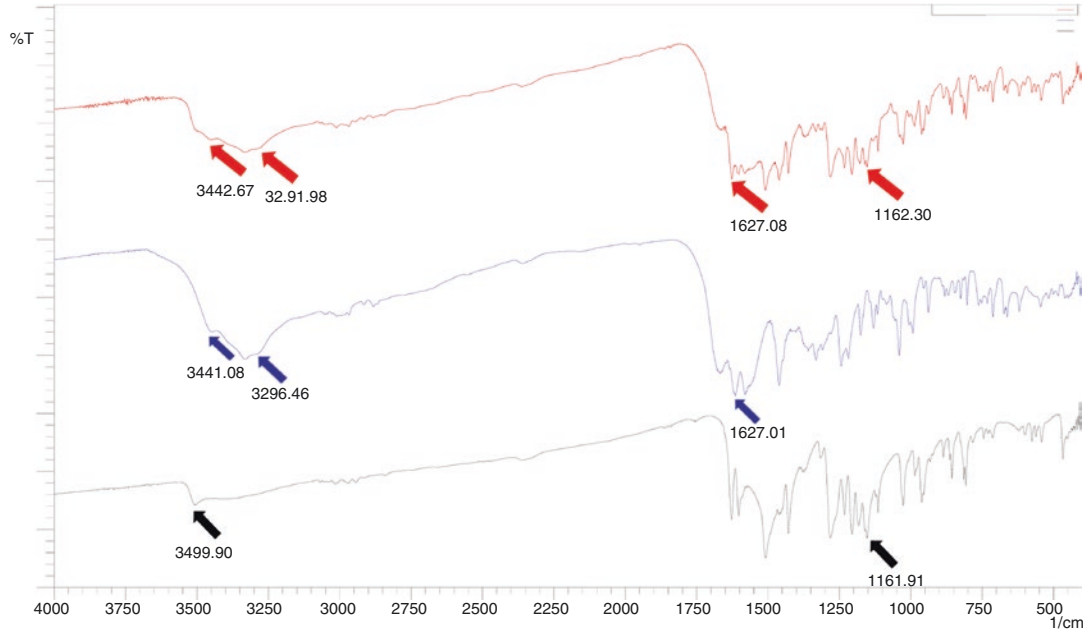



**Fig. 2** FTIR spectra of CUR, DOX, and their physical mixture

### 3.1.2 Saturation Solubility Studies

In order to maintain the sink conditions, it is essential that the drug should have sufficient solubility in the release media. Since CUR is poorly soluble in water, solubility determination was carried out using 0.25, 0.5, 0.75, and 1.0% of SLS in SWF and phosphate buffer (pH 7.4 and 8). The saturation solubility of CUR in different

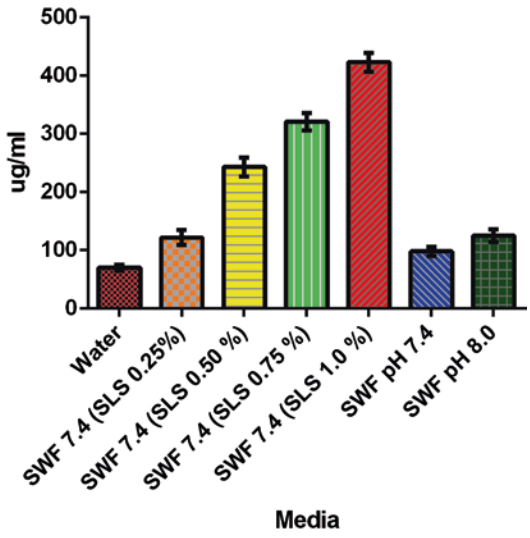



**Fig. 3** Saturation solubility of CUR in various dissolution media

media is given in Fig. 3. The results indicated that solubility of CUR increased with increase in SLS concentration in the medium and maximum solubility (422 ± 8.6 µg/mL) was found in media containing 1% w/v of SLS.

## 3.2 Simultaneous Analytical Method Development for the Estimation of CUR and DOX Using UFLC

By corroborating with the ICH guidelines for its linearity, range, accuracy, precision, sensitivity, and robustness, the UFLC method incorporated in this study was ensured of its validity. Placebo and test samples, with their concentrations equated to that of the standard, were injected into the UFLC to ensure the specificity of the method. To test for linearity, standard solutions (five) were prepared for CUR (10–2000 ng/mL) and DOX (20–2000 ng/mL) in mobile phase. Every solution injection was triplicated and the linear regression curve for both the molecules was drawn. A standard quantity of each molecule was added that corresponds to three concentrations (50, 100, and 150%) of the assumed nominal drug concentration with excipients for the determination of the method's accuracy. Care was taken to perform this method in triplicate and received similar processing as that of the sample solution. Recoveries of CUR and DOX were determined. The method's precision was verified by performing six independent comparisons of the test sample versus standard. By ensuring that a different analyst evaluated the samples on different days, intermediate precision was achieved. Deliberate variations in chromatographic conditions (mobile-phase flow rate was changed from 1.0 to 0.8 mL/min and 1.2 mL/min while organic strength was altered by ±2% units) ensured the robustness of the method.

The effect of composition of the mobile phase on the retention time of CUR and DOX was thoroughly investigated by trial-and-error method. Mobile-phase composition of ACN:5 mM Pot. dihydrogen orthophosphate (65:35 v/v) was found to be optimum to separate the CUR and DOX. The CUR was eluted at 5.8 min and DOX was eluted at 2.3 min (Fig. 4).

The present UFLC method was optimized specifically considering the placebo incorporated in this study (CSNPs). No interference was observed in the chromatograms obtained for the placebo. With an $R^2$ value >0.995, good linearity was ensured at five levels of CUR and DOX. The slope and intercept of the calibration curve were 212.3 and 1047.8 for CUR, and 9.2 and 497.6 for DOX, respectively. The LOD and LOQ for CUR were found to be 3 and 10 ng/mL. The LOD and LOQ for DOX were found to be 5 ng/mL and 15 ng/mL, respectively. Accuracy was assessed by spike recovery, in which the % recoveries of analytes at each level ($n$ = 3) were found to be 97.47 and 95.38% for CUR and DOX, respectively. The recoveries of analytes at each level were found well within the acceptable criteria of bias, ±5% (Tables 1 and 2). The intra- and inter-assay precision ($n$ = 6) was confirmed since the %C.V. were well within the target criterion of ≤3% (Tables 3 and 4).

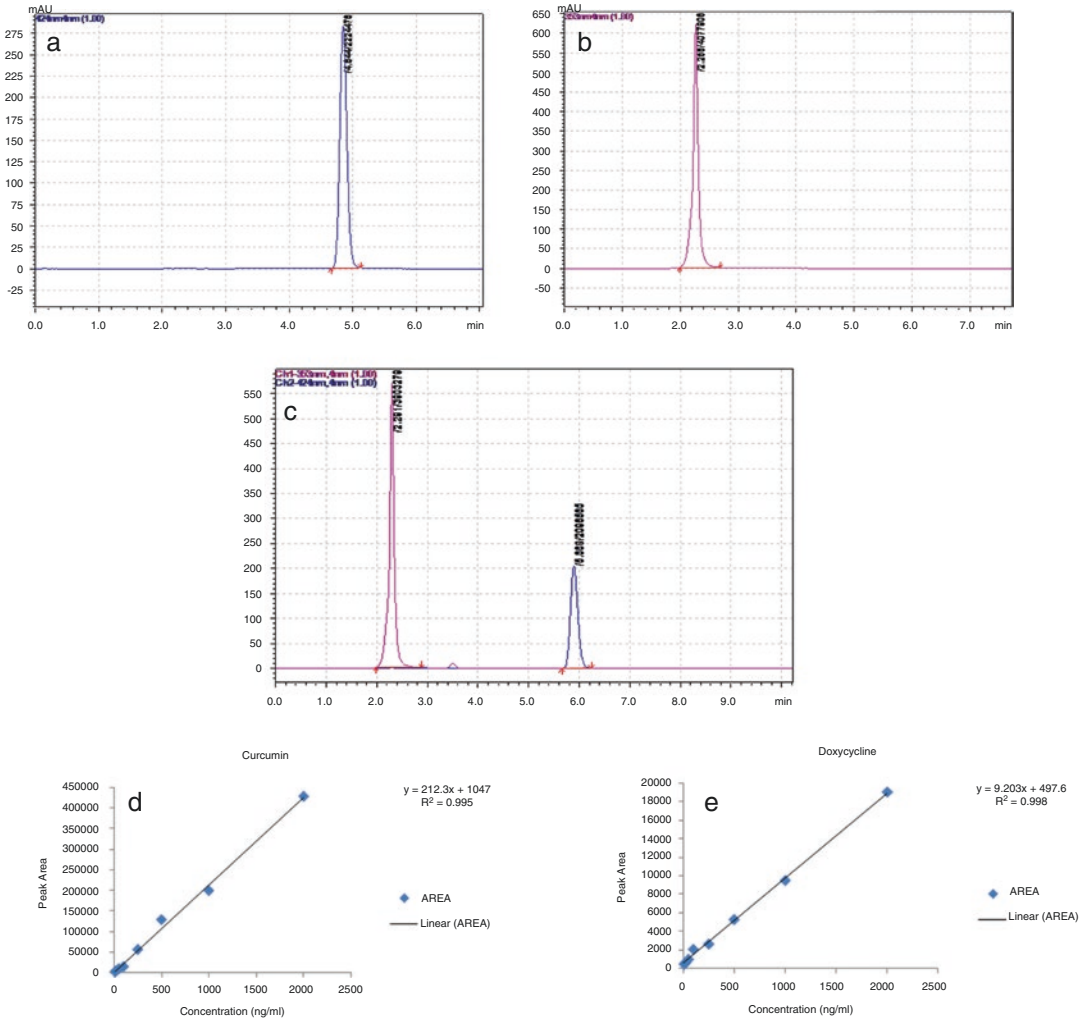



**Fig. 4** Analytical method development of CUR and DOX; chromatogram of (**a**) CUR, (**b**) DOX, (**c**) CUR, and DOX; calibration curve of (**d**) CUR and (**e**) DOX

**Table 1** Precision studies: inter-day precision-1

| | Curcumin (ng/mL) | | | Doxycycline (ng/mL) | | |
|---|---|---|---|---|---|---|
| # | 10 | 100 | 1000 | 10 | 100 | 1000 |
| 1 | 9.46 | 98.34 | 987.45 | 10.16 | 98.76 | 992.87 |
| 2 | 9.76 | 98.56 | 987.88 | 10.21 | 99.56 | 991.83 |
| 3 | 9.53 | 97.35 | 989.57 | 9.87 | 99.76 | 993.56 |
| 4 | 9.73 | 98.85 | 992.93 | 9.76 | 96.84 | 995.03 |
| 5 | 9.1 | 98.53 | 994.87 | 9.87 | 98.56 | 996.87 |
| 6 | 9.36 | 98.16 | 995.81 | 9.85 | 96.87 | 997.45 |
| Mean | 9.49 | 98.298333 | 991.41833 | 9.9533333 | 98.391667 | 994.60167 |
| SD | 0.2457641 | 0.5189766 | 3.6102544 | 0.1846799 | 1.2747143 | 2.2453366 |
| %C/V | 2.5897167 | 0.5279607 | 0.3641505 | 1.8554578 | 1.2955511 | 0.2257524 |

**Table 2** Precision studies: inter-day precision-2

| # | Curcumin (ng/mL) | | | Doxycycline (ng/mL) | | |
|---|---|---|---|---|---|---|
| | 10 | 100 | 1000 | 10 | 100 | 1000 |
| 1 | 9.87 | 99.01 | 987.74 | 9.29 | 97.43 | 998.76 |
| 2 | 9.76 | 98.63 | 989.92 | 9.38 | 98.23 | 997.76 |
| 3 | 9.79 | 98.86 | 994.54 | 9.35 | 99.36 | 996.76 |
| 4 | 9.41 | 98.13 | 996.56 | 9.28 | 99.76 | 998.48 |
| 5 | 9.86 | 98.11 | 995.43 | 9.25 | 96.29 | 994.86 |
| 6 | 9.33 | 99.46 | 995.38 | 9.15 | 99.76 | 996.87 |
| Mean | 9.67 | 98.7 | 993.26167 | 9.2833333 | 98.471667 | 997.24833 |
| SD | 0.2374026 | 0.5248238 | 3.5597102 | 0.0809115 | 1.4150536 | 1.4247585 |
| %C/V | 2.4550425 | 0.5317364 | 0.3583859 | 0.8715778 | 1.437016 | 0.142869 |

**Table 3** Accuracy studies for CUR

| # | Actual concentration (ng/mL) | Amount added (ng/mL) | Amount found | % Nominal | AVG | SD | %CV |
|---|---|---|---|---|---|---|---|
| 1 | 10 | 10 | 19.76 | 98.8 | 97.09 | 2.85 | 2.94 |
| | | | 18.76 | 93.8 | | | |
| | | | 19.73 | 98.65 | | | |
| 2 | 100 | 100 | 198.76 | 99.38 | 99 | 0.54 | 0.55 |
| | | | 196.76 | 98.38 | | | |
| | | | 198.45 | 99.225 | | | |
| 3 | 1000 | 500 | 1458.87 | 97.258 | 96.45 | 0.88 | 0.91 |
| | | | 1432.62 | 95.508 | | | |
| | | | 1448.76 | 96.584 | | | |

**Table 4** Accuracy studies for DOX

| # | Actual concentration (ng/mL) | Amount added (ng/mL) | Amount found | % Nominal | AVG | SD | %CV |
|---|---|---|---|---|---|---|---|
| 1 | 10 | 10 | 18.65 | 93.25 | 94.18 | 2.00 | 2.17 |
| | | | 18.75 | 93.75 | | | |
| | | | 18.01 | 90.05 | | | |
| 2 | 100 | 100 | 194.76 | 97.38 | 97.89 | 0.50 | 0.51 |
| | | | 195.83 | 97.915 | | | |
| | | | 196.75 | 98.375 | | | |
| 3 | 1000 | 500 | 1398.76 | 93.25 | 94.34 | 1.14 | 1.21 |
| | | | 1413.76 | 94.25 | | | |
| | | | 1432.87 | 95.53 | | | |

## 3.3 Optimized Chromatographic Conditions

Stationary phase: Hibar C18 (250 × 4.6 mm i.d., 5 µ), mobile phase: ACN: potassium dihydrogen orthophosphate, mobile phase ratio: 65:35, flow rate: 1.0 mL/min, sample volume: 20 µL using Rheodyne 7725i injector, detection λ: 230 nm (CUR) and 425 nm (DOX), pH: 3.0, buffer strength: 5 mM, data station: LC-20 AD (PDA), retention time of CUR: 5.8 ± 0.1 min, and retention time of DOX: 2.3 ± 0.1 min.

### 3.4 Preparation of CUR-CSNPs Using Ionic Gelation Method

Due to the poor water solubility and stability of CUR, initially, it was first fabricated into chitosan nanoparticles using ionic gelation method and then incorporated into scaffold. Various process parameters have been identified in preparing CUR-CSNPs. Among these stirring time, chitosan and TPP concentrations have been taken into consideration for preparing CUR-CSNPs since these three parameters will highly influence the entrapment efficiency, particle size, zeta potential, and morphology of nanoparticles. In this ionic gelation method, TPP addition into CS solution resulted in the spontaneous formation of CSNPs. Constant stirring was maintained to break down the formed chitosan particles into nanoparticles. The ionic interactions between the negatively charged phosphate groups of TPP (P3O105−) and positively charged amino groups of CS (—$NH_3^+$) resulted in the formation of CSNPs. The pH of CS solution was adjusted to 5 to enhance more protonation on the —$NH_3^+$ of CS to ensure maximum electrostatic interactions with TPP (34). To ensure optimization of CSNPs' physical properties, CS:TPP weight ratios, stirring time, and drug entrapment (processing variables) were investigated. A stock solution of CUR was prepared in absolute ethanol at a concentration of 1 mg/mL. CUR was loaded by incorporating the required volume of CUR solution into the CS solution (Fig. 5).

### 3.5 Characterization of CUR-CSNPs

#### 3.5.1 Determination of Particle Size, Polydispersity Index (PDI), Zeta Potential, EE, and Morphology

The effects of stirring times and CS:TPP quantity ratio on CUR-CSNPs size are demonstrated in Fig. 6. From the results it was observed that 30 min of stirring time did not produce total interaction of TPP with CS. Conversely, in comparison with a stirring time of 30 min, a 1-h stirring time ensured a marginal reduction in the size of CSNPs. However, maintaining a 1-h stirring time yielded a negligible increase in size when the weight ratio of CS:TPP was maintained at 6:1. This may have been a consequence of the greater quantity of CS entrapped within the nanoparticles and subsequently led to the larger CSNP formation. On the other hand, it was observed that further stirring beyond 1 h of all CSNPs prepared irrespective of the CS:TPP weight ratios resulted in an increase in size of the

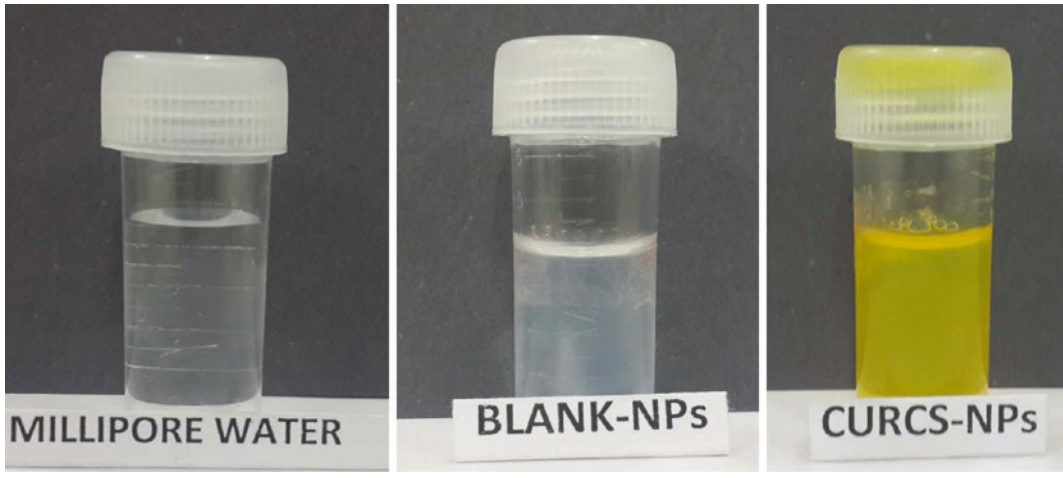



**Fig. 5** (Left) Millipore water. (Middle) Blank CSNPs. (Right) CUR-loaded CSNPs

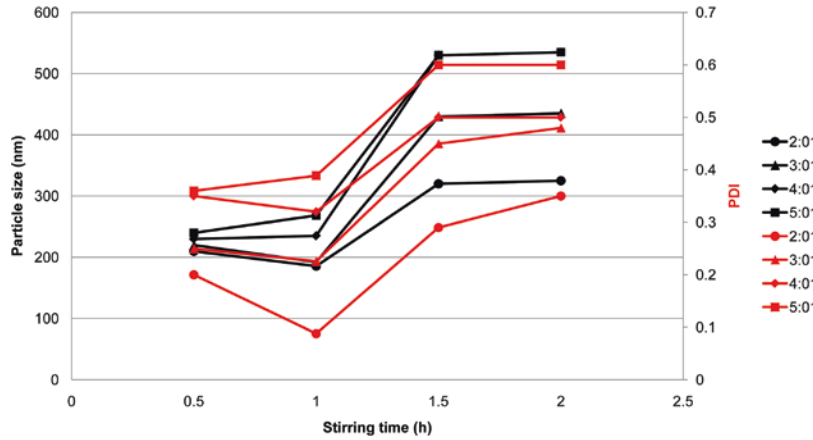



**Fig. 6** Effect of stirring time on particle size and zeta potential of CUR-CSNPs

**Table 5** Effect of CS:TPP weight ratio on various properties of CUR-CSNPs

| Formulation | Chitosan: TPP ratio | Curcumin (μg) | Particle size (nm) | Zeta potential (mV) | Polydispersity index (PDI) | Entrapment efficiency (%) | Amount encapsulated (μg) |
|---|---|---|---|---|---|---|---|
| A | 2:1 | 800 | 185.7 | +22.7 | 0.088 | 62.82 ± 1.3 | 502.64 ± 3.2 |
| B | 3:1 | 800 | 192.2 | +27.5 | 0.226 | 75.41 ± 1.8 | 603.28 ± 4.9 |
| C | 4:1 | 800 | 235.1 | +30.3 | 0.323 | 78.99 ± 1.1 | 631.92 ± 3.3 |
| D | 5:1 | 800 | 268.4 | +31.6 | 0.389 | 80.43 ± 0.9 | 643.44 ± 5.6 |

nanoparticles. This may be attributed to the formation of a constant frequency of interaction between the CS and TPP. Calvo et al. reported similar results when they evaluated the effects of fixed ratios of CS towards gradual increase in molecular weights of the cross-linker polyethylene oxide [33, 41]. Therefore, from these results it may be understood that agglomeration of smaller CSNPs might be the reason for the formation of larger NPs upon prolonging the stirring times during preparation. Hence these results indicate that a stirring time between 1 and 2 h is optimum.

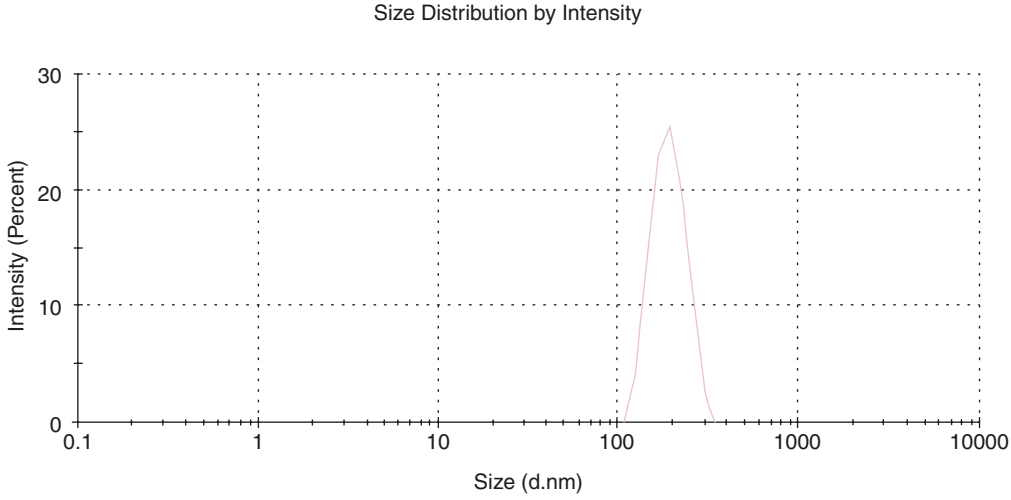
Coming to the effect of particle size related to polymer ratio it is evident from Table 5 that larger CSNPs were formed when the CS:TPP ratio was higher. The increase in the particle size was observed with an increase in the concentration of CS. The reports for CUR-CSNP particle size are shown in Figs. 7, 8, 9, and 10.

The PDI is a very important parameter, which measures the width of the size distribution in a sample of particles. A desired optimal value of PDI should be closer to 0 (0 being for monodispersed particles). Low PDI values also indicate the relative homogenous nature of the dispersion. The data of PDI keeping TPP constant are shown in Table 5. Increasing the amount of CS leads to an increase in the PDI values (Fig. 6).

The prepared CSNPs possessed zeta potentials ranging from +22.7 mV (±1.87 mV) to +31.6 mV (±1.20 mV) (Figs. 7, 8, 9, and 10). As a consequence of the protonation of free amino groups, CS possessed a positive charge and therein the CSNPs were conferred with the same charge too. Irrespective of charge, the zeta potential needs to be around ±30 mV to ensure a stable nanosuspension. In our study, encapsulation efficiency of CUR-CSNPs reached up to 83.43 ± 0.9% (Table 5). The advantage of better encapsulation efficiency lies in the fact that a greater quantity of the molecule gets deposited at the target site and enhances its residency there. Analyzing the results demonstrated the direct proportional behavior of encapsulation efficiency with CS concentration at constant CUR (800 μg) and TPP concentration. The high encapsulation efficiency

**a**

| | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|
| Z-Average (d.nm): 185.7 | Peak 1: | 190.2 | 100.0 | 39.75 |
| Pdl: 0.088 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.671 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality: Good | | | | |




**b**

| | | Mean (mV): | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): 22.7 | Peak 1: | 22.7 | 100.0 | 6.69 |
| Zeta Devation (mV): 6.69 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 2.39 | Peak 3: | 0.00 | 0.0 | 0.00 |
| Result quality: Good | | | | |

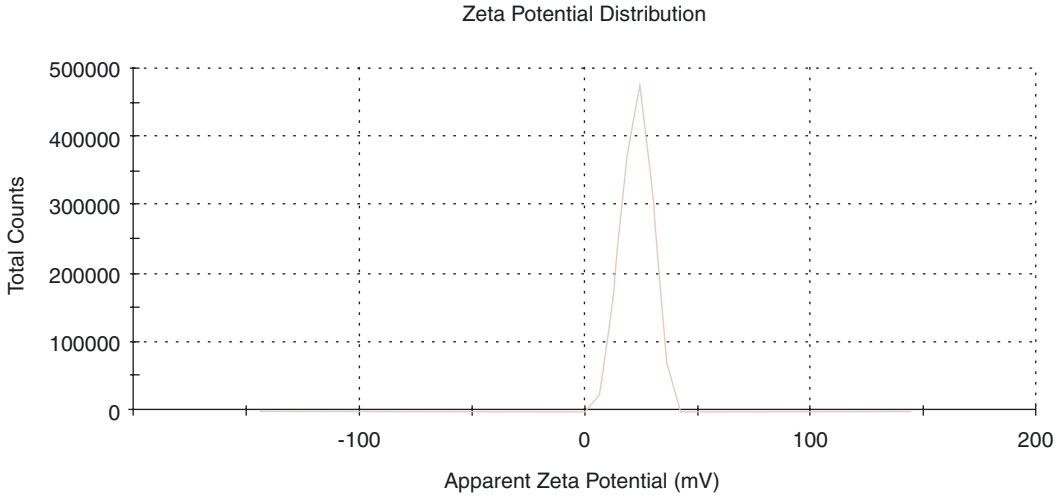
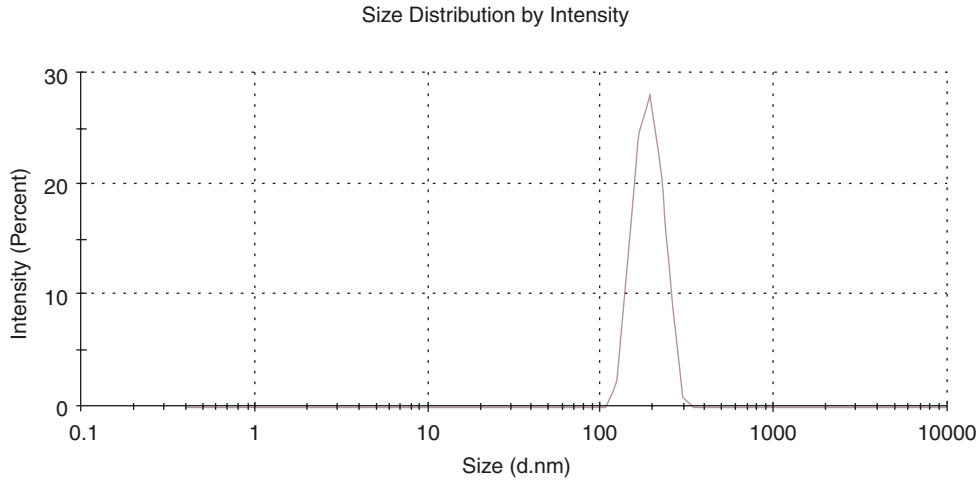



**Fig. 7** Formulation A: (**a**) CUR-CSNP size distribution by intensity using dynamic laser light scattering (DLS). (**b**) Zeta potential distribution of CUR-CSNPs

**a**

| | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|
| Z-Average (d.nm): 185.7 | Peak 1: | 190.2 | 100.0 | 39.75 |
| Pdl: 0.088 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.671 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality: Good | | | | |




**b**

| | | Mean (mV): | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): 27.5 | Peak 1: | 27.5 | 100.0 | 6.23 |
| Zeta Devation (mV): 6.23 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 2.150 | Peak 3: | 0.00 | 0.0 | 0.00 |
| Result quality: Good | | | | |

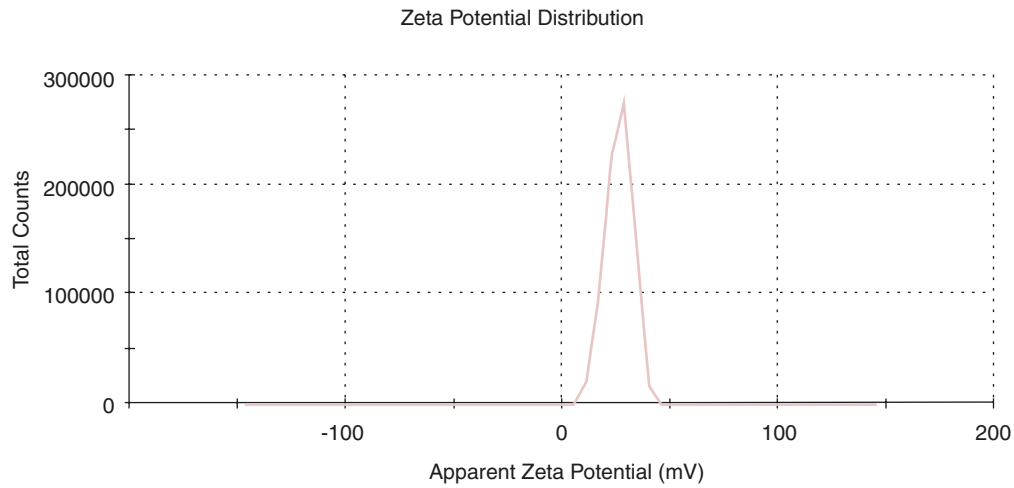
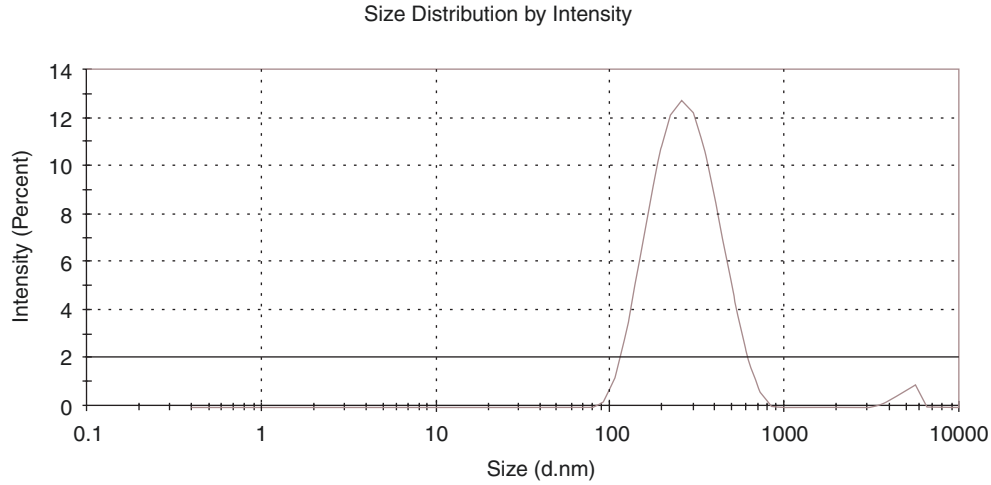



**Fig. 8** Formulation B: (**a**) CUR-CSNP size distribution by intensity using dynamic laser light scattering (DLS). (**b**) Zeta potential distribution of CUR-CSNPs

**a**

| | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|
| Z-Average (d.nm): 235.1 | Peak 1: | 284.6 | 97.9 | 120.7 |
| PdI: 0.323 | Peak 2: | 4937 | 2.1 | 645.3 |
| Intercept: 0.939 | Peak 3: | 0.000 | 0.0 | 0.000 |
| Result quality: Good | | | | |




**b**

| | | Mean (mV): | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): 30.3 | Peak 1: | 30.3 | 100.0 | 8.55 |
| Zeta Devation (mV): 8.55 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 1.19 | Peak 3: | 0.00 | 0.0 | 0.00 |
| Result quality: Good | | | | |

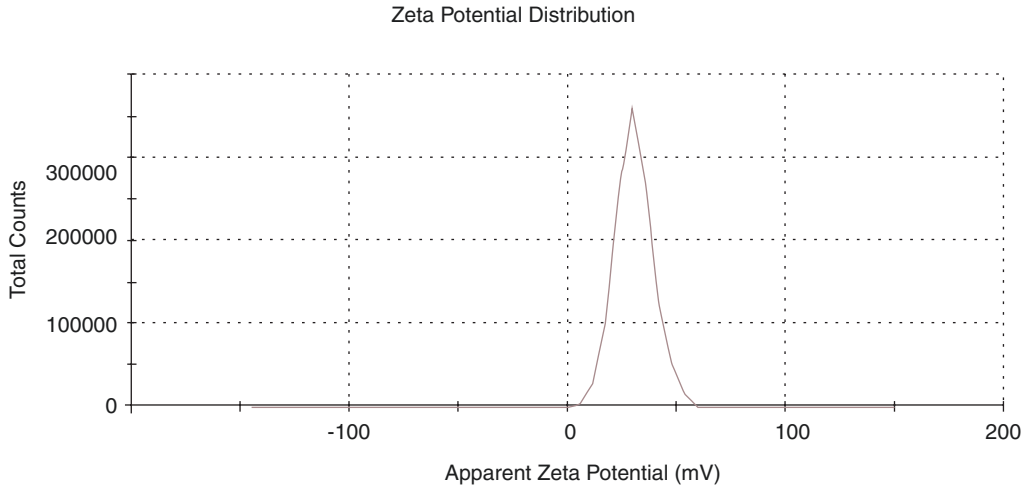
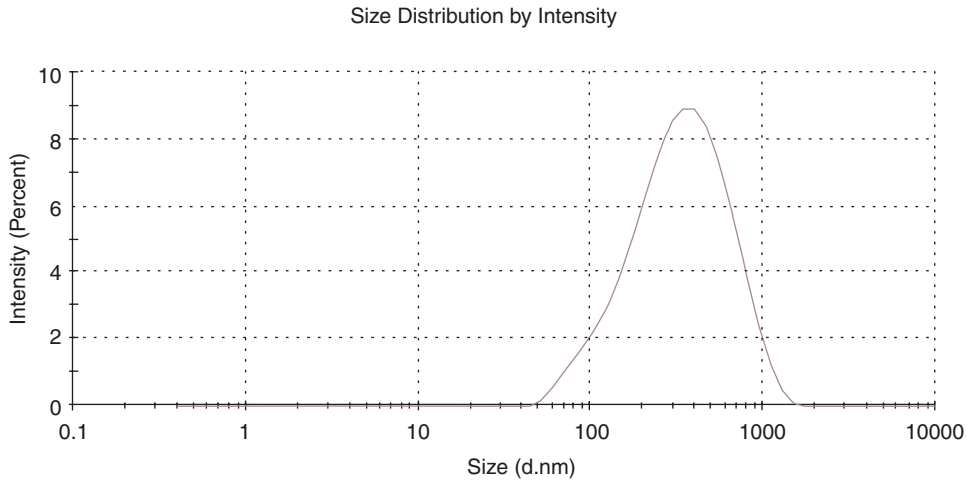



**Fig. 9** Formulation C: (**a**) CUR-CSNP size distribution by intensity using dynamic laser light scattering (DLS). (**b**) Zeta potential distribution of CUR-CSNPs

**a**

| | | Size (d.nm): | % Intensity: | St Dev (d.n... |
|---|---|---|---|---|
| Z-Average (d.nm): 268.4 | Peak 1: | 384.2 | 100.0 | 234.6 |
| PdI: 0.389 | Peak 2: | 0.000 | 0.1 | 0.000 |
| Intercept: 0.696 | Peak 3: | 0.000 | 0.0 | 0.000 |

Result quality: Good




**b**

| | | Mean (mV): | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): 31.6 | Peak 1: | 31.6 | 100.0 | 13.2 |
| Zeta Devation (mV): 13.2 | Peak 2: | 0.00 | 0.0 | 0.00 |
| Conductivity (mS/cm): 1.52 | Peak 3: | 0.00 | 0.0 | 0.00 |

Result quality: Good See result quality report

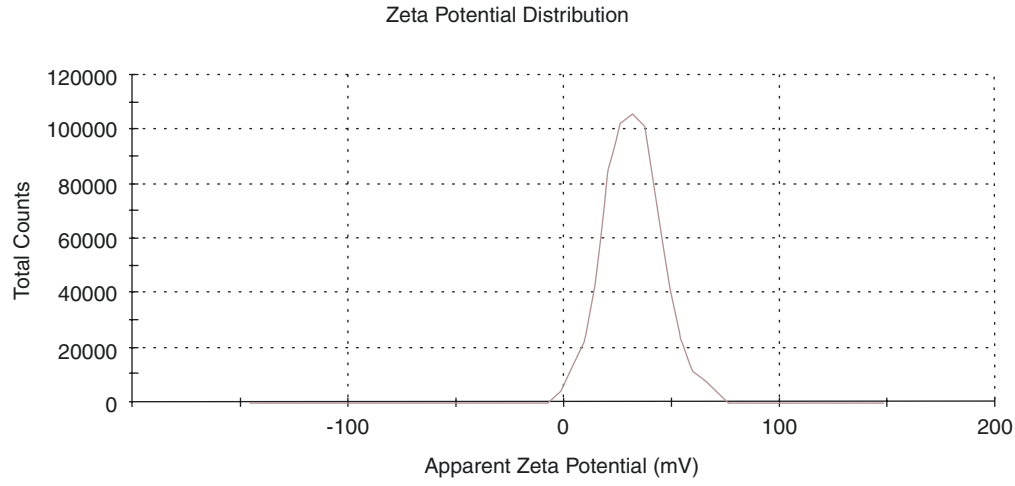



**Fig. 10** Formulation D: (**a**) CUR-CSNP size distribution by intensity using dynamic laser light scattering (DLS). (**b**) Zeta potential distribution of CUR-CSNPs

of the Cur-CSNP nanoparticles can be attributed to several factors. First, the higher amount of CS has higher ability of ionic gel formation which prevents the CUR movement to the external phase and increases in the drug encapsulation efficiency, hence the drug loading. Second, the presence of large numbers of amine groups facilitates electrostatic interaction between the cationic groups located on the amino groups of the CS and the negatively charged anionic CUR. Finally, the lipophilic nature of CUR leads to a marginal loss of drug into the external aqueous phase at the time of formulation.

In conclusion, a particle size of <200 nm with good entrapment efficiency has better control in drug release and also has cell interaction (cell infiltration) nature. Zeta potential of near to or more than +30 mV is necessary for good stability. Further, the positive zeta potential of CUR-CSNPS helps in better adherence of scaffold to the negatively charged biological membranes. Considering the above points the CUR-CSNPs of formulation B has been selected as a standard batch for further evaluations.

The SEM analysis was performed to study the morphological characteristics of the prepared CSNPs and it was found that the nanoparticles had a spherical morphology. CUR-CSNP nanoparticles have showed the typical spherical shape with uniform size distribution (Fig. 11).

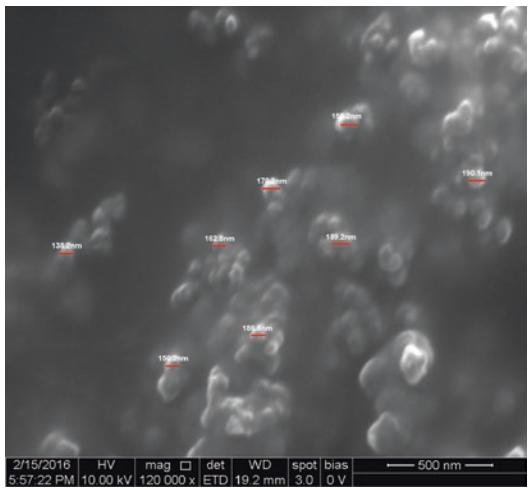



**Fig. 11** CUR-CSNP size and morphological characterization using scanning electron microscopy (SEM)

### 3.5.2 FTIR Analysis

FTIR spectra of CS, CUR, and CUR-loaded CSNP nanoparticles are shown in Fig. 12. According to the spectra, two characterization peaks, 1028 $cm^{-1}$ of $\nu$ (C—O—C) and 1627 $cm^{-1}$ of $\nu$ (aromatic OH), existed in the spectrum of CUR and three characterization peaks (1030 $cm^{-1}$ of $\nu$ (C—O—C) and of 1588 $cm^{-1}$ $\nu$ ($NH_2$) existed in the spectrum of CS. In comparison with CUR, a different spectrum was observed for CUR-loaded CS-TPP nanoparticles and new sharp peak appeared at 1655 $cm^{-1}$ $\nu$ (C—N); it can be supposed that the amine groups of CS were linked with hydroxyl groups of CUR in nanoparticles. Evident interactions between the phosphate and protonated CS amine groups were witnessed in the FTIR spectra for NPs that were correlated to stretching near 1509 $cm^{-1}$. Another peak at 1153 $cm^{-1}$ was indicative of TPP groups being linked to CS by means of intermolecular interactions.

### 3.5.3 DSC and XRD Studies

To understand the physicochemical changes of CUR encapsulated in CSNPs, DSC and XRD analysis was performed. In DSC analysis, CUR exhibited a characteristic endothermic peak at 182 °C, due to its crystalline nature, while the CUR-CSNPs presented no peak in this region (Fig. 13). These results suggest that crystalline form of CUR was converted into amorphous form, which has more solubility and stability. Further, the absence of CUR characteristic peak in CUR-CSNPs indicates that the drug was incorporated into the CS matrix.

In XRD analysis of CUR (Fig. 14), numerous peaks were observed in the 2θ range of 10–30° inferring its crystalline nature. However, in the CSNPs of CUR, no such crystalline peaks were found. This again confirms the amorphous or disordered crystalline phase of CUR in the CSNPs. As seen in the XRD analysis, modification of crystallinity is essential because this was strongly associated with drug incorporation and drug release rate. This data also suggests that the CUR will remain entrapped in the polymer during the shelf life [42, 43].

### 3.6 Scaffold Preparation and Characterization

The schematic representation of fabrication of nanohybrid scaffolds is shown in Fig. 15. Thermally triggered fibrillogenesis of a collagen–alginate blend leads to the formation of opaque composite hydrogel with good elastic property. The subsequent freeze-drying of this composite hydrogel leads to the formation of porous 3D scaffolds. The 1:1 ratio COL:ALG is taken as ideal for the better stability and formation of scaffolds as per study reported by Sang et al. [34].

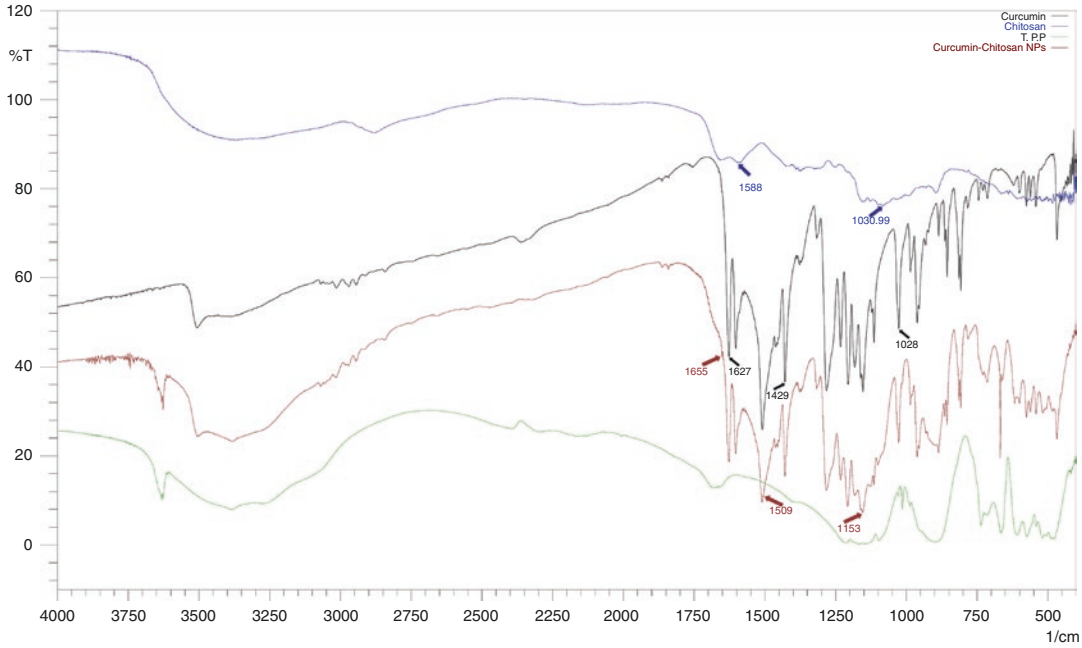



**Fig. 12** FTIR spectra of CUR, CS, TPP, and CUR-CSNPs

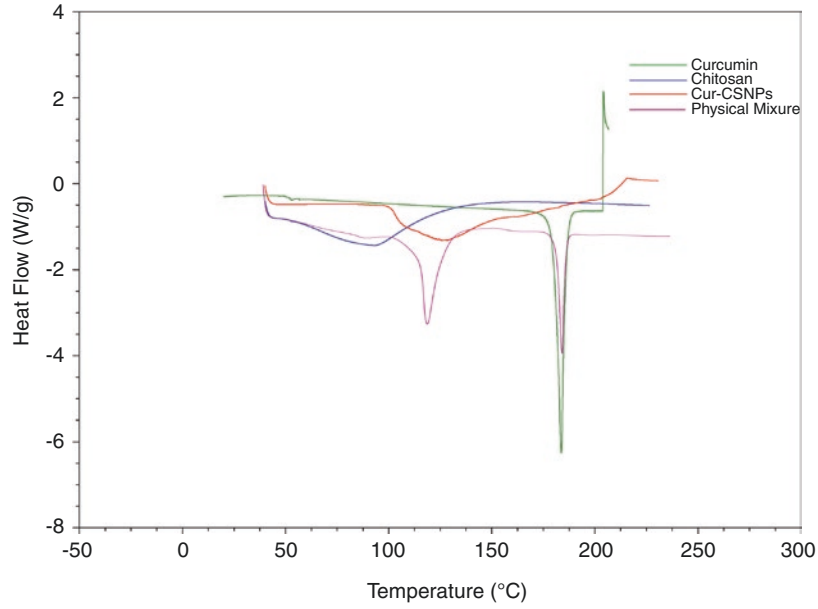



**Fig. 13** Differential scanning calorimetric (DSC) profiles of curcumin, chitosan, and CUR-CSNPs

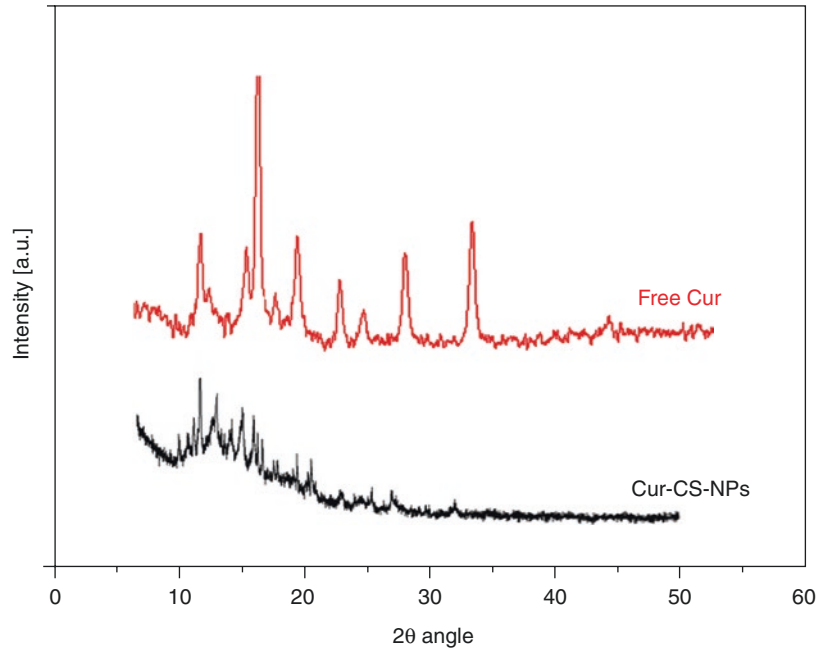



**Fig. 14** X-ray powder diffraction (XRD) patterns of free curcumin and CUR-CSNPs

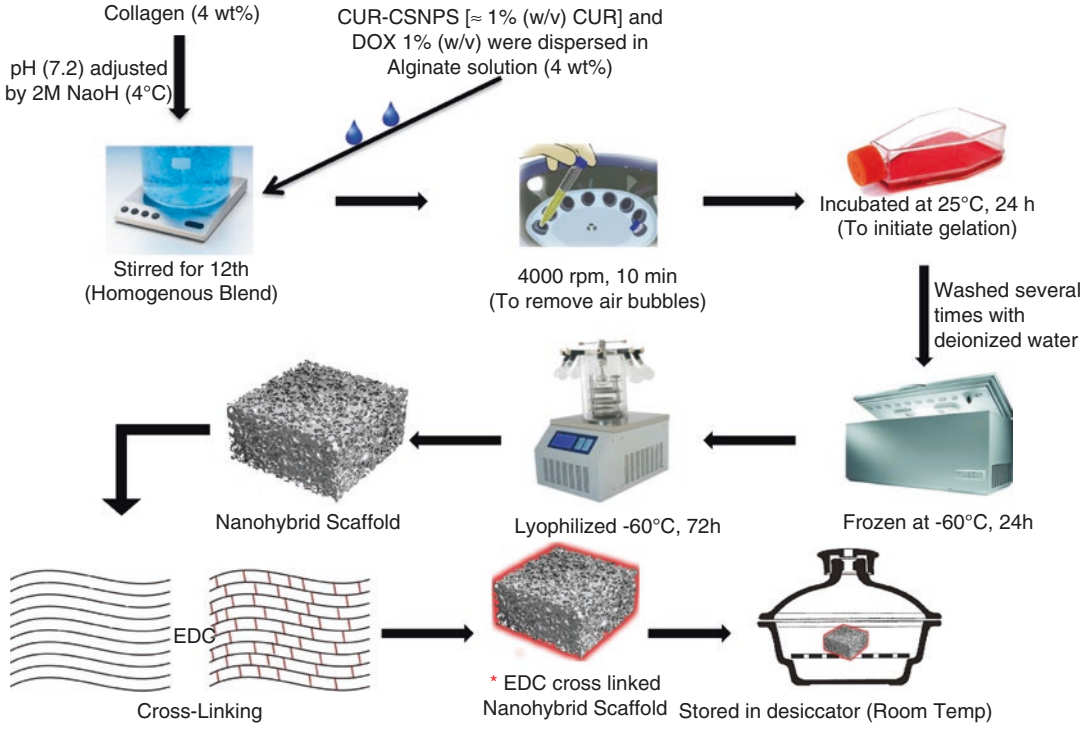



**Fig. 15** Fabrication of nanohybrid scaffolds

### 3.6.1 Matrix Morphology Using Scanning Electron Microscopy

SEM morphology of both COL/ALG scaffold and nanohybrid scaffold is shown in Fig. 16. The appearance of CUR-CSNPs incorporated in COL–ALG biocomposite confirmed the homogenous distribution of CUR-CSNPS throughout the scaffold. The SEM image of both the scaffolds indicated the porous architecture with a geometry ranging from 50 to 250 μm in size. The existence of interconnected porous like appearance between the COL–ALG strands could be significant for cell attachment, proliferation, and migration for tissue regeneration [44]. Furthermore, the porous structure of scaffolds is likely to aid in improved oxygen permeability to the wounds.

### 3.6.2 Differential Scanning Calorimetry Studies

Figure 17 shows the DSC pattern of COL, ALG, and nanohybrid scaffold. The thermogram values of COL, ALG, and their blended scaffold were found to be 39.5 °C, 121.3 °C, and 78.6 °C, respectively. These results clearly indicate that native COL is highly unstable at body temperature; therefore it needed to be cross-linked to stabilize the COL. The process of stabilizing COL (cross-linking) involves the creation of links between individual strands of COL. This inhibits degradation of the COL by proteases (e.g., matrix metalloproteinase) and prolongs its presence in the wound [45]. In nanohybrid scaffolds the melting temperature of COL was shifted to 78.6 °C indicating its stabilization by EDC cross-linking. The higher transition temperature suggests that scaffolds have high stability at high-temperature environment than pure COL. Similar results were also observed in case of ALG. Pure ALG has shown a melting temperature of 121.3 °C, whereas in scaffold the temperature transition occurs at 182 °C. Thermal stability also controls the durability of scaffolds [46].

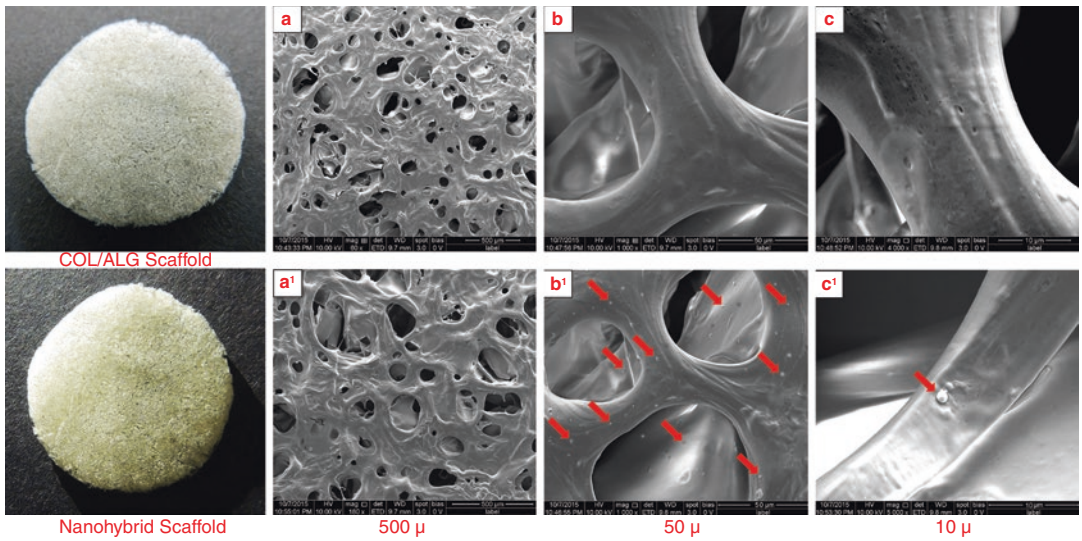



**Fig. 16** SEM images of COL–ALG scaffolds (**a**, **b**, **c** without nanoparticles) and nanohybrid scaffolds (**a**$^1$, **b**$^1$, **c**$^1$ with nanoparticles)

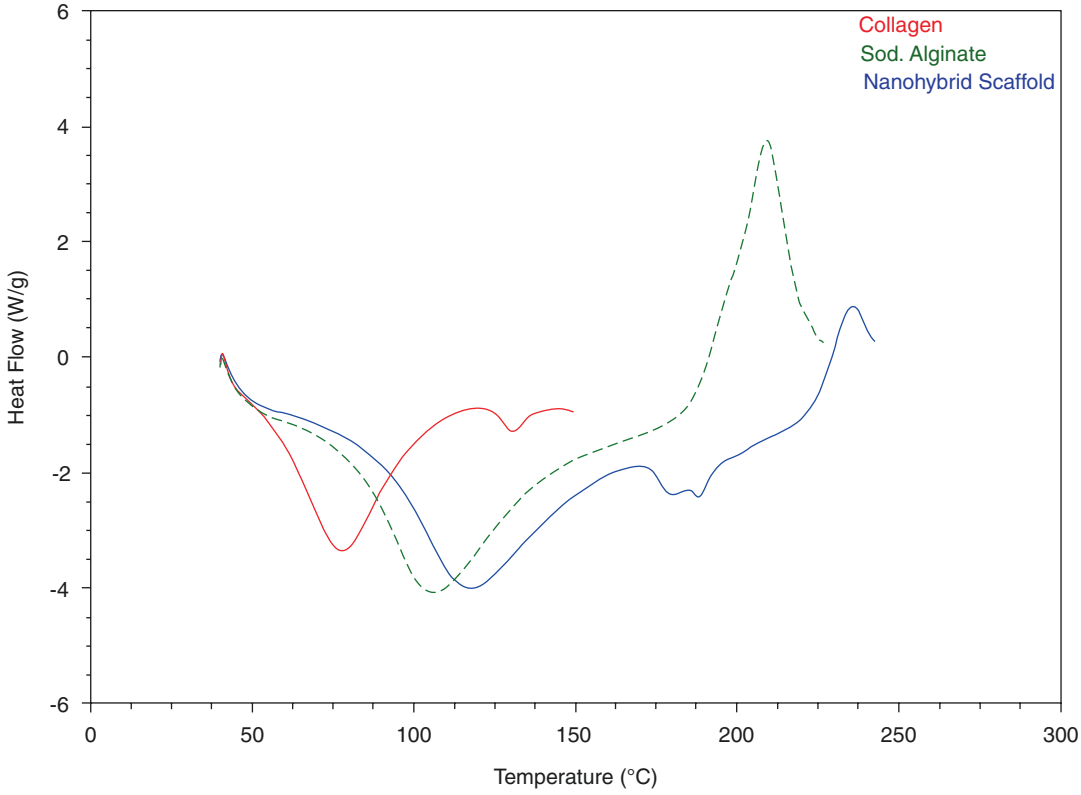



**Fig. 17** Differential scanning calorimetric (DSC) profiles of COL, ALG, and nanohybrid scaffolds

### 3.6.3 Tensile Strength Measurement

Figure 18 shows the typical stress–strain curves for the pure COL, COL–ALG scaffold, and nanohybrid scaffolds at a constant stretching velocity of 0.5 mm $s^{-1}$. The nanohybrid scaffold showed a maximum strength (1.72 ± 0.93 MPa) due to the cross-linking with EDC/NHS. However, the strength of the COL–ALG scaffold was 0.82 ± 0.59 MPa, which indicates that this scaffold was not having enough strength due to lack of cross-linking even though it comprises natural polymer ALG. The tensile strength of COL scaffold is very less indicating that it is not ideal for tissue engineering application. The initial slope of the stress–strain curve was converted into Young's

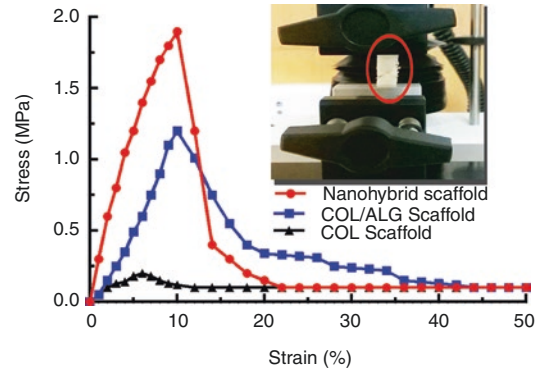



**Fig. 18** Stress–strain curves for the COL, COL–ALG, and nanohybrid scaffolds

modulus (Fig. 19). These results demonstrate that the mechanical properties of nanohybrid scaffold are highly improved compared with those of the pure COL scaffold and COL–ALG scaffold.

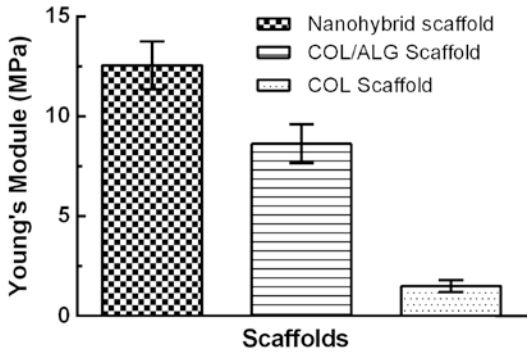



**Fig. 19** Comparison of Young's modulus among the COL, COL–ALG, and nanohybrid scaffolds

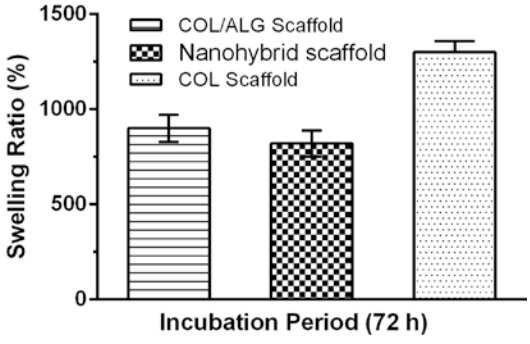



**Fig. 20** Swellability ratio of nanohybrid scaffolds before and after the EDC cross-linking treatment in simulated wound fluid (SWF), pH 7.4, maintained at 37 °C

### 3.6.4 Swelling Behavior

Swelling behavior of nanohybrid scaffolds was observed before and after cross-linking treatment (Fig. 20) in SWF at pH 7.4. The swelling ratio of cross-linked and non-cross-linked scaffolds was found to be 820 and 910%. After cross-linking of COL–ALG scaffold (nanohybrid scaffold), the swelling ratio was slightly reduced (90%) probably due to the formation of strong molecular interactions by EDC cross-linking [19]. These results are in agreement with the results obtained by Sang et al. [34] using COL–ALG scaffolds. Nevertheless, higher water absorption could lead to loss of physical integrity and therefore loses its stability during the cell culture process and for in vivo application.

### 3.6.5 Matrix Degradation Studies

The biodegradability tests were carried out for the nanohybrid scaffolds before and also after the cross-linking treatment. Pure COL scaffold was also studied as control. The scaffolds degraded gradually after incubation in the collagenase solution for 7 days. All the scaffolds degraded swiftly in the first initial day with an identical enzymatic degradation rate, after which the degradation proceeded at a reduced speed for next 4 days (Fig. 21). Conversely, after the COL–ALG scaffold was cross-linked to EDC (nanohybrid scaffold), the rate of degradation and degradation degree were considerably dropped, suggesting improved resistance to enzymatic degradation.

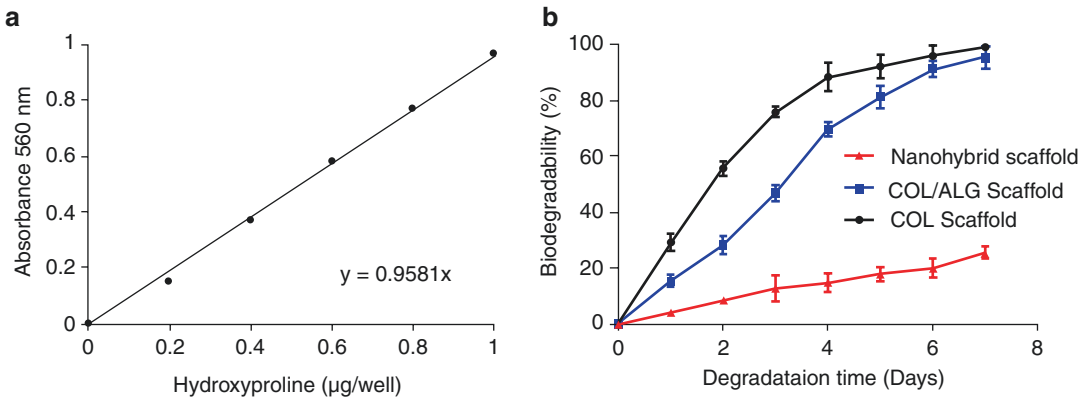



**Fig. 21** In vitro biodegradation studies: (**a**) Hydroxyproline standard curve. (**b**) Enzymatic degradation curves of different scaffold treatment in pH 7.4 PBS containing 265 Umg − 1 collagenase at 37 °C

Specifically, after incubation for 7 days, the cross-linked nanohybrid scaffold produced the least degradation degree of 25.30%, in contrast to the non-cross-linked COL–ALG and COL scaffolds that showed 95.60% and 99.2% degradation, respectively.

### 3.6.6 In Vitro Drug Release Studies

Drug release investigations from both the CUR-CSNPS and nanohybrid scaffold were performed to evaluate the degree of drug release regulated by the nanoparticles and scaffolds. In vitro release behavior of CUR from CSNPs and nanohybrid scaffold in SWF, pH 7.4, at 37 °C is showed in Fig. 22. The drug release from the CSNPs demonstrated a biphasic trend characterized by swift release for 24 h that was accompanied by a sustained-release phase. In the initial 3 h, 21.71 ± 2.90% of drug became available from the CSNPs, while nanohybrid scaffold merely released 8.2 ± 3.43% of drug. Post 4 h, the scaffold's drug release improved by 28.63 ± 3.54% after 12 h when compared to 41.99 ± 3.10% release from CSNPs. It was apparent that the scaffold delayed the initial drug release that can be as outcome of the time taken for wetting the scaffold under initial condition. After 24 h of study 36.21 ± 2.98% from nanohybrid scaffold and more than 50% from CSNPs of CUR were released. At 72 h of evaluation 81.06 ± 3.32% and 56.24 ± 4.05% of drug were available from CSNPs and nanohybrid scaffold, respectively. It will be favorable only if the delivery system (scaffold) sustains the release of drug in the duration of treatment. The drug release of 94.66 ± 5 0.23% from the nanohybrid scaffold at 360 h will ensure effective drug release over 14 days and above for the treatment.

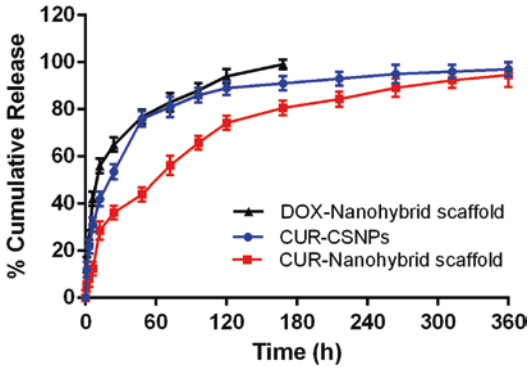



**Fig. 22** In vitro drug release profile of CUR and DOX from nanohybrid scaffold as well CUR release from CSNPs in simulated wound fluid, pH 7.4, maintained at 37 °C (mean ± SD, $n = 5$)

### 3.6.7 In Vitro Drug Release Kinetics

After in vitro drug release studies the data obtained was fitted into various release kinetic models like zero order, first order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas. The values are presented in Table 6. The drug release from CUR-CSNPs was found to have higher $r^2$ values for the Peppas model which shows that the release of CUR from the CSNPs was due to drug diffusion and polymer erosion. However, the drug release from nanohybrid scaffold was found to have higher $r^2$ values for the first-order and Higuchi model which shows that the release of drug from the scaffolds was concentration dependent (control release). The DOX

**Table 6** Different kinetic models applied on release behavior of CUR and DOX from NPs and scaffolds

| | | Release kinetics | | | | |
|---|---|---|---|---|---|---|
| | | Zero | Higuchi | Peppas | First | Hixson-Crowell |
| | | 1 | 2 | 3 | 4 | 5 |
| CUR-CSNPs | Slope | 0.510 | 7.369 | 0.446 | −0.011 | 0.016 |
| | $r^2$ | 0.7556 | 0.9345 | 0.9921 | 0.9216 | 0.8718 |
| CUR-nanohybrid scaffold | Slope | 0.480 | 6.557 | 0.574 | −0.008 | 0.012 |
| | $r^2$ | 0.8953 | 0.9875 | 0.9655 | 0.9807 | 0.9602 |
| DOX-nanohybrid scaffold | Slope | 0.500 | 7.314 | 0.410 | −0.014 | 0.019 |
| | $r^2$ | 0.7138 | 0.9042 | 0.9486 | 0.9771 | 0.9212 |

release from nanohybrid scaffold follows first-order model which indicates that the drug release was dose dependent.

### 3.7 In Vitro Cytotoxicity Studies

The in vitro cytotoxicity of the nanohybrid scaffold has shown that the introduction of the test material to the growth medium did not induce cytotoxicity against mouse NIH 3T3 fibroblasts, and the cell viability was at the level of the control (Fig. 23). The fibroblasts grew uniformly in the pores of the nanohybrid scaffold structure. This finding may be explained by the excellent biocompatibility, swelling ability, porous nature of the nanohybrid scaffold, as well as even distribution of COL, to which cells have high affinity. The obtained results suggest that the cells could proliferate and maintain their fibroblast morphology while in contact with the scaffold. This clearly indicates that the developed nanohybrid scaffolds are cell friendly and biocompatible since the scaffold is made of natural polymers which are biodegradable. Further, chitosan's positive surface charge enables it to effectively support cell growth [47].

### 3.8 In Vitro Antimicrobial Studies

In diabetes the combination of hyperglycemia, vascular insufficiency, and peripheral neuropathy leads to the development of diabetic wound. If untreated, the wounds become infected and spread to deeper layers which leads to formation of gangrene and ultimately results in amputation. An infected diabetic wound precedes ~60% of amputations. DFIs are polymicrobial in nature. *S. aureus*, *P. aeruginosa*, *E. coli*, and MRSA are the most important pathogens in DFIs since they occupy about 70% in the mixed infections. Hence, antimicrobial studies have been performed against these four different microbes to study the effect of CUR and DOX on them.

The results for antimicrobial studies are shown in Figs. 24 and 25. These results clearly indicate that DOX was significantly ($p < 0.001$) more effective than CUR against the bacteria present in DFIs and also DOX has also shown broad-spectrum inhibitory effect against all microorganisms than CUR. This may be due to the fact that even though CUR is a good antibacterial agent its minimum inhibitory concentration is very high as compared with synthetic antibiotic drugs, and this property makes the CUR ineffective to be used as

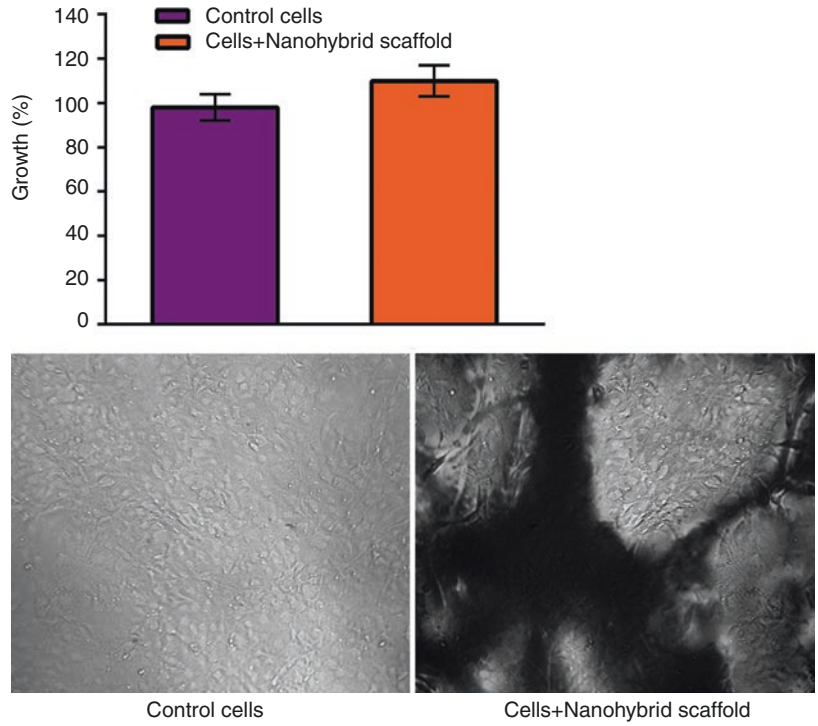



**Fig. 23** Optical microscopy images (150×) of fibroblast 3T3-L1 cells (negative control) and fibroblasts cultured in the presence of the nanohybrid scaffold

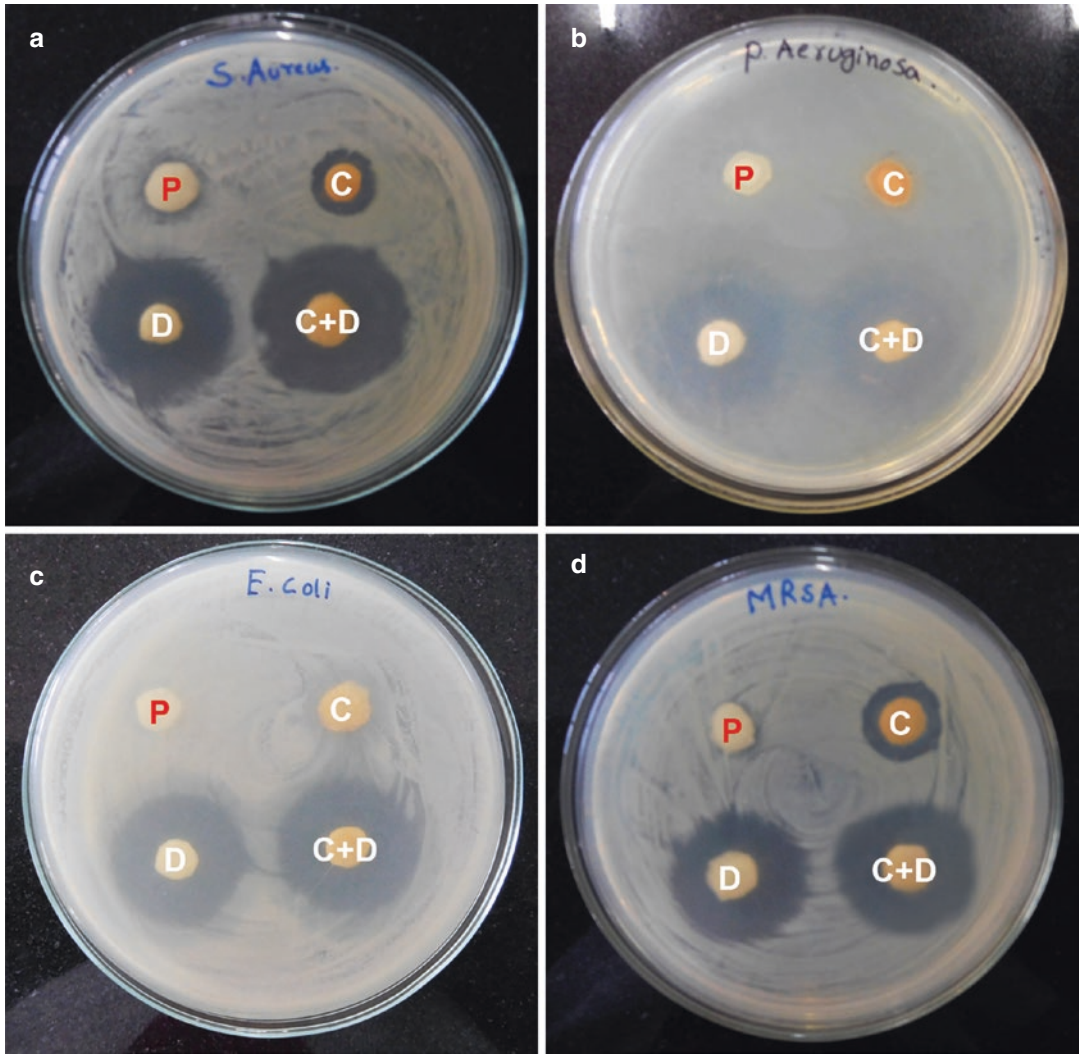



**Fig. 24** Disc diffusion studies of composite scaffolds against (**a**) *S. aureus*, (**b**) *P. aeruginosa*, (**c**) *E. coli* (**d**) MRSA, where *P* represents placebo scaffold (COL–ALG, control), *C* represents CUR-CSNP-loaded scaffold, *D* represents DOX scaffold, *C* + *D* represents nanohybrid scaffold

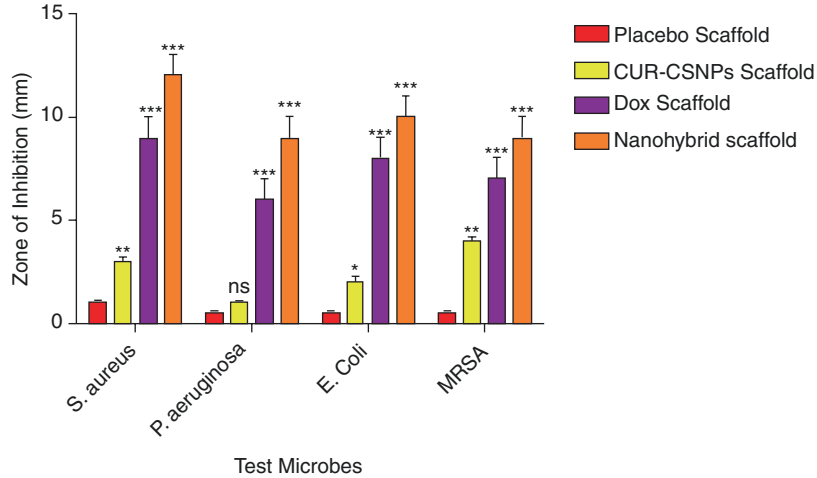



**Fig. 25** Comparative antibacterial activity of composite scaffolds against *S. aureus*, *P. aeruginosa*, *E. coli* MRSA. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus placebo group

an antibacterial agent especially topically as that of available antibiotics. From the results it can also be observed that CUR is more active against gram-positive bacteria (*S. aureus* an MRSA) rather than on gram-negative bacteria (*P. aeruginosa*, *E. coli*). This may be attributed to the structure and constitution of the cell membrane. Upon coming into contact with CUR, gram-positive (outer peptidoglycan layer) and gram-negative bacteria (outer phospholipid membrane) undergo varied types of interaction. These results also further demonstrate that the combination of CUR and DOX has synergistic effect ($p < 0.001$) compared to individual treatments. These observations can be compared to published results that CUR possesses a synergistic effect with important antibiotics such as vancomycin, cefixime, and tetracycline against various gram-positive and gram-negative microbes.

## 3.9 Skin Irritation Studies

The results of the skin irritation study are shown in Table 7. Edemal scores of all formulations are similar during all the time points. There was no evidence of erythema, eschar, or edema formation (Table 7) (Fig. 26) throughout the entire 72-h period of observation. The calculated guideline-based primary irritation index (PII) was 0.0 (i.e., the test scaffolds are classified as nonskin irritant item under ISO-10993 international standard) [48].

**Table 7** Mean erythemal and edemal scores of various scaffolds at the end of 24, 48, and 72 h ($n = 3$)

| | Erythemal scores | | | Edemal scores | | |
|---|---|---|---|---|---|---|
| Formulation | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| Control | 0 | 0 | 0 | 0 | 0 | 0 |
| COL–ALG scaffold | 0 | 0 | 0 | 0 | 0 | 0 |
| Nanohybrid scaffold | 0 | 0 | 0 | 0 | 0 | 0 |

The mean erythemal and edemal scores were recorded on the basis of degree of severity: no erythema/edema = 0, slight erythema/edema = 1, moderate erythema/edema = 2, severe erythema/edema = 3
Primary irritation index (PII): nonirritant = 0–0.4, slightly irritant = 0.5–1.9, moderately irritant = 2–4.9, strongly irritant = 5–8

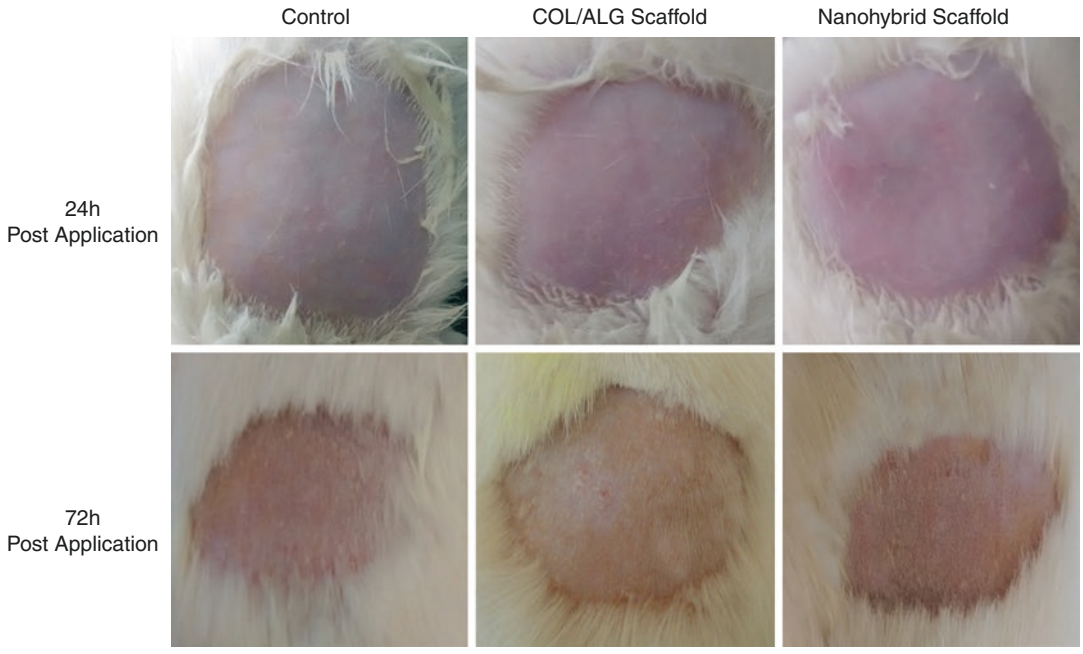



**Fig. 26** Skin irritation studies of various scaffolds at the end of 24, 48, and 72 h ($n = 3$)

## 3.10 In Vivo Diabetic Wound Healing Studies

In this study STZ-induced diabetic rat model was used to study the diabetic wound healing. The characteristic signs of diabetes (high levels of glucose, polyuria, polyphagia, polydipsia, and loss of body weight) were observed from the second day of STZ administration. In order to maintain uniformity in the animals included in the study, a minimal threshold was established to ensure the least error occurrence. Therefore, 400 mg/dL was maintained as the minimal threshold and only those animals exhibiting a blood glucose level higher than this were included into the study.

### 3.10.1 Wound Contraction

Wound contraction was analyzed in each group as a percentage of the reduction in wounded area at days 3, 7, 14, and 21 (Fig. 27). In this study, nanohybrid scaffold- and CUR-CSNP scaffold-treated wounds contracted significantly faster than the wounds from the control-, COL–ALG-, and DOX scaffold-treated groups. The mean percentage of wound contraction of the nanohybrid scaffold (98.1 ± 3.4% at day 21, $p < 0.001$) and CUR-CSNP scaffold (83.5 ± 5.6% at day 21, $p < 0.01$) treated group was significantly higher compared with that of the control (36.6 ± 6.3% at day 21,) and COL–ALG (52.4 ± 5.8% at day 21, $p < 0.05$) and DOX scaffold treated (61.6 ± 4.4% at day 21, $p < 0.05$) groups after wound creation (Fig. 28).

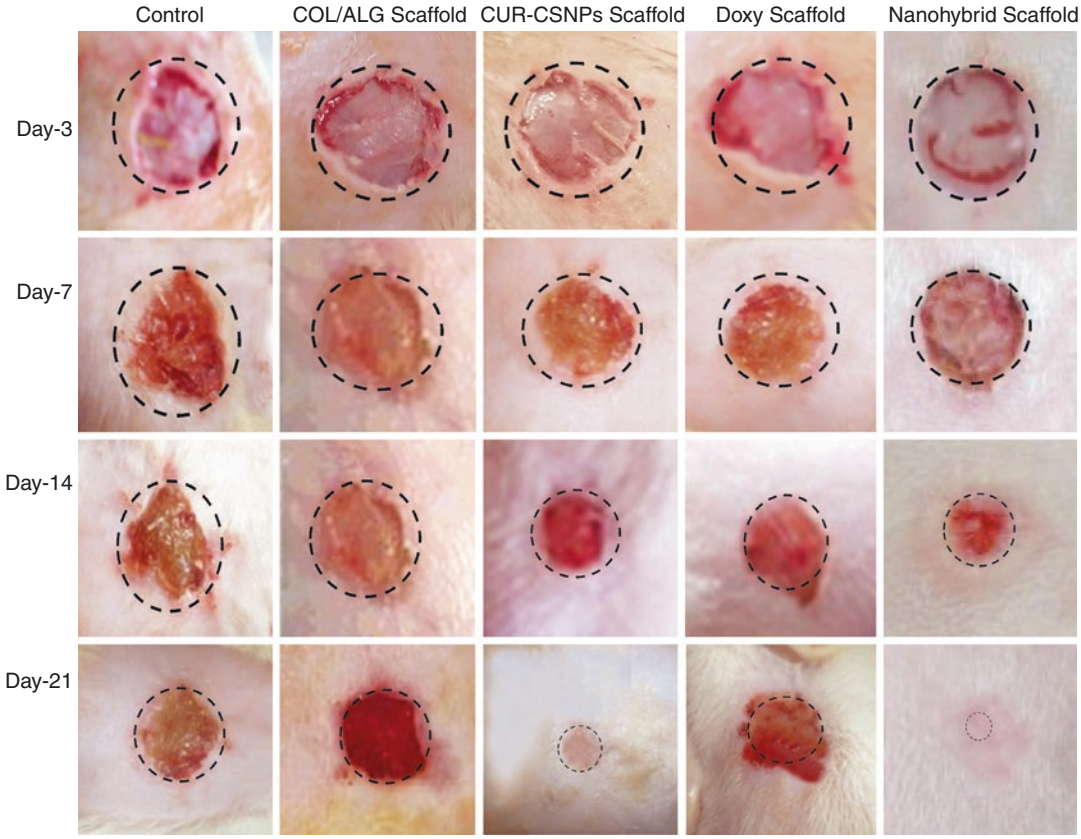



**Fig. 27** Digital photographs of wound healing and re-epithelialization in control-, COL–ALG-, CUR-CSNP-, DOX-, and nanohybrid scaffold-treated groups on days 3, 7, 14, and 21 post-wounding

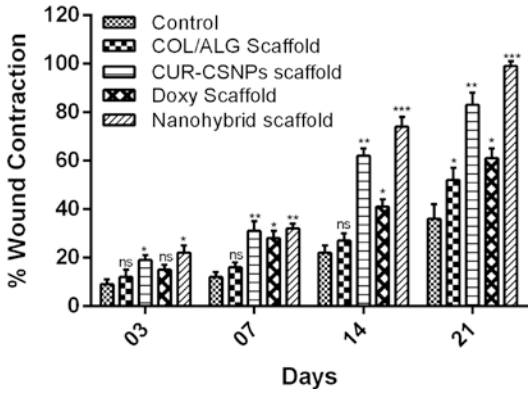



**Fig. 28** Wound contraction (%) in the control-, COL–ALG-, CUR-CSNP-, DOX-, and nanohybrid scaffold-treated groups on days 3, 7, 14, and 21 post-wounding. Data are expressed as means ± SD ($n = 3$). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus other group(s) on the same day

### 3.10.2 ELISA Assay for TNF-α and IL-10

The results of TNF-α production are shown in Fig. 29. The production of TNF-α was increased in control- and COL–ALG-treated groups up to 7 days of post-wounding followed by a slight reduction in TNF-α levels in both the groups on days 14 and 21. On day 3 the protein levels of TNF-α were significantly decreased in CUR-CSNP scaffold (1254 ± 87 pg/mL, $p < 0.05$) and nanohybrid scaffold (852 ± 98 pg/mL, $p < 0.01$) treated groups when compared to control- and COL–ALG-treated groups. There was no significant decrease of TNF-α levels observed in DOX scaffold-treated group on day 3. On days 14 and 21 the levels of TNF-α were highly decreased in nanohybrid scaffold-treated groups (14th day: 546 ± 83 pg/mL, $p < 0.001$

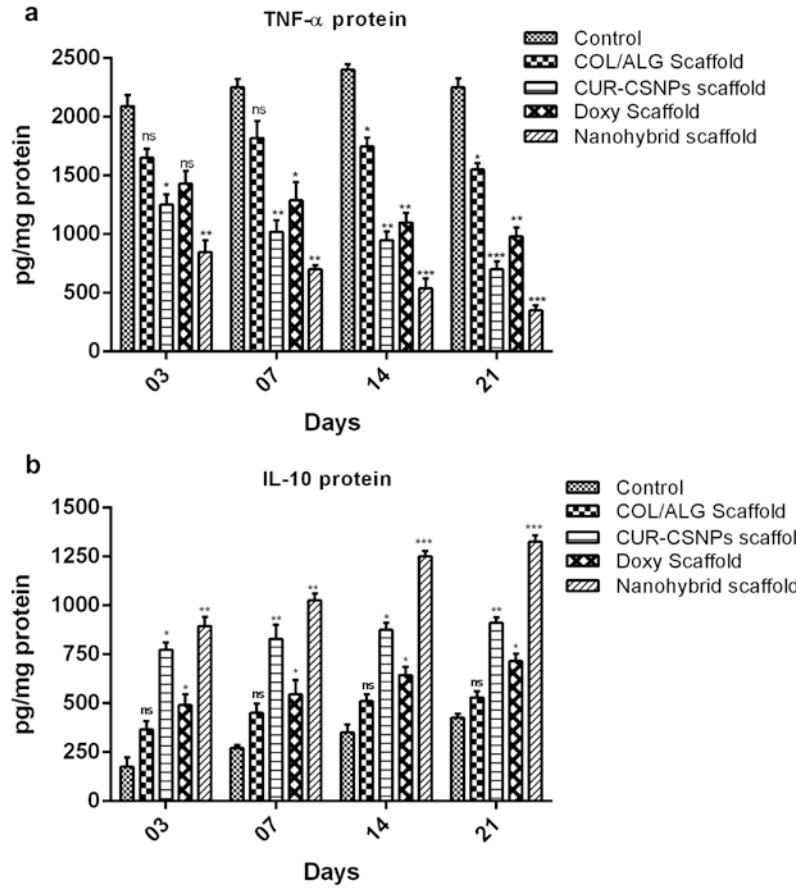



**Fig. 29** Effect of different scaffolds on protein levels of (**a**) TNF-α (**b**) IL-10 on various days. Data are expressed as means ± SEM ($n = 3$). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to control, respectively, on the same day

21st day: 365 ± 45 pg/mL, $p < 0.001$) when compared to other groups.

The results of IL-10 production are shown in Fig. 29. There was no significant increase in IL-10 production; it was decreased in control- and COL–ALG-treated groups up to 21 days of post-wounding. On day 3 the protein levels of IL-10 were significantly increased in CUR-CSNP scaffold (773 ± 39 pg/mL, $p < 0.05$), DOX scaffold (490 ± 55 pg/mL, $p < 0.05$), and nanohybrid scaffold (894 ± 56 pg/mL, $p < 0.01$) treated groups when compared to control- and COL/ALG-treated groups. The similar results were also observed on day 7 also. On days 14 and 21 the levels of IL-10 were markedly increased in nanohybrid scaffold-treated groups (14th day: 1253 ± 28 pg/mL, $p < 0.001$ 21st day: 1,325 ± 34 pg/mL, $p < 0.001$) when compared to other groups.

On all the days (3, 7, 14, and 21) the relative protein expression of TNF-α was significantly decreased and IL-10 was significantly increased by CUR-CSNP-, DOX-, and nanohybrid scaffold-treated groups compared to control and COL–ALG groups. Interestingly the CUR-CSNP- and nanohybrid scaffold-treated groups showed markedly lower levels of TNF- α and higher levels of IL-10 when compared to DOX scaffold-treated groups on all the days. This indicates the potential anti-inflammatory activity of CUR when compared to DOX.

### 3.10.3 Western Blot Analysis of MMP-9

Figure 30 shows the Western blot results for MMP-9 protein expression. It was found that MMP-9 expression is not decreased in control- and COL–ALG-treated groups. On the other hand the CUR-CSNP-, DOX-, and nanohybrid scaffold-treated groups showed gradual decrease in MMP expression. The results are in agreement with published results that both CUR and DOX have potent inhibitory activity against MMP-9. Among the groups nanohybrid scaffold-treated group has shown marked decrease of MMP-9 expression.

Figure 31 shows the relative protein expression of MMP-9 with respect to GAPDH as con-

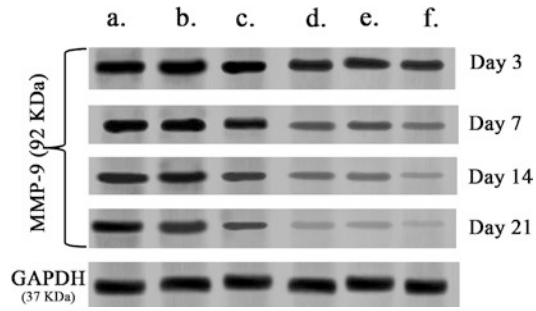



**Fig. 30** Representative Western blot analyses for the expression of MMP-9 in the skin wound lysate samples of various scaffold-treated groups. (**a**) Marker-, (**b**) control-, (**c**) COL–ALG-, (**d**) CUR-CSNP-, (**e**) DOX-, and (**f**) nanohybrid scaffold-treated groups on days 3, 7, 14, and 21 post-wounding. MMP-9 protein loading was normalized to that of GAPDH (bottom)

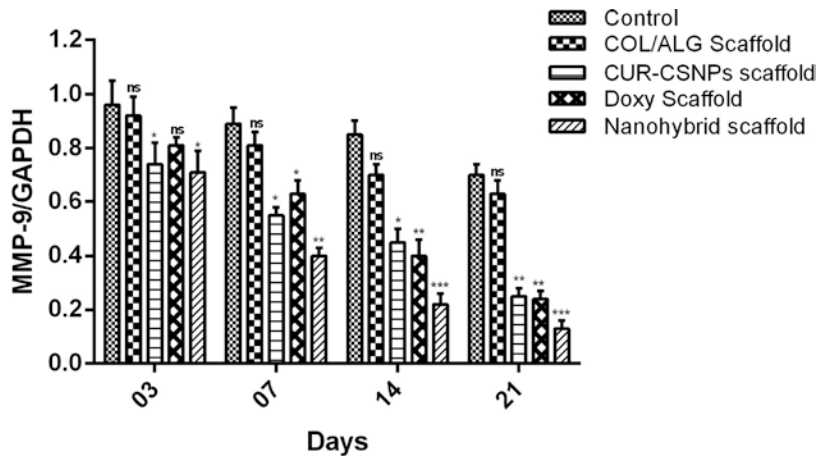



**Fig. 31** Relative expressions of MMP-9 with respect to GAPDH which was used as internal control. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to control

trol. The results clearly indicate that CUR-CSNP-, DOX-, and nanohybrid scaffold-treated groups have shown significant inhibition of MMP-9 protein expression compared to control- and COL–ALG-treated groups. These results suggest that CUR and DOX could promote diabetic wound healing by inhibition of MMP-9 activity. To confirm the above findings histopathological studies were conducted.

### 3.10.4 Histopathological Observations

The H&E-stained wound sections of all groups on days 3, 7, 14, and 21 post-wounding are shown in Fig. 32. On day 3 inflammatory cells are present in all groups of wound sections which represent chronic stage of inflammation. However the presence of fibroblasts was observed in all the groups except control that indicates that the presence of collagen and porous structure of scaffolds infiltrates the fibroblasts. An important function of 3D ECM is its ability to modulate cell infiltration and adhesion, which is highly hinge on the presentation of cell-adhesion ligands and the pore size of the matrix [49]. Pro-inflammatory cytokines and proteases are primarily involved in persistent inflammation and degradation of ECM in diabetic wounds [3]. They play a major role in various cascades of wound healing like debridement, angiogenesis, and epithelialization remodeling of scar [50]. The expression/levels of these cytokines and proteases vary in acute and chronic wound healing stages. In chronic wounds their levels were elevated and prolonged expression leads to persistent inflammatory and delayed wound healing in diabetics [15]. Hence, controlling the levels of inflammation is a critical part of diabetic wound healing. Similarly, in this study persistent inflammation was observed as inflammatory cells in the wound sections of control- as well as COL–ALG scaffold-treated groups that leads to impaired granulation tissue formation and delayed wound closure.

On day 7 of post-wounding, the inflammatory cells are still persisting in all the groups, comparatively more in control-, COL–ALG-, and DOX scaffold-treated groups. The nanohybrid-treated group has shown well-formed granulation tissue with fibroblasts and little collagen deposition with negligible inflammatory cells. On postoperative day 14 of

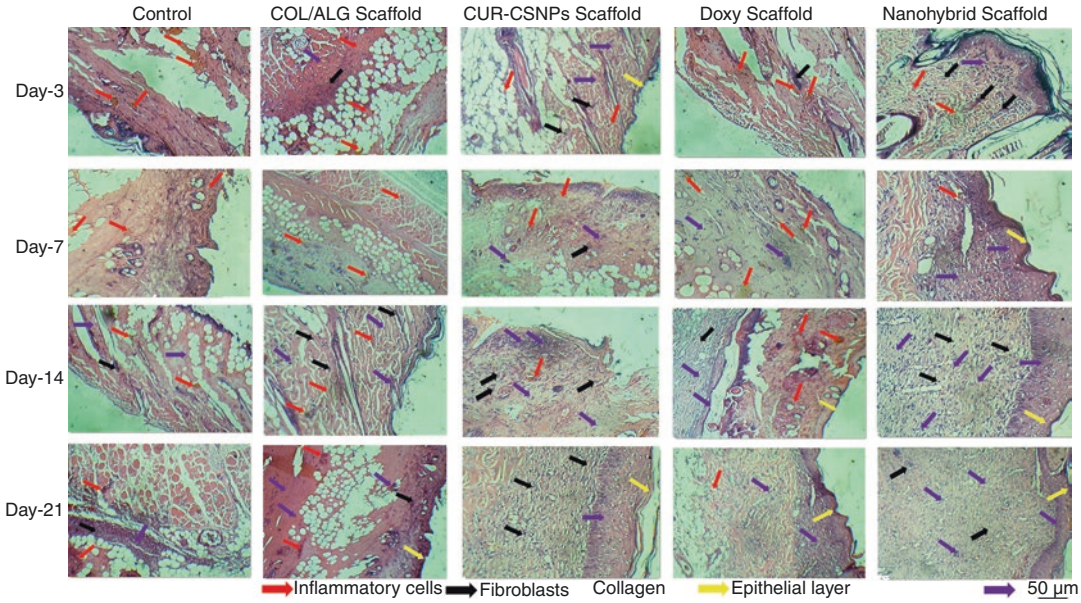



**Fig. 32** Hematoxylin and eosin (H&E)-stained histopathological view of granulation tissues of control-, COL–ALG-, CUR-CSNP-, DOX-, and nanohybrid scaffold-treated groups on days 3, 7, 14, and 21 post-wounding. (40× magnification and scale bar 50 µm)

wounding the wound sections of control and COL–ALG groups showed the presence of fibroblasts and little collagen deposition with still the presence of inflammatory cells. However, in CUR-CSNP- and nanohybrid scaffold-treated groups a thick granulation tissue was formed with fibroblasts, compact collagen deposition, and a new epithelial layer. There is also no inflammatory cells in both the groups. On the other hand DOX scaffold-treated group still shows the presence of inflammation cells but with granulation tissue formation. On day 21, the wound sections of the control- and COL–ALG scaffold-treated groups still showed the marked presence of inflammatory cells with some fibroblasts. The thick granulation formation was still not evident in the wound sections of both the groups. The wound sections of the DOX scaffold-treated group showed a good number of fibroblasts with marked collagen synthesis; however, the collagen deposition lacks compactness. However the wound sections of the CUR-CSNP- and nanohybrid scaffold-treated group showed a compact extracellular matrix covered by a thick epithelial layer. This may be due to the fact that even though collagen infiltrates the fibroblasts to proliferate in scaffold-treated group, the existence of chronic inflammation obstructs further fibroblast proliferation and collagen deposition. These results are in agreement with the findings previously reported by Kant et al. [3] who demonstrated that CUR has potential anti-inflammatory properties in treating diabetic wounds. Furthermore, several reports have shown that CUR has anti-inflammatory, antioxidant, anti-infective, angiogenic, and nerve healing properties in treating the wounds [26]. In addition, the presence of chitosan in the form of NPs in nanohybrid scaffold gradually depolymerizes to release N-acetyl glucosamine which initiates fibroblast proliferation and helps in ordered collagen deposition. The CSNPs also provide a slow and sustained release of CUR that has anti-inflammatory action for a prolonged time. In conclusion, the strong antioxidant and anti-inflammatory properties of CUR make this molecule a potential contender in tissue regeneration of diabetic wounds [3]. However, due to its hydrophobicity, its clinical application is greatly restricted [51]. Hence, in this study CUR was encapsulated into polymeric CSNPs to increase its solubility and control the release. In conclusion, the histopathological observations in compliance with ELISA and Western blot analysis reveal that the groups treated with CUR alone or CUR and DOX combination reduce the inflammation significantly, thereby promoting collagen deposition and wound healing. The results also further indicate that CUR alone or in combination with DOX is comparatively more effective in reducing the inflammation than DOX alone.

## 4 Discussion

Patient education, blood sugar control, wound debridement, advanced dressing, off-loading, surgery, and advanced therapies are still the standard care of therapies for treating diabetic wounds. Various treatment strategies have been developed for the treatment of diabetic wounds, but unfortunately no single treatment fulfills the needs necessary for treating this condition due to its complex, multifactorial pathophysiology [2]. As a number of biochemical shortcomings/variations eventually cause diabetic wounds, a single treatment strategy holds no promise. Hence, multi-mechanism-based products to plug the loopholes involved in diabetic wounds need to be developed. Even though the pathophysiology of diabetic wound is multifactorial the long-term inflammation accompanied by chronic infections with improper tissue management is the principal factor that impairs wound healing [4].

In this context, the extensive literature survey was carried out about various drugs and excipients that can be used in the management of diabetic wounds. Based on the literature review the drugs CUR and DOX have been selected since they have anti-inflammatory and antibacterial effects. On the other hand excipients such as COL, CS, and ALG have been selected since they have a potent role in tissue regeneration and have been extensively studied for tissue management purposes.

Since two drugs have been selected for this research compatibility studies between these two drugs have been evaluated using DSC and FTIR studies. In compatibility studies using DSC and FTIR the results confirmed that both CUR and DOX are compatible with each to formulate a product.

Saturation solubility studies have performed to provide sink conditions and determine the drug release pattern of CUR. These studies demonstrated that CUR has shown maximum solubility (422 ± 8.6 μg/mL) in media containing SWF with 1% w/v of SLS and is best suitable for conducting in vitro drug release studies. Addition of surfactant to the medium improves the solubility of the drug by facilitating the micelle solubilization at solid/liquid interface. Higher concentrations of the surfactants (up to 3%) are used to provide the sink conditions for poorly water-soluble drugs.

The developed UFLC method for simultaneous estimation of both the drugs indicates that the method was accurate. Additionally, robustness study revealed that small changes did not alter the retention times, retention factor, and resolutions more than 4% and therefore it would be concluded that the method conditions are robust and can be used to determine the drug concentration.

Due to the poor water solubility and stability of CUR, initially, it was fabricated into nanoparticles and then incorporated into scaffold. An in-depth literature survey has been done regarding various methods and materials used to prepare CSNPs. The literature study reveals that ionic gelation method is the most commonly used method for preparing CSNPs since it is a simple and easy method and requires aqueous environment for nanoparticle preparation. Further, it is a mild method achieved without applying harmful organic solvent, heat, or vigorous agitation that is damaging to sensitive drugs. It could efficiently retain the drug molecules during preparation. TPP was chosen as cross-linking agent rather than other cross-linking agents like glutaraldehyde and pluronic, because they are toxic. Hence, ionic gelation method was chosen for preparing CUR-loaded CSNPs.

Using ionic gelation method, CUR-CSNPs of 192.2 nm size and +27.5 mV zeta potential with an EE of 75.41 ± 1.8% have been prepared successfully. A particle size of <200 nm with good entrapment efficiency has better control in drug release and also has cell interaction (cell infiltration) nature. Zeta potential of near to or more than +30 mV is necessary for good stability. Further, the positive zeta potential of CUR-CSNPs helps in better adherence of scaffold to the negatively charged biological membranes. These results are also further conformed to SEM analysis of nanoparticles where the SEM images revealed that CUR-CSNPs are spherical in shape with an average size of 200 nm.

In DSC and XRD analysis of CUR-CSNPs, the conversion of crystalline form of CUR to amorphous form was observed which can increase the solubility and stability of CUR. Modification of crystallinity is essential because this was strongly associated with drug incorporation and drug release rate. This data also suggests that the CUR will remain entrapped in the polymer during the shelf life [42, 43].

SEM morphology of the nanohybrid scaffold verified the uniform dispersion of CUR-CSNPs all through the scaffold. The SEM image of both the scaffolds indicated the porous architecture with a geometry ranging from 50 to 250 μm in size. The existence of a web of poriferous appearance between the COL–ALG strands could be significant for cellular adhesion, ontogeny, and movement for tissue re-formation [44]. The nanoparticle-loaded cross-linked web shall lend 3D structural integrity to the scaffold, as the COL–ALG is primarily concerned with the biological component. Furthermore, the porous structure of scaffolds is likely to aid in improved oxygen permeability to the wounds.

The DSC and tensile strength measurement of scaffolds revealed that the EDC cross-linking greatly enhanced the mechanical properties of nanohybrid scaffolds compared to COL and COL–ALG scaffolds which indicates its better in vivo stability.

In swellability studies the prepared nanohybrid scaffolds showed optimum swelling. However, COL scaffold and COL–ALG have

shown a higher swellability ratio compared to nanohybrid scaffold because of their hydrophilic properties and high porous structure. This higher water absorption could lead to loss of physical integrity and therefore loses scaffold stability during the cell culture process and for in vivo application. The swelling ability of a scaffold is often reflected by its water uptake. This property has been proved to be imperative for the absorption of body fluids and for transfer of cell nutrients and metabolites inside the scaffold [44]. The results reveal that the nanohybrid scaffold was stable and holds the ability to absorb water sufficient for tissue engineering applications, particularly in fabrication of wound dressing biomaterials. These results are in compliance with biodegradation studies of scaffolds where the nanohybrid scaffolds show resistance in degradation for the collagenase enzyme compared to COL and COL–ALG scaffold. These results further indicate the increased stability of nanohybrid scaffolds.

In vitro drug release studies the CUR-CSNPs impregnated in COL–ALG scaffold have shown much retention of drug up to 15 days than that of CSNPs alone which have the drug release of about 90% in 7 days. Controlled drug delivery mediated by nanohybrid scaffold can be beneficial in limiting the inflammation for an extended time and also minimizing the rate of reapplication that contributes to effective wound healing and treatment. On the other hand, more than 50% of DOX was released at 24 h from scaffold. This initial burst release and greater fraction of DOX are essential to control the infection load and to exert immediate chemoprophylaxis on wound bed [31, 52].

The in vitro cytotoxicity of the prepared scaffolds was performed on 3T3-L1 fibroblasts as they regulate protein synthesis in the ECM [53]. They also play a critical role in supporting key processes in normal wound healing (fibrin clot degradation, ECM regeneration, and collagen redevelopment to help in wound contraction [54]). The obtained results suggest that the cells could proliferate and maintain their fibroblast morphology while in contact with the scaffolds. The cell viability and compatibility of nanohybrid scaffolds were demonstrated since the scaffold is made of natural polymers which are biodegradable and biocompatible.

In patients with DFIs narrow-spectrum antibiotics may be used for minor infections, preferably through oral route, and for severe infections broader spectrum antibiotics have been prescribed which are most often administered intravenously, because the infections are severe. However, both routes of administration are insufficient in treating DFIs since the presence of microvascular diseases leads to insufficient blood supply to the wound area and antibiotic therapy is associated with frequent adverse effects and development of resistance. Achieving the right therapeutic drug concentration is the key of a successful antibiotic therapy. An alternative to this could be topical antimicrobial therapy. This mode of therapy could afford many hypothetical advantages such as improved localized drug concentrations and bypassing the adverse effects of antibiotics at the systemic level. Drugs such as silver sulfadiazine, neomycin, polymyxin B, gentamicin, metronidazole, and mupirocin have been studied by this direction for soft-tissue infections in other areas; however, no reports exist confirming their potential in DFIs. The results obtained through this study seem promising and deem further exploration in antimicrobial agents necessary. Hence, in this study it was proposed to use the selected drugs CUR and DOX topically. Further, due to the increase of resistance by bacteria, there is an urgent need to identify and assess alternative antimicrobials, or their combinations including those from plant origin with low human cytotoxicity. Considering the above points in vitro antimicrobial activity of CUR and DOX has been performed against various bacteria present in DFIs. The results reveal that DOX was more effective than CUR and also DOX has shown broad-spectrum inhibitory effect against all microorganisms than CUR. This can be attributed that even though CUR is a good antibacterial agent its minimum inhibitory concentration is very high as compared with synthetic antibiotic drugs, and this property makes the CUR ineffective to be used as an antibacterial agent as that of available antibiotics. These results also further

demonstrate that the combination of CUR and DOX has synergistic effect compared to individual treatments. From in vitro antibacterial studies it can be concluded that combination of CUR and DOX topically is necessary to have synergistic activity in treating microbes associated with DFIs which is beneficial and essential for improving quality of life in diabetic patients. Further it is suggested that CUR alone had very less antibacterial activity and may not be recommended for the treatment of DFIs.

A formulation's irritation index could stunt its use and compliance in patients. Hence the skin irritation test performed is validated as its reactivity would provide better insight into the formulation's discomforting features. The skin irritation potential of prepared scaffolds was tested on the rat skin by performing Draize primary skin irritation test. The results of this study reveal that no erythema or edema in any of the scaffold-treated groups was observed. This may be due to the fact that the scaffold is made up of natural substance like COL, ALG, chitosan, and CUR. Further, during the preparation of the scaffolds the pH was adjusted up to 7 (neutral) and hence may be no skin irritation was observed.

Impaired wound healing in diabetes is multifactorial and includes neuropathic, vascular, immune function, and biochemical abnormalities [55, 56]. Experimental studies indicate that these complex pathogenic features in impaired diabetic wound healing observed in diabetic patients can be simulated experimentally in animal models (chemical-induced and genetic diabetic rats or mice) [55, 57]. However, the STZ-based diabetes model has its own set of complications [57–61]. Hence the incorporation of this model to observe the diabetic wound healing effects of various composite scaffolds seems significant. Post-single injection of high-dose STZ in rats initiates the type 1 diabetes after 2 or 3 days. This is due to the selective destruction of pancreatic β-cells that inhibits the synthesis and secretion of insulin.

Diabetic wounds are characterized by significant levels of pro-inflammatory cytokines and proteases [62] which have the potential cause for diabetic wound chronicity [63]. This can be further exacerbated by susceptibility of open wounds to infection. Protease activity is by specific group of proteases called MMPs. These MMPs are responsible for ECM degradation in chronic wounds. Among the MMPs it is believed the MMP-2 and MMP-9 are necessary for both remodeling and re-epithelialization phases of wound healing. However, earlier research indicates that their expressions in chronic wounds like diabetic wounds have been increased leading to ECM degradation and worsening of the chronic wounding [64]. MMP-9 has been classified as a major protease of chronic wound fluid, with marked heightened activity levels in chronic wounds when compared to acute wounds [62]. These studies also indicate that MMP-9 inhibition may have a better therapeutic effect than general MMPs [MMP-1, 2 and 8] in progressing long-term wounds into a healing state [65].

TNF-α and IL-10 are among the many mediators that are active during the inflammatory phase of wound healing. Overproduction of pro-inflammatory cytokines (TNF-α) and suppressed levels of anti-inflammatory cytokines (IL-10) are associated with chronic inflammatory condition in wounds. High levels of TNF-α inhibit angiogenesis and cell proliferation and migration in diabetic wounds and increase apoptosis levels. Further, TNF-α, cytokine, influences the synthesis of collagen as well as MMPs [66]. TNF- α levels were approximately 100-fold higher in chronic wound fluids when compared to levels in acute wounds, indicating an imbalance of pro-inflammatory cytokines within the chronic wound [67]. Since both the increased expression of TNF-α, MMP-9, and decreased levels of IL-10 contribute to poor wound healing in diabetics, agents that decrease the levels of TNF-α, MMP-9, and increase the IL-10 expression levels may be useful to cure the impaired wound healing in diabetic patients. Hence in this study the pro-inflammatory cytokine TNF-α, anti-inflammatory cytokine IL-10, and protease MMP-9 have been chosen as markers to investigate the effects of CUR and DOX in diabetic wound healing.

The anti-inflammatory effects of CUR have been extensively studied in various *in vitro* and

in vivo studies. However, there was no data on the potential role of CUR alone or in combination with other anti-inflammatory drugs in treating diabetic wounds. In particular, no studies have reported till date the anti-inflammatory effects of CUR and DOX either alone or in combination in diabetic wound healing. In order to elucidate the in vivo anti-inflammatory effects of both the drugs at the wound site in diabetic rats, we measured the expression/levels of TNF-α, IL-10, and MMP-9 on different days in the granulation/healing tissues. CUR and DOX caused a marked reduction in the expression/levels of TNF-α, MMP-9, and increased expression of IL-10 in the cutaneous wounds of diabetic rats. Our results demonstrate time-dependent decrease in the levels of TNF-α and MMP-9. CUR caused marked decrease in TNF-α and MMP-9 expressions compared to DOX. Interestingly, when the combination of CUR with DOX (nanohybrid scaffold) was used both the expressions of TNF-α and MMP-9 were reduced significantly in comparison with single-drug treatment. The results showed that CUR and DOX combination significantly decreased the expression/levels of TNF-α and MMP-9 in a time-dependent manner compared to single-drug treatment alone. Similar kind of results was also observed with increased levels of IL-10 expressions. Further, these results were also supported by histology findings.

CUR and DOX combination significantly reduced the inflammation and appears to protect collagen from degradation in chronic wounds. Histopathological examination with H&E staining showed the marked collagen synthesis and fully regenerated epithelial layer with advancement in all three stages (proliferation, remodeling, and maturation) after topical application of nanohybrid scaffolds > CUR-CSNP scaffold > DOX scaffold compared to control- and COL–ALG scaffold-treated wounds which seem to have a constant infiltration of neutrophils and polymorphonuclear cells. The results also further indicate an unorganized and poorly defined dermis in wounds treated with control and COL–ALG in contrast to those in the nanohybrid scaffold > CUR-CSNP scaffold > DOX scaffold-treated wounds demonstrating dermis regeneration through the appropriate manner of formation. Such features are essential for the stipulated dressing to enhance the healing process in the wound. The results of this study suggest that CUR and DOX combination may have synergistic therapeutic effects on impaired wound healing associated with diabetics via the mechanisms of inhibiting TNF- α, MMP-9, expressions and upregulation of IL-10 expressions. The findings of this study showed markedly decreased expression/levels of TNF-α, MMP-9, and increased expression of IL-10 in all groups, comparatively better in CUR-CSNP- and nanohybrid scaffold-treated groups in comparison to control and COL–ALG groups. They also supported cell adhesion, proliferation, and decreased degradation of collagen at the wound site which was evident from H&E staining. Significantly decreased expressions of pro-inflammatory cytokine TNF-α, protease MMP-9, and increased expression of anti-inflammatory cytokine IL-10 in this study suggest that drug-treated scaffolds efficiently controlled the inflammation levels and tissue damage for better granulation tissue formation. The results also further indicate that the activities of these two compounds were synergistic. These data suggest that the nanohybrid scaffold having the combination effect of CUR and DOX is a promising novel wound dressing for diabetic wounds. However, further animal studies should be done in future on larger animal groups to confirm the potential use of nanohybrid scaffold in diabetic wounds.

## Conclusions

The prepared novel composite nanohybrid scaffolds satisfied the properties of an ideal diabetic wound dressing in terms of mechanical strength, swelling, porosity, biodegradation, biocompatibility, controlled release, cell adhesion, and proliferation with antibacterial and anti-inflammatory properties which are crucial for tissue regeneration in diabetic wounds. Hence, this study suggests that the synergistic combination of CUR, DOX (anti-inflammatory and antibacterial), chitosan

(controlled drug carrier, wound healing), COL (established wound healer), and ALG (biomaterial for regenerative medicine) is a promising strategy to address various pathological manifestations of diabetic wounds and has better wound healing capability.

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# Risk Factors for Lower Extremity Amputation in Patients with Diabetic Foot Ulcer

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## 1 Introduction

Lower extremity amputations (LEAs) are historically known as a treatise for lifesaving surgery in battle war or trauma; currently, it is mainly concerned with the diabetic foot [1]. Studies showed that the incidence of overall LEA ranges from 46.1 to 9600 per 100,000 and major amputation ranges from 5.6 to 600 per 100,000 diabetic patients [2]. This surgical procedure, more often as the result of neglected diabetic foot ulcers (DFUs), was reported to occur at a rate of 15 times higher among diabetic patients than in person who do not have diabetes [3]. The global view, which reveals more than 1 million annual LEAs—one every 30 s—is even more troubling, particularly since the International Diabetes Foundation (IDF) predicts that current global prevalence of diabetes will burgeon from 285 million to reach 435 million by 2030 [4]. Therefore, the number of patients with diabetic foot, and consequently the number of candidates for LEA will increase gradually over time rather than decrease. With this in mind, it becomes clearer that preserving the LEA is itself the achievement of important goal [4, 5].

Diabetic-related LEA is performed for various indications; the most commonly cited were gangrene (84%), severe soft-tissue infection and osteomyelitis (63%), and nonhealing ulcer (55%) [6]. The strings of events that results in LEA usually begins with an innocuous-appearing foot ulcer [7]. A new-onset DFU, as reported by Moulik et al. [8], has 11–29% LEA rates within 5 years despite treatment. Whether an ulcer will heal or proceed to stage where LEA be necessary, there were factors that exist along with the resolution and progression of the initial ulcer [7, 9–11]. Therefore, a causal pathway for amputation has been introduced, where diabetics develop foot ulcers and then have certain periods before a severe DFU leads to amputation [7]. Within the frame of this pathway [7], clinicians have an important role (and a great opportunity) to consider any causal factors and comorbidities related to LEA, thus understand the element that may improve limb viability whenever an ulcer occurs in diabetic patients [10, 11].

The purpose of this chapter is to review the analytic or experimental studies concerning risk factors for LEA in patients with DFU. Generally, two broad categories have been discerned, that is metabolic and non-metabolic risk factors. These factors are either clinical or nonclinical entities; some of these factors are modified, or

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can be treated, however, some cannot be modified at all. Understanding the dynamic between these two risk factor categories is expected to shed light on what can be done to preserve or salvage the limb.

## 2 Foot Complications and Amputation in Diabetes

The diabetic foot can be defined as a foot that exhibits any pathology that results directly from diabetes-related complications in the lower extremity. The decisive factors for the etiology of these complications are diabetic neuropathy and peripheral arterial disease [12, 13]. Furthermore, diabetic foot is one of the most common complications associated with diabetes. Singh et al. [14] reported that the lifetime risk that a diabetic patient will acquire foot lesions (ulcers/gangrene) has been estimated at 15–25% and the corresponding annual incidence to be 1.0–4.1%. In mixed cohort studies, the majority (65–85%) of DFUs will heal either spontaneously or with treatment, but it can be a slow process, often taking more than a year, and over half will get recurrent ulceration within 12 months. Twenty percent of these subsets will be necessary for hospitalization, and in the most unfortunate event, a LEA surgery must be undertaken [8, 12, 15–17]. Major as well as minor LEA in diabetic patients has several objectives: (1) to eradicate the presence of gangrene or necrotic tissue, (2) to control infection, (3) to relieve pain, and (4) to regain ambulation, allowing the patient to return to an independent existence in the community [18]. The loss of a lower extremity or even part of it greatly impacts quality of life. The impact of diabetes-related LEA will ultimately result in an increased risk for further ulceration, infection, another amputation, and even death, making them a considerable public health problem [4–6, 10, 14].

## 3 The Clinical Importance of Risk Factors Analysis in Diabetic-Related Amputation

Examination of the risk factors of LEA in DFU is important for a number of reasons. First, the impact of DFU with resulting amputation is so enormous, and the prevention relies on the identification of high-risk patients [4]. Second, the presence of a foot ulcer is the single biggest risk factor for LEA in persons with diabetes [7, 10, 19]. Analytical studies [7, 19, 20] have shown risk factors for LEA related to diabetes to be similar to those for ulceration and the same factors involved in ulceration can have at least contributory roles in LEAs. Once ulcers develop, they are practically exposed with the other various risk factors with different importance in ulcers progression. Third, there is no single risk factor; rather it is a complex interrelationship between diabetes-specific factors, general environment, quality of healthcare service, and personal commitment to disease prevention. Such analysis is particularly important in the study of complex conditions, of which diabetes-related amputation is one of the examples [21, 22]. Further, we need to sort out the modifiable risk factors of the many factors, to understand the relative importance or contribution from other risk factors.

## 4 Causal Pathway for Amputation

Foot ulcers as the primary cause of LEA are considered as the most preventable complication in patients with diabetes, but often viewed as a minor problem and underestimated by the patient, yet frequently impact the patient's survival [4, 12, 14, 22]. In a continuing effort to better understand factors associated with LEA in diabetic

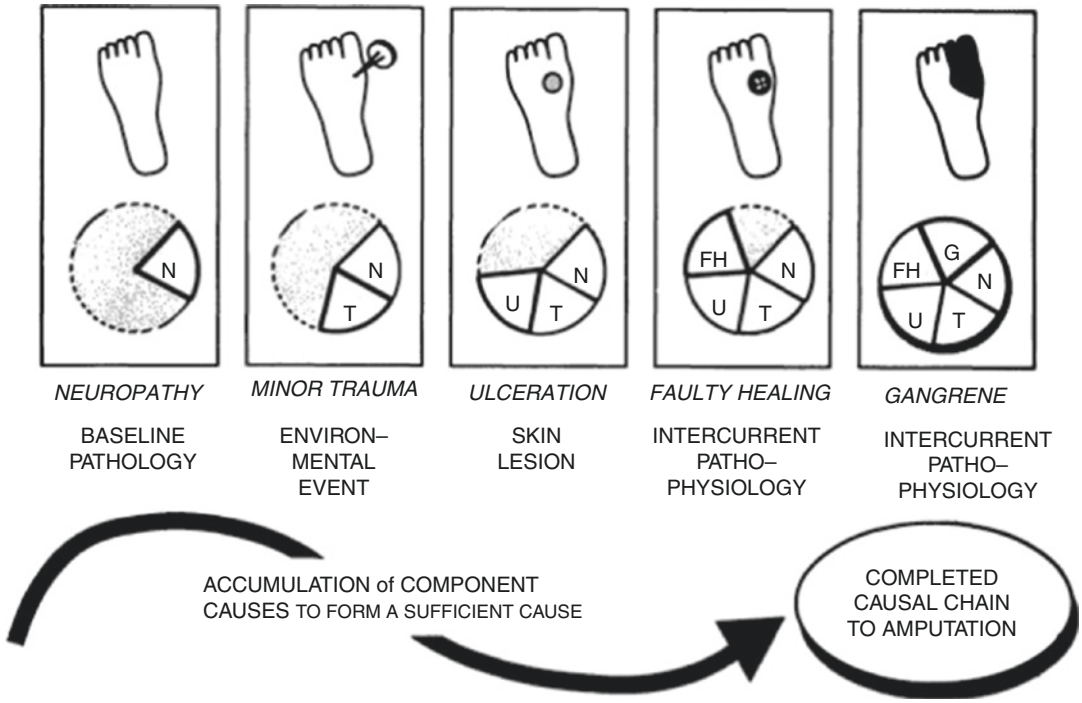



**Fig. 1** Representation of causal pathway to individual amputation, which includes essential contribution from underlying diabetes-related pathophysiology, initiating event, formation of foot lesion, and subsequent healing complications. Eventual occurrence of gangrene is the terminal event of this causal chain, which requires the participation of all preceding components before becoming sufficient to cause amputation. Theoretically, amputation could have been avoided by elimination of any component cause before convergence of causal chain (Reprinted with permission from Diabetes Care, Vol. 13, No. 5, May 1990)

foot, causal pathways relating various common factors have been described [7]. This pathway incorporates major risk factors of ischemia and neuropathy with specific component causes and sufficient causes of LEA, illustrated in Fig. 1. Component causes are risk factors that are essential components, but not independently sufficient, in the causal sequence to cause amputation. A sufficient cause is a causal pathway to disease containing a complete set of risk factors that inevitably produce the outcome. There can be a number of sufficient causes with various combinations of component causes that produce the outcome, in this respect, the LEA [9–11]. According to the proximity of the factors in the causal chain leading to LEA, the terminology also differs. Figure 2 delineates a causal framework in which risk factors (intermediate and distant risk factors) interact to form the sufficient cause (true risk factors) of LEA, i.e., severe DFU, gangrene, or septic foot. The critical public health massage imparted from this model is preventing the occurrence of a component cause so that it stops entering the path, thus renders other components unable to produce the sufficient cause and ultimately LEA [10].

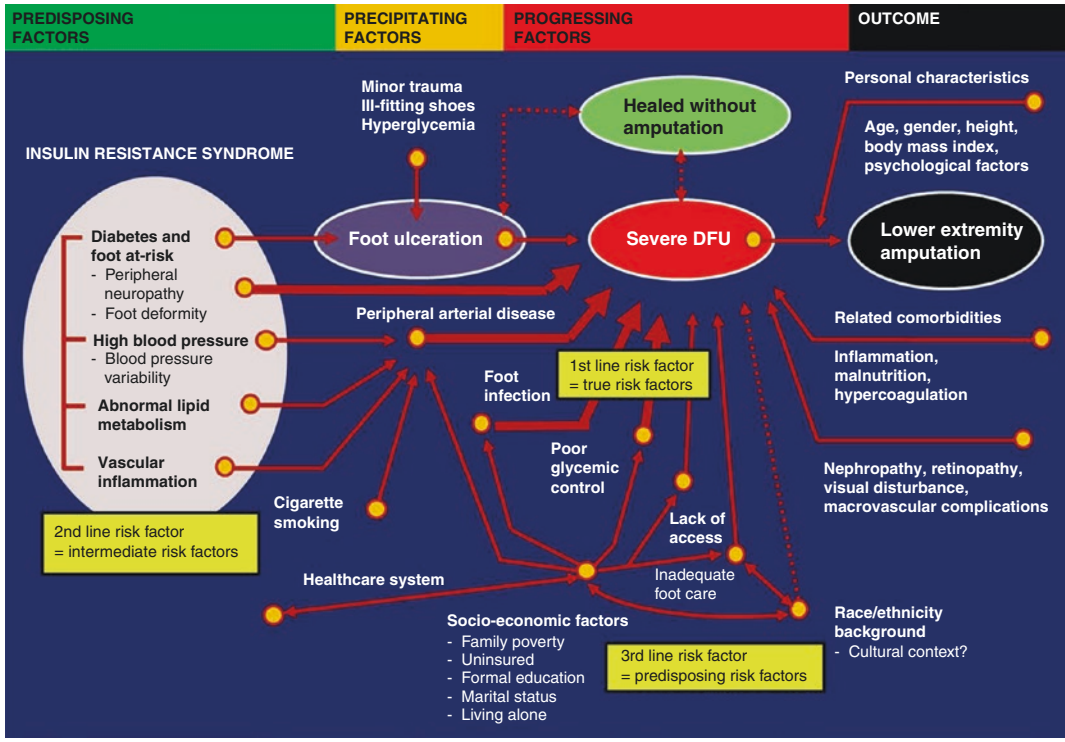



**Fig. 2** Conceptual framework and interrelationship between risk factors for diabetic LEA. It is not possible to determine whether a specific element was responsible for the observed outcome. In DFU, the risk factor exposure has an indirect effect on the likelihood of the event, except for PAD that can directly cause acute arterial occlusion. The figure also shows true risk factors (first line or proximal risk factors) that precipitate LEA are distinguished from intermediate and predisposing risk factors, which have a remote and complex association with the disease. The risk factors should be evaluated in terms of the individual patient rather than weighing the risk factors separately

## 5 Exploring Risk in Lower Extremity Amputation

The literature review has highlighted the difficulty in making comparison between studies due to the dissimilarity in methodology, demographics, research design, and definitions to described amputation in diabetics [21–25]. Inconsistencies between published studies are also reflected by the inclusion or exclusion criteria of diabetic patients. Most of the studies of LEA have been conducted in population that have a high prevalence of diabetes (summarized in Table 1) [26–40]; others use foot at risk [41–45] as the starting point to their observation. However, there is plenty of data on this outcome in patients with preexisting DFU (summarized in Table 2) [8, 16, 17, 46–66]. Various risk groups (in certain ethnicity [29, 32, 38, 39, 67–69], neuropathic or neuroischemic ulcers [8, 17], dialysis patients [70–73]) have been analyzed by different studies to be associated with an increase in LEA in patients with diabetes. Different studies often use different data sources to identify LEA. Several studies has used hospital amputation events by either clinical records or electronic databases [27, 30, 32, 36–40, 46, 52, 58, 61, 66, 74–76], while others relied on coding expertise [42, 77–79]. Yet others have studied cohorts of patients attending a program designed to prevent amputation [20, 67, 68, 80–84].

The approach employed to understand the risk rather than incidence rate is somewhat different [21]. The published studies that we

**Table 1** Comparison of significant risk factors for LEA from selected publications on diabetes population

| Characteristic and risk factors | Study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Nelson et al., Pima Indians [26] | Moss et al., Wisconsin study [34] | | Selby and Zhang, San Francisco [30] | Lehto et al., type 2 DM (Finland) [31] | Lee et al., type 2 DM (Oklahoma Indians) 31] | | Reiber et al., Male veterans [27] | Mayfield et al., (Pima Indians) [32] |
| | | Early onset | Late onset | | | Men | Women | | |
| Design | Cohort | Cohort | | Nested case-control | Cohort | Cohort | | Case-control | Case-control |
| Population | Diabetes, population-based | Diabetes, population-based | | Diabetes, population-based | Diabetes, population-based | Diabetes, population-based | | Diabetes, population-based | Diabetes, population-based |
| Data collection | Exam | Self-report, exam | | Chart review | Exam | Exam | | Chart review, exam | Chart and photographic review |
| Sample size | 4399 | 1210 | 1780 | 150 + 278 | 1044 | 332 | 543 | 80 + 236 | 63 + 183 |
| Analysis | Mantel-Haenszel | Logistic regression | | Logistic regression | Cox regression | Cox proportional hazard model | | Logistic regression | Logistic regression |
| Measure | Incidence rate ratio | OR | | OR | RR | Rate ratio | | OR | OR |
| Male vs. female | Adjusted | NS | 2.8 | Matched | Adjusted | NA | NA | NA | 6.5 |
| BMI | – | NS | NS | NS | NS | – | – | – | – |
| Ethnicity | Matched | – | – | NS | – | Matched | Matched | – | – |
| Age (per × years) | Adjusted | 2.0 per 10 | NS | Matched | Adjusted | NS | NS | Adjusted | 1.3 per 5 |
| Smoking | NS | NS | NS | NS | NS | – | – | – | – |
| Diabetes duration | – | – | – | – | – | – | – | – | – |
| Glycemic control (per × units) | – | 1.4 per 2% HbA1c | 1.5 per 2% HbA1c | 1.75 per glucose score | 2.5 for FPG > 13.4mMol/L 2.5 for HbA1c > 10.7% | 1.08 per mMol/L | NS | Adjusted | 1.6 per 50 mg/dL |
| Insulin use | – | NS | NS | – | – | 2.56 | NS | – | – |
| Retinopathy | 2.1 | 1.4 per 2 steps | NS | 3.68 | 3.6 | 3.19 | 3.33 | Adjusted | 4.6 |
| Renal disease | 2.2 | – | – | – | NS | NS | NS | Adjusted | 4.6 |
| Proteinuria | – | NS | 4.3 | NS | 1.3 | NS | NS | – | NS |

(continued)

**Table 1** (continued)

| Characteristic and risk factors | Study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Nelson et al., Pima Indians [26] | Moss et al., Wisconsin study [34] | | Selby and Zhang, San Francisco [30] | Lehto et al., type 2 DM (Finland) [31] | Lee et al., type 2 DM (Oklahoma Indians) 31] | | Reiber et al., Male veterans [27] | Mayfield et al., (Pima Indians) [32] |
| | | Early onset | Late onset | | | Men | Women | | |
| Hypertension (per mmHg of sBP or dBP) | NS | 2.1 per 10 dBP | NS | 1.02 per 1 sBP | NS | 1.15 per 10 sBP | 1.28 per 10 dBP | – | NS |
| Cholesterol | NS | – | – | NS | 1.8 for >6.2 mMol/L | NS | 1.18 per mMol/L | 6.4 for HDL <1.3 mMol/L | NS |
| Neuropathy | 2.0 patellar reflex 12.8 VPT | – | – | 4.05 | 4.3 Achilles tendon reflex 2.7 VPT | – | – | 12.9 VPT 5.1 Touch sensation | 2.1 any diagnosis |
| Hypoalbuminemia | – | – | | | – | – | – | – | – |
| Inflammatory markers | – | – | – | – | – | – | – | – | – |
| Ulcer size | – | – | – | – | – | – | – | – | – |
| Ulcer depth | – | – | – | – | – | – | – | – | – |
| Peripheral arterial disease | 4.8 Medial artery calcification | – | – | – | 3.9 absent pulses 2.1 femoral bruit | – | – | – | 2.1 any diagnosis |
| Foot infection | | | | | | | | | |
| Foot deformity | – | – | – | – | – | – | – | – | 2.1 |
| Ulcer (or LEA) history | – | 10.5 | 4.6 | – | – | – | – | – | 2.1 |
| No outpatient education | – | – | – | – | – | – | – | 16.5 | – |

**Table 2** Comparison of significant risk factors for LEA from selected publications on DFU patients

| Characteristic and risk factors | Study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Yesil et al. Turkish study [50] | | Gershater et al. [17] | | Winkley et al. [47] | Hennis et al., Caribbean population [39] | Zubair et al. [54] | Namgoong et al. [60] | Pemayun, et al. Semarang, Indonesia [61] |
| | All LEA | Major LEA | Neuropathic | Neuroischemic | | | | | |
| Design | Cohort | | Cohort | | Cohort | Prospective case-control | Cohort | Cohort | Case-control |
| Population | DFU, hospital-based | | DFU, hospital based | | DFU, population-based | DFU, population-based | DFU, hospital-based | DFU, hospital-based | DFU, hospital-based |
| Data collection | Self-report, exam, photographic review | | Self-reported, exam | | Exam | Exam | Self-reported, exam | Self-reported, exam | Chart review |
| Sample size | 510 | | 2480 | | 253 | 200 + 104 | 162 | 837 | 47 + 47 |
| Analysis | Logistic regression | | Logistic regression | | Cox regression | Logistic regression | Logistic regression | Logistic regression | Logistic regression |
| Measure | OR | | OR | | HR | OR | OR or RR | OR | OR |
| Male vs. female | NS | NS | NS | 1.5 | NS | 1.54 | NS | NS | Matched |
| BMI | NS | NS | – | – | – | NS | – | – | Matched |
| Ethnicity | – | – | – | – | – | – | – | – | – |
| Age | 1.73 | NS | NS | NS | NS | NS | NS | NS | Matched |
| Smoking | 1.41 | 2.04 | | | NS | NS | NS | NS | – |
| Diabetes duration | NS | NS | 2.6 | 1.8 | NS | 1.06 | – | NS | NS |
| Glycemic control | NS | NS | – | – | NS | 1.24 | NS | NS | 20.47 HbA1c ≥8% |
| Insulin use | NS | NS | – | – | NS | 3.13 | – | – | NS |
| Retinopathy | NS | NS | 1.8 | NS | NS | | NS | NS | NS |
| Renal disease | NS | NS | 2.6 | NS | NS | – | 2.24 nephropathy 3.48 SCr > 1.5 mg/dL | 2.53 Nephropathy 8.68 on dialysis | NS |

(continued)

**Table 2** (continued)

| Characteristic and risk factors | Study | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Yesil et al. Turkish study [50] | | Gershater et al. [17] | | Winkley et al. [47] | Hennis et al., Caribbean population [39] | Zubair et al. [54] | Namgoong et al. [60] | Pemayun, et al. Semarang, Indonesia [61] |
| | All LEA | Major LEA | Neuropathic | Neuroischemic | | | | | |
| Proteinuria | – | – | – | – | – | | – | | – |
| Hypertension (per mmHg of sBP or dBP) | NS | NS | – | – | – | NS | 2.83 | NS | 3.67 |
| Cholesterol | – | – | – | – | – | – | | – | 5.58 Triglycerides ≥150 mh/dL |
| Neuropathy | NS | NS | Adjusted | Adjusted | NS | 2.74 lower-limb reflex 31.9 monofilament 1.07 VPT | 2.24 any diagnosis | NS | NS |
| Anemia | 1.83 | 2.26 | – | – | – | – | 3.46 | Hb 0.64 | NS |
| Hypoalbuminemia | 2.25 | 2.51 | – | – | – | – | – | NS | NS |
| Inflammatory markers | 5.25 CRP 3.87 ESR | 3.08 CRP 5.68 ESR | – | – | – | – | 2.80 leukocytosis | NS | NS |
| Ulcer size | | 3.97 | – | – | 1.99 | – | – | NS | – |
| Ulcer depth | 7.83 | 9.06 | – | – | 3.6 | – | 3.7 deep ulcer | 11.67 Bone | NS |
| Peripheral arterial disease | 6.17 | 13.2 | 2.5 Rest pain | 2.0 Rest pain 1.8 Claudication 1.7 Toe pressure | NS | 2.28 claudication 3.88 Low ABI 19.2 absent pulses | 6.95 | – | 12.97 ABI <0.9 |
| Foot infection | 4.5 | 3.6 | 4.8 | – | – | 34.5 | 4.52 biofilm infection 3.7 osteomyelitis | – | NS |
| Foot deformity | – | – | – | 1.6 | – | | – | NS | – |
| Ulcer (or LEA) history | – | | 3.3 | – | – | 149.4 | | NS | NS |
| No outpatient education | – | | – | – | – | NS | | – | – |

reviewed include population cohort [8, 19, 28, 33, 34, 36, 38, 40, 47, 60, 84–87], hospital cohort [8, 16, 17, 50, 59, 70, 88, 89], and case-control studies [27, 30, 32, 36, 37, 39, 44, 49, 58, 61, 77, 82, 90]. Risks are calculated using logistic regression in most of the series; however, some papers use survival analysis [8, 17, 52, 57, 62, 74, 79, 86, 87] and meta-analysis on HBA1c [91, 92] and effect of gender [93] to describe LEA risk in diabetic patients. Although the reported variable and risk assessment may vary, some findings are shared in the majority of these reports. Lower-level LEA were more common in diabetes [22]; it is actually important to report major LEA separately as they are undertaken for more serious disease and are associated with more consequences. However, our chapter focuses on any LEA and not separately on minor and major amputations. Both are considered together in this chapter otherwise stated with notification. Priority was given to papers that convey the statistical measures of the risk in the form prevalence of risk (PR), odds ratio (OR), relative risk (RR), or hazard ratio (HR) and its respecting confidence interval (95% CI).

## 6 Risk Factors for Lower Extremity Amputation

Metabolic risk factors emphasized on diabetes health history as the primary disease, atherosclerotic risk factors, and several nutritional or biochemical parameters. The non-metabolic risk factors are foot-related pathology, diabetes comorbidities, and patient's general characteristics. Sociodemographic and environmental factors can be regarded as extrinsic (nonclinical) factors, also included in the non-metabolic risk category.

### 6.1 Metabolic Risk Factors

#### 6.1.1 Diabetes Health History

**Type of Diabetes**

Diabetes is a metabolic disorder with differing etiology, pathophysiology, manifestation, and treatment approach in individuals with type 1 and type 2. Majority of DFU patients were type 2, while type 1 constituted at least 5–10% of the cases [30, 43, 85], and reflects the general pattern of diabetes demography. Patients with both type 1 (11 to 20.7-fold) and type 2 diabetes (15-fold) demonstrated notably elevated risk of LEAs in comparison with general population of similar age [40]. The cumulative 14-year incidence of first DFU was 7.2% in type 1 and 9.9% in type 2 diabetes and the presence of DFU was found to increase the LEA risk with OR of 3.19 (95% CI 1.71–5.95) in type 1 diabetes and OR of 3.56 (95% CI 1.84–6.89) in type 2 diabetes [34]. Ethnic background may attenuate the risk, because in Pima Indians, the age, diabetes duration, and sex adjusted RR for LEA in type 1 diabetes was 11.4, while 3.8 in type 2 diabetes. In type 1 diabetes, the main risk factors for LEA were vascular complications, serum cholesterol, and triglycerides. In type 2 diabetes, key risk factors for LEA, apart from diabetes duration, were retinopathy, poor glucose control, and triglyceride [85].

**Clinical Duration of Diabetes**

Diabetes diagnosis usually marks the onset of clinical disease; however, disease process could have been ongoing prior to diagnosis, especially in type 2 diabetes. A population-based LEA data from Rochester, Minneapolis, indicated that the incidence of LEA following diabetes diagnosis was 8% at 20 years and 11% at 30 years [94]. The association between diabetes duration and LEA was significant in many studies, either in diabetes population [26, 28–34, 38, 39] or in clinical settings with DFU [17, 44, 52, 70, 95], though this was not the case in all studies [48, 50, 57, 61, 63, 66, 74]. Yusof et al. [44] reported wherein diabetes duration of more than 10 years significantly increased the risk for amputation (OR 63.1, 95% CI 7.6–507.4; $p < 0.001$). The positive association between diabetes duration and risk of LEA is shown to increase exponentially 1.2-fold for every increase in diabetes duration [79]. Moreover, duration of diabetes related to the risk of LEA was independent of the degree of hyperglycemia [31, 33]. An increased duration of diabetes appears to provide important information on LEA risk among DFU patients, as the occurrence of chronic

complications as micro- and macroangiopathy, which is none other than the LEA risk factors per se, is increasingly prevalent in both long-standing type 1 and type 2 diabetes [33].

### Level of Glycemic Control

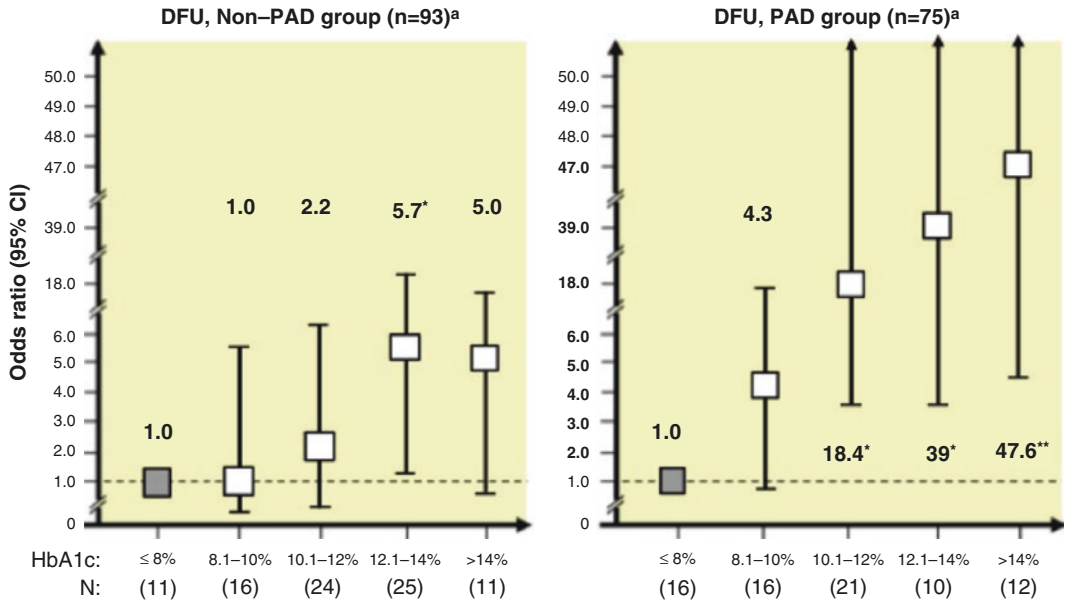
Chronic hyperglycemia as indicated by high HbA1c value has been shown to be an independent risk factor for LEA [27, 30, 52, 61, 70, 74, 79, 86]. In studies not reporting HbA1c association with LEA, Nelson et al. [26] and Hamalainen et al. [33] used blood glucose findings, others used fasting blood glucose (FBG) level [26, 29, 31, 49], while Adler et al. [19] and Moss et al. [34] used a categorical variable of glycated hemoglobin. High FPG represent a catabolic state, with a negative balance as a consequence of gluconeogenesis from protein breakdown. This is problematic because synthesis of proteins such as fibroblast and collagens has an integral part in wound healing. One study by Christman et al. [96] also emphasizes the HbA1c value is even better than the baseline wound size to predict the rate of healing in DFU. Hyperglycemia per se can also precipitate spontaneous foot lesion, including gangrene, for about two factors compared with those with lower glucose levels [94]. This certainly puts the importance of managing hyperglycemia, not merely focusing on wounds of the lower extremity.

According to American Diabetes Association (ADA), the recommended level of HbA1c should be less than 7% (grade A) for people with diabetes and can be achieved with nutritional and pharmacological therapy [97]. High HbA1c (>13.4%) and high FPG level are shown to be associated with a twofold higher risk of amputation compared with well-controlled diabetes [31]. Even moderately poor control (HbA1c > 7 or 7.5%) of diabetes contributes to a significantly higher risk of amputation [52, 74, 75]. In a study by Pscherer et al. [52], Kaplan–Meier survival analysis showed that LEA was independently associated with higher HbA1c value, and the HR for LEA associated with HbA1c value >7.5% was 1.20. Among the risk factors identified from our local study in Semarang, HbA1c > 9% had the strongest effect on the occurrence of LEA (OR 20.47, 95% CI 3.12–134.31; $p < 0.001$). A previous meta-analysis also demonstrated that HbA1c was a significant factor affecting LEA in diabetic patients such that for every 1% increase in HbA1c, there is an associated 26–36% increase of LEA [91, 92].

Despite encouraging evidence, HbA1c level in some studies showing opposite results to that of earlier studies [50, 54, 56, 57, 63, 90] could be due to the effect of compounding variables. One should remember that HbA1c serves as a marker for average blood glucose levels for a few months prior to measurement; it does not reflect the real risk of amputation. However, this is the most modifiable major factor related to LEA, so it must remain the focus of attention for every patient with diabetes. Some of the factors that cause less good glycemic control are disobedient to prescribed treatment [17, 98], infrequent HbA1c screening [58], and missing clinic appointment [90, 99]. Further study identified that the magnitude of LEA risk associated with hyperglycemia is thought to be mediated by several other major factors such as the presence of PAD, see Fig. 3 [100]. High HbA1c also leads to infection susceptibility, affecting leukocyte function, and associated with poor wound healing which maybe another reason for LEA [99].

### Insulin Treatment

Controlling glucose levels, receiving diabetes treatment, and attending nursing visit are important aspects of LEA prevention in DFU patients. Additional risk factors concerning therapeutic regimen have been identified, with insulin treatment being more predictive [29, 58]. Usual (prehospital) treatment with insulin was associated with an increased 1.2- to 2.5-fold likelihood of LEA in some studies [29, 39, 42, 58, 86]. The current explanation of this finding attributes to the progression of advanced diabetes requiring glucose control with insulin injections. Insulin is generally needed after long diabetes duration associated with poor glycemic control, with both being major risk factors for micro- and macroangiopathic complications [9]. Usual insulin treatment thus might be considered more as a marker of the disease severity and long-standing diabetes rather than an independent LEA risk factor in its own right.




[a] Logistic regression; adjusted for age, blood pressure, BMI and neuropathy
*p* value for trend vs. lowest quintile:
* p <0.05, *** p <0.01

**Fig. 3** Risk of LEA in foot ulcers as indicated by level of HbA1c and the presence of PAD (ABI value <0.9). Using HbA1c <8% as a reference category, a progressive increase in LEA risk can be shown as HbA1c quintiles in DFU patients with and without PAD. It seems to have a particular negative in the latter patient group, those with PAD. Source: Naibaho RM, Pemayun TGD. Joglosemar Endocrinology Scientific Meeting XVII in conjunction with Semarang Endocrine and Metabolic Meeting 2016

### 6.1.2 Atherosclerosis Risk Factors

Major risk factors for the development of atherosclerosis are cigarette smoking, lipoprotein abnormalities, and high blood pressure. Hypertension and dyslipidemia are nearly always seen in a syndrome including insulin resistance, and obesity, which are intervening factor in neuroischemic ulcer and LEA. These factors are assumed to be similarly atherogenic in diabetic individuals.

#### Hypertension and Blood Pressure Variability

Hypertension has also been implicated as a risk factor for LEA in some studies [29, 30, 34, 50, 54, 61]. This variable was found to be one of the most important factors that had a significant relationship with LEA. Lee et al. [29] reported that elevated systolic blood pressure (sBP) was a significant risk factor for men, while elevated diastolic blood pressure (dBP) was a significant predictor for women. It should be noteworthy to apprehend that the impact of hypertension is not instant and there is also a positive correlation between the risk and duration of hypertension (OR 1.04; $p = 0.049$) [64]. Magalhaes et al. [101] reported that amputees had higher sBP than non-amputees and demonstrated increased arterial stiffness in those submitted to LEA. Arterial stiffness is also associated with the occurrence of PAD, which involved gradual reduction in blood flow to the lower extremity secondary to atherosclerotic process. This last condition will be discussed further below, in lower extremity arterial disease subchapter.

Although the causal relationship between hypertension and atherosclerosis is so obvious, some studies assessed this measure reported no statistically significant association between blood pressure and diabetic-related LEA [26, 27, 31–33,

39]. Hypertension is nearly always seen in a syndrome including insulin resistance, obesity, dyslipidemia, and coronary disease, which are intervening factors in neuroischemic ulcer and LEA [69]. In addition to the duration and severity of the sBP level, it is evident that day-to-day variability in blood pressure may also affect the risk of LEA. Excessive sBP variability creates endothelial dysfunction and correlates with the severity of PAD, which in turn has downstream effect on DFU and increased the risk of LEA [102]. The International guideline [97] advocated is for blood pressure management in diabetics to be less than 130 over 80 mmHg, and the cardiovascular risk assessment should be less than 15%. Regarding blood pressure, both its level and variability are potential risk factors for LEA which would be likely to bring benefit from intervention.

#### Lipid Effects

Lipoprotein abnormalities, which include elevated levels of plasma triglycerides, very-low-density lipoprotein (VLDL), cholesterol, and low-density lipoprotein (LDL) cholesterol and decreased levels of high-density lipoprotein (HDL) cholesterol, may be more prevalent in diabetics than in nondiabetic individuals. Lipoprotein abnormalities have long been associated with PAD [103], but there are few data evaluating direct effects on risk for LEA. Total cholesterol was a significant risk factor for LEA in women in one study by Lee et al. [29] and low level of HDL (subfraction 3) were reported to be a statistically significant risk factor for LEA in the Seattle WA study [27]. Hypertriglyceridemia was also an independent risk factor for LEA in a large cohort of patients with diabetes within a US health claim database [104]. In a study by Ikura et al. [105] from Japan, low HDL cholesterol value also had a role as a clinical predictor for the incidence of LEA in patients with DFU. There was an inverse relationship between HDL and LEA resulting from DFU, with a 1 mMol/L increment in HDL associated with a 67% lower risk of minor and 88% major LEA. The underlying mechanisms are still not clear; potential explanations include HDL's effects related to immunomodulation and acute immunosuppression in infection.

#### Smoking Habit

Interestingly, among diabetic patients, smoking does not seem to be constantly associated with recurrent foot ulcers [106] or risk of amputation from DFU [26–28, 30, 31, 33, 38, 47, 57, 69]. These data conflict with information in nondiabetics, which clearly indicates that smoking is a risk factor for claudication and arterial disease [107]. Smoking was a risk factor for LEA, however, in only four studies [34, 50, 58, 79]. In an Iranian case-control study, Kogani et al. [58] found that the adjusted OR for amputation among smokers was 3.44 (95% CI, 1.45–8.13; $p = 0.005$). In The Wisconsin Epidemiologic Study of Diabetic Retinopathy [34], smoking is a LEA risk factor among people with younger onset of diabetes. Thus, although cigarette smoking is a strong risk factor for PAD in both diabetic and nondiabetic [107, 108], smoking does not appear to contribute substantially to the excess risk for LEA in diabetes. Regardless of whether smoking affects lower limb complications, diabetic patients who smoke have all-cause mortality twice than that of nonsmoking diabetic patients [108] and should be strongly advocated to cease this habit.

#### Lipoprotein (a)—Lp(a)—and homocysteine (Hcy)

Both Lp(a) and Hcy are mostly genetically committed and play a role in the development of some diabetic complications such as coronary disease, stroke, and PAD. One study, conducted by Gazzaruso et al. [109], demonstrated that high Lp(a) and Hcy levels are associated with the development of ischemic or neuroischemic diabetic foot, while low Lp(a) levels appear to be associated with delayed wound healing in patients with DFU. One can speculate that among patients with DFU, the alteration of Lp(a) and hyperhomocysteinemia could have some problem to obtain an adequate wound healing. The mechanisms by which this could happen are unclear.

### 6.1.3 Hypoalbuminemia and Micronutrient Deficiency

Data provided evidence that 80–85% of LEA are the result of chronic ulceration and faulty wound healing [6, 9, 10]. In addition to arterial disease,

healing of wounds in individuals with diabetes poses several special concerns due to metabolic deficiencies associated with the disease, the disease-specific deficiencies in immune system, the oft-associated relative malnutrition [110, 111], and oxidative stress [112]. Therefore, physiologic parameters of poor wound healing, such as plasma albumin <3.5 g/dL and low plasma zinc and certain vitamin as vitamin C levels, have also been identified as risk factors for nontraumatic amputation in patients with diabetes [27].

Serum albumin is used as a measure to evaluate nutritional status of human body but may also reflect catabolic state and marker for the stress response in the severely ill patients. In patients with DFU, low serum albumin was reported to be associated with increased amputation risk; the reported risk was around 1.2 to 2.5-factor compared to patients with normal serum albumin value [44, 50, 53]. Lipsky et al. [89] reported that low serum albumin (< 2.8 g/dL) was independently related to LEA in diabetic foot infection (DFI). Low albumin levels were also found to be associated with poor wound healing after Syme's amputation leading to higher level of amputation in patients with DFU [113]. It is currently accepted that the threshold serum albumin necessary to support wound healing in the DFU is 3.0 g/dL [114].

Zinc is well established to be vital in wound repair. Zinc, a trace element, also has antioxidant activity of the cysteine-rich metalloproteins to modulate cytoprotection against reactive oxygen species and bacterial toxin [115]. Only one case-control study to date has reported the association between low zinc level and LEA; indeed the zinc levels are lower in serious foot infection [116] and diabetic amputees than in the non-amputees [27]. Zinc level below the threshold value gives OR of 5.1 (95% CI 1.9–13.7) for the likelihood to develop a LEA [27]. Vitamin C is also among vital micronutrients which plays a role in tissue regeneration and wound healing. A deficiency can occur very quickly in DFU patients whose requirements are increased. Reiber et al. [27] measures vitamin C deficiency also carry a certain risk for LEA (OR 2.1, 95% CI 1.2–3.6). Other elements have also been exploratory variables, such as Cuprum or Vitamin E; they were not found to be predictive of LEA [60].

### 6.1.4 Anemia

As with many chronic diseases, anemia is found in diabetes but is frequently unrecognized. Anemia in DFU can be found unrelated to chronic blood loss, malnutrition, iron deficiency, or renal function which may be explained by an underlying inflammatory process [117, 118]. Khanbai et al. [117] reported an inverse correlation between hemoglobin level and diabetic foot progression. A recent study from Brazil reported that more than 80% of DFU patients who submitted to amputation had hemoglobin levels <11 g/dL and present as the most significant risk factor for LEA (OR 5.57, 95% CI 2.90–10.69; $p < 0.0001$), along with the presence of PAD and old age [66]. It is probably due to diminished tissue oxygen delivery; anemia can complicate the ischemic state in the presence of PAD, leading to impairment in wound healing [118] and poor infection control. Though its relationship with diabetic foot complication is not completely clear, its association with the higher risk of both overall [41, 46] and major amputation [53, 115] is well established. These results reinforce that diabetic patients must be routinely screened and treated for anemia, although a question exists in regard to the benefits of treatment of mild anemia in DFU patients [117].

### 6.1.5 Markers of Inflammation

The traditional markers in this setting incorporate C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). In DFU patients, Yesil et al. [50] from Turkish cohort study found that baseline acute phase reactants, especially CRP and ESR, were related to LEA. Acute phase response in DFU mostly depends on limb ischemia, severity of infection, and the presence of osteomyelitis [119]. Lymphocyte count has long been suggested as a laboratory index of nutritional status and immunological competence [111, 114]. Its association with LEA was established is the study by Leung et al. [46]. The accepted threshold marker for lymphocyte function is a total lymphocyte count of 1500, and lymphopenia may describe immunopathy in diabetes [110]. Based on previous studies, neutrophil-to-lymphocyte ratio (≥3.8) has been

indicated as effective predictive marker for major LEA in critical limb ischemia [120]. In diabetic foot, this thesis has been investigated by Yapici et al. [121] from Turkey. Yapici et al. showed that neutrophil-to-lymphocyte ratio was significantly higher in DFU patients with osteomyelitis and independent predictor for determining progression to LEA. Other marker that has been studied was fibrinogen. Data suggested that patients with DFU have higher fibrinogen levels than those without ulcers [122]. Two studies have revealed significant association between fibrinogen and DFU severity as reflected by University of Texas classification [119] and Wagner system [123], thus able to predict LEA.

#### 6.1.6 Other Metabolic Risk Factors

Apart from lipids and blood pressure, other markers of vascular risk may have a bearing on the incidence of diabetic complications, such as serum uric acid, a final product of purine metabolism, and total bilirubin. A study involving a Chinese population reported that elevated uric acid level was a significant and independent risk factor for DFU in female Chinese patients with Type 2 DM [124]. In Finnish cohort study by Hamalainen et al. [33], it turns out that serum uric acid is one of the discriminators between diabetic patients who will experience a LEA or not. This finding is still a novel interest; however, one possible explanation is the close relationship between hyperuricemia towards peripheral neuropathy [125] and peripheral arterial disease (PAD) in the lower extremity [126, 127]. As frequently encountered complications in diabetic patients, peripheral neuropathy and PAD count among the main factors causing foot ulceration and LEA.

Low plasma bilirubin levels have also been related to the risk of amputation in type 2 diabetic patients (HR 1.38 per 5 uMol/L decrease in bilirubin concentration; 95% CI 1.05–1.81, $p = 0.019$) after multiple adjustments. This association was observed between total bilirubin and total LEA, as well as for major but not minor amputation [128]. The hypothesis for this finding was that several actions attributed to bilirubin have antioxidant and anti-inflammatory properties that may protect from atherosclerosis [129]. Further studies are needed to clarify the specific role for serum uric acid and bilirubin in the pathogenesis of diabetic foot amputation.

### 6.2 Non-metabolic Risk Factors

#### 6.2.1 Foot-Specific Risk Factors

##### Lower Extremity Arterial Disease

Vascular insufficiency, affecting large vessel (macroangiopathy) or small vessel (microangiopathy) is a major factor resulting in reduced blood supply to ulcers and the lower extremity. Involvement of microvasculature is not only dependent on the underlying arterial circulation, but may also be critically influenced by other factors, including skin integrity, mechanical effects of repetitive pressure, and tissue edema [130]. Microvascular and macrovascular disease in the lower extremity are referred to as PAD; in diabetes, PAD is known to involve distal arteries more than proximal ones. PAD in the diabetic foot is associated with the most severe adverse outcomes, including lower probability of healing, longer healing times, higher probability of ulcer recurrence, greater risk of amputation, and potentially higher mortality [48, 106, 131]. The importance of peripheral arterial function, as measured by low ankle-brachial index (ABI), low $TcPO_2$, low toe pressure index, absent or diminished lower extremity pulses, angiography or history of revascularization and its relationship to LEA, was assessed in many studies [19, 26, 27, 31–33, 39, 46, 48, 49, 52, 55–57, 61, 64, 69, 70, 77, 82, 90, 132] and found to independently predict LEA in each.

Screening diabetic patients for PAD often includes a measurement of the ABI [130–133]. The threshold to define PAD was the ABI value below 0.9, and there is an inverse relationship between ABI reading with the corresponding LEA risk. When measured using hand-held Doppler apparatus, ABI readings between 0.45 and 0.70 will reveal a moderate increased risk (OR 4.3, 95% CI 1.8–10.3), if compared with

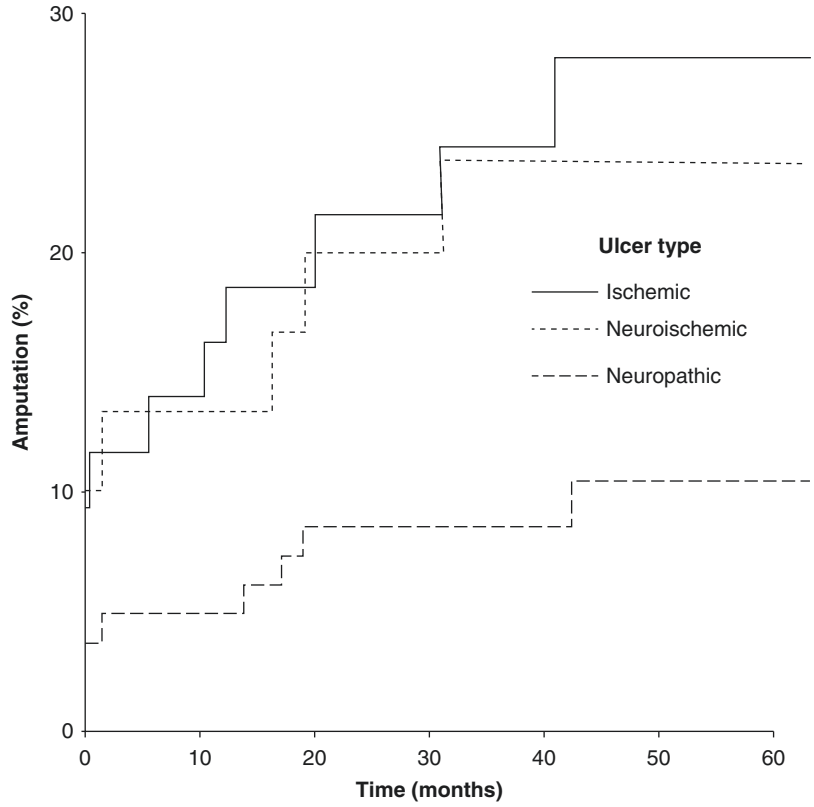



**Fig. 4** Cumulative amputation rates for foot ulcers of various etiologies (Reprinted with permission from Diabetes Care, Vol. 20, No. 2, February 2003)

those who had ABI value above 0.70. Furthermore, in severe PAD when ABI readings are below 0.45, the risk of getting LEA is increased substantially (OR 55.8, 95% CI 14.9–209) [27]. The presence of cutaneous ischemia as reflected by low $TcPO_2$ also appeared to be a strong independent (OR 161, 95% CI 55.1–469) association of LEA with PAD [27]. The medial artery calcification (MAC) in the dorsalis pedis, which results in high pressure ankle and high ABI, is another manifestation of PAD in the diabetic foot [26, 69, 134]. Everhart et al. [134] studied 4553 diabetic patients in a 20-year longitudinal study and reported a 5.5-fold increased rate of LEA compared with patients with diabetes but without MAC. Thus, not only low, but also high ABI are prognostic in predicting LEA.

From many epidemiological studies, it is clear that the key clinical risk factors for LEA in DFU are the complications of PAD and peripheral neuropathy. Accordingly, DFU can present several types such as purely ischemic, purely neuropathic, or a combination of the two, namely, neuroischemic [8, 13, 17, 43]. Patients with ischemic disease, either in an isolated form or associated with neuropathy presented the greatest risk for LEA. A study published by Moulik et al. [8] provides outcome for patients with different type of DFUs. Those with ischemic or neuroischemic ulcers have a much higher probability of amputation compared with pure neuropathic, 5-year amputation rates were 25–29% (Fig. 4). PAD in foot ulcers has frequently been associated with poorer healing outcomes, especially when associated with infection [48] or high HbA1c [100], explaining the higher probability of ending in LEA. The risk factors for LEA from multivariable analysis in ischemic or neuroischemic DFU were duration of diabetes, uremia, pedal edema, foot deformity, toe pressure <30 mmHg, sign of ischemia such as intermittent claudication and rest pain, multiple ulcers, and male sex (Table 3) [17].

**Table 3** Factors related to LEA in DFU associated with PAD, either pure ischemic or neuroischemic ulcers [17]

| Exploratory variables | Odds ratio | 95% CI | *p* value |
|---|---|---|---|
| Diabetes duration >23 years | 1.88 | 1.15–3.50 | 0.011 |
| Uremia | 2.43 | 1.33–4.45 | 0.004 |
| edema | 2.51 | 1.79–3.54 | <0.001 |
| Foot deformity | 1.69 | 1.08–2.63 | 0.021 |
| Toe pressure <30 mmHg | 1.70 | 1.20–2.40 | 0.003 |
| Intermittent claudication | 1.88 | 1.25–2.82 | 0.002 |
| Rest pain | 2.06 | 1.45–2.98 | <0.001 |
| Multiple ulcers | 2.92 | 1.90–4.49 | <0.001 |
| Non-compliant | 2.15 | 1.26–3.66 | 0.005 |
| Male sex | 1.51 | 1.06–2.15 | 0.021 |

### Ulcer's Characteristics

The ulcer size could be used as the predictor of the outcome in DFU. Smaller wound size is associated with faster wound healing [135], while larger ulcers will require a longer considerable time to heal [48, 136]. Ulcer size was identified as one of the dominant risk factors for predicting LEA resulting from DFU [47, 50, 62, 136]. In the EURODIALE study [48], ulcer size >5 $cm^2$ vs. < 1 cm is an important predictor of poor wound healing (OR 3.88). Two previous studies has reported the positive association between ulcer size to greater possibility of infection take event and longer duration of ulcer associated with a larger foot ulcer and more possibility that LEA would be undertaken [136, 137]. With respect to the depth of the wounds, Armstrong et al. [138] showed that amputation risk was 11-fold higher when ulcer has penetrated to bone. Winkley et al. [47] found that in patients who underwent amputation with their first DFU episode, the depth of the ulcer was the only explanatory factor significantly associated with amputation (HR 3.34, 95% CI 1.17–9.56; $p < 0.001$), excluding those with severe PAD, i.e., ABI < 0.5.

The ulcer's characteristic that is most dramatically associated with a healing failure is increasing wound duration, besides larger size and the grade or severity of the wound [136, 139]. A number of authors have demonstrated that wound chronicity represents a risk factor for subsequent nonhealing DFU and LEA, notably those reported by van Battum et al. [132] and Margolis et al. [140]. Van Battum et al. [132] demonstrated that the ulcer duration of more than 3 months before enrollment have portend an incidence risk of LEA (OR 1.67–2.61) when compared to ulcers with shorter duration. These wounds are prone to infection or colonization due to immunopathy. It is clear that the DFU of longer duration had a greater bacterial diversity with possible resistant microorganism and is associated with biofilm production, which are risk factors for amputation in diabetic foot [54, 139]. In a study of 1666 diabetic patients, ulcers of greater than 30 days duration have an increased risk of clinically relevant infection [141]. However, in the presence of deeper ulcer, thus wound duration and wound size were not indicator of amputation risk [141, 142].

### Diabetic Foot infection

An infected DFU precedes about two-thirds of LEA, and is surpassed only by gangrene as indication for this surgical procedure [6, 7, 143]. Furthermore, infection leads to microthrombi formation, causing further ischemia, necrosis, and progressive gangrene [143]. Massive infection (osteomyelitis and deep-seated sepsis) and wet gangrene are the most common factors leading to amputation [144]. The independent risk factors for LEA were, in decreasing order of HR: positive probe-to-bone test, deep ulcer, elevated CRP levels, and the presence of periwound or pretibial edema. The presence of non-purulent exudate, foul smell, and fever independently also predicted any amputation. Larger ulcer size and presence of PAD were also independent predictors of both minor and major amputation, with HRs between 1.81 and 3 (95% CIs between 1.05 and 6.6) [62]. Patients with the abovementioned characters should be treated aggressively with intravenous antibiotic(s) without the need of waiting for the signs of sepsis to appear.

Type of DFI also determines the prognosis as surgical site infection is reported to have four-times more risk associated with diabetic amputation compared with infected ulcer or cellulitis [89]. Similarly, Chen et al. showed that patients with DFU that are accompanied by necrotizing fasciitis are at high LEA risk and similar to

patients with high-grade DFU [145]. Specific pathogen isolated from culture specimen may predict the outcome of DFU. Gram negative microorganisms are known as antibiotic-resistant microorganisms and carries a higher risk for severe infection with resultant LEA (OR 1.8, 95% CI 1.08–3.02; $p = 0.002$) [139]. The development of a foul odor also indicates worsening infection and may indicate the presence of anaerobes. In a study of DFI conducted by Indian microbiologist, predictors of LEA in patients with DFU are male patients with microvascular complications, associated with PAD, deep ulcer, osteomyelitis, high leukocyte count, have previous antibiotic use, and biofilm production.

Recently, Pickwell et al. [62] utilized IWGDF's classification system to categorize the severity of infection to categorize DFU patients with higher risk for amputation. In comparison with mild infection, the presence of a moderate infection increased the HR for any amputation by a factor of 2.15 (95% CI 1.25–3.71). For severe infection, the HR for any amputation increased to 4.12 (1.99–8.51). It is noteworthy that most patients with DFU do not have leukocytosis [61, 88, 115], which define a severe infection according to IDSA-IWGDF criteria. Therefore one should not depend on white counts alone as a measure of the seriousness of the foot infection, though several authors claimed leukocytosis was associated with the need for amputation [50, 62, 88, 89]. The worst scenario leading to LEA is DFI in the presence of PAD. Patients with infection and ischemia are nearly 90 times more likely to receive a midfoot or higher amputation compared with patients in less advanced wound stages (76.5% vs. 3.5%, $p < 0.001$) [138]. These combinations pose a challenging problem to clinicians because foot ischemia certainly appears to be associated with an increased severity of an infection and the unreliability of healing after the ulceration ensues [48, 77].

### Peripheral Neuropathy of the Foot

There are three different outcomes of peripheral neuropathy on the lower extremity: sensory loss, motor nerve damage, and autonomic response neuropathy [12, 146]. The impact on LEA risk is in large part due to loss of peripheral sensation. An array of measures was used to quantify sensory peripheral neuropathy associated with amputation risk: insensitivity to the 10-g Semmes-Weinstein monofilament (SWM), motor nerve conduction velocity of the sural nerve, vibration perception threshold (VPT), absent or diminished bilateral vibration sensation, and absent Achilles tendon and patellar reflexes [146]. There are nine studies that reported a significant association between one or more measures of peripheral neuropathy and LEA [26, 27, 30–33, 39, 56, 132]. In studies that did not report this association, peripheral neuropathy was not measured directly [29, 34, 57, 69]. Other researchers who found negative association have evidently assume that the effect of neuropathy on LEA risk could be more dependent on the presence of infection and PAD [44, 46–48, 50, 61, 75].

The prediction of LEA by measuring sensory loss using monofilament was included in the study by Carrington et al. [147] who identified foot ulceration, motor nerve conduction velocity, and peripheral nerve and vascular insufficiency as predictors of amputation. They found that monofilament test (OR 5.18; 95% CI 1.96–13.68; $p = 0.001$) was one of the best tests which predicted LEA. In the cohort study by Lehto et al. [31], two measures of peripheral neuropathy were significantly associated with amputation: bilateral absence of Achilles tendon reflex (RR 4.3, 95% CI 2.5–7.3) and bilateral absence of vibration sense (RR 2.7 (95% CI 1.6–4.7). The ability to feel deep vibration was traditionally assessed using tuning forks of 128 Hz, or using biothesiometry to quantify the vibration perception threshold (VPT). The study by Shearer et al. [148] states that reduced VPT cohort had three and a quarter times for LEA than the normal cohort. They suggested that the probability of LEA is dependent on DFU.

Foot ulcers with underlying neuropathy are accounted for 45–60% of patients, while up to 45% have neuropathic and ischemic components [8, 17, 43]. In those who have developed a neuropathic DFU, the most important determinants of risk of amputation are the depth of the tissue involved by the ulcer and the existence of infec-

**Table 4** Factors related to LEA in DFU associated with peripheral neuropathy [17]

| Exploratory variables | Odds ratio | 95% CI | *p* value |
|---|---|---|---|
| Type 2 diabetes | 1.94 | 1.08–3.49 | 0.026 |
| Duration of diabetes 8–15 years | 1.91 | 1.11–3.28 | 0.020 |
| Duration of diabetes 15–23 years | 2.66 | 1.43–4.95 | 0.002 |
| Non-retinal eye disease/ visual impairment | 1.85 | 1.16–2.85 | 0.009 |
| Uremia | 2.62 | 1.39–4.96 | 0.003 |
| edema | 2.07 | 1.40–3.05 | <0.001 |
| Previous amputation | 3.33 | 1.54–7.22 | 0.002 |
| Walking disability | 1.71 | 1.16–2.52 | 0.007 |
| Rest pain | 2.52 | 1.66–3.83 | <0.001 |
| Plantar ulcer | 4.13 | 2.34–7.28 | <0.001 |
| Deep infection | 4.80 | 3.13–7.34 | <0.001 |

tion [27, 31, 32]. In the study by Gershater et al. [17], in the mulltivariate model, deep foot infection, site of ulcer (plantar for foot ulcer, metatarsal head), and comorbidity (non-retinal eye disease, end-stage renal disease, edema, walking disability) were related to minor or major amputation in nonischemic patients (Table 4).

### Foot Deformity

Foot deformities are notoriously common in the diabetic patients with peripheral neuropathy. Lavery et al. [149] reported that patients with foot deformity combined with neuropathy will increase the risk of ulcer by 1.7 times and similar risk to LEA. Charcot's foot is the classic diabetic foot deformity, affecting more than 16% of those with a history of ulcer [94]. The presence of a DFU alone in a person with DM increases the risk of LEA 3.6 – 7 times relative to non-diabetic patients [45, 95], and ulcers concomitantly with Charcot's arthropathy will heighten the risk of LEA by a factor of 12 compared to Charcot arthropathy without ulcer. Foot deformity such as Charcot arthropathy itself does not necessarily pose an escalation of LEA risk unless complicated by an ulcer [95]. Other foot deformities such as hallux valgus, limited metatarsophalangeal and ankle mobility, or calluses are more to do with an increased risk of ulcer, though it also indirectly increases the likelihood of amputation [7, 149].

### Ulcers Location

As far as the location of the foot lesion is concerned, those in the midfoot and heel or hindfoot ulcers heal the worst [60, 150]. In a large cohort study of patients with diabetes, Gershater et al. [17] found that ulcers on the heel of neuropathic origin had higher rates of both major LEA compared with toe(s) and plantar ulcers. Furthermore, heel lesions were also significantly more frequent in patients requiring LEA reamputation than in those who did not (HR 2.69; $p = 0.05$) [151]. The significant predictors of healing of heel ulcers include adequate circulation to the heel area, particularly the posterior tibialis artery [152]. The locations of DFU in a study from Korea were reported to be concentrated in forefoot area in toe(s) (56%), which revealed the highest amputation rate (70%) of all procedures [64]. Monteiro-Soares et al. [59] found that the odds of toe(s) DFU were 2.3 to develop minor, but not major LEA. Perhaps toe(s) are more vulnerable to devitalization; they can rapidly become nonviable, particularly since toe(s) are very small organ with minimal tissue substance. The risk factors for toe(s) amputation were male sex, osteomyelitis, foot abscess, diabetic retinopathy, and those with congestive heart failure [153].

### Previous Foot Problems

A history of ulceration heightens the risk of further ulceration and amputation, and it may be explained that patients with a history of ulceration possess all the risk factors necessary to produce another ulceration [154] and also LEA, see Fig. 5. Following one LEA, there is a 50% incidence of a serious contralateral foot lesion within 2 years and a 50% incidence of contralateral amputation within 2–5 years. Apelqvist et al. [15] found a recurrence rate of DF amputation of 34% after 1 year and 70% after 5 years. This can be explained by the progression of the peripheral neuropathy and PAD and the continuing presence of additional risk factors which lead to the first ulceration. Neuropathy increases the risk of LEA by 1.7-fold, and the risk increases to 12-fold if there is deformity (itself a consequences of neuropathy) and 36-fold if there is a history of ulceration or amputation [138]. A history of a prior foot ulceration is associated with

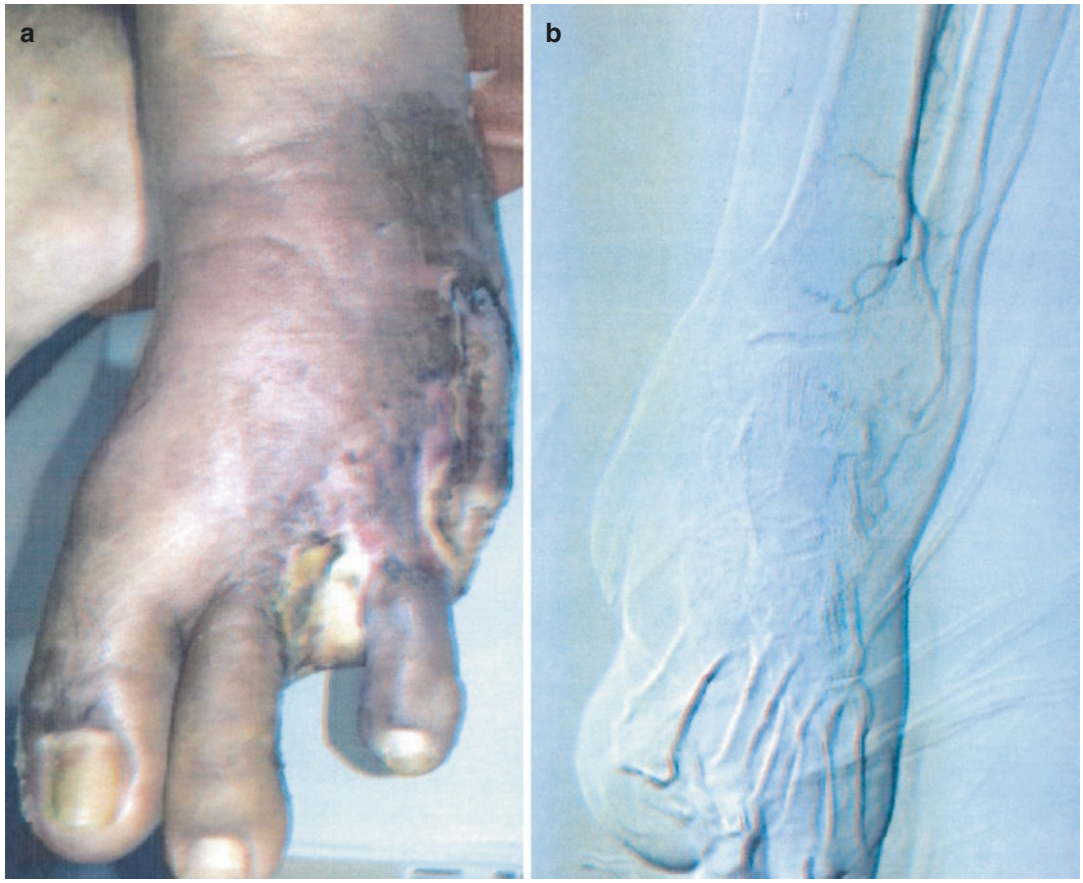



**Fig. 5** (**a**) Compound risk factors in a patient: a 44-year-old diabetic male with osteomyelitis, foot abscess, and prior partial foot amputation. (**b**) Digital subtraction angiogram of the patient, showing minimal flow to foot where both plantar and pedal arch are occluded. Bypass reconstruction is not feasible (Courtesy of Tjokorda Gde Dalem Pemayun, M.D., Ph.D., Dr. Kariadi General Hospital, Semarang, Indonesia)

2- to 10-fold to 30-fold higher risk of amputation [17, 27, 70]. Clearly, this is a risk factor that must be considered in any diabetic foot assessment program.

### 6.2.2 Diabetes-Associated Comorbidities, Other Than Arterial Disease and Peripheral Neuropathy

The risk of diabetic foot disease is associated with comorbid conditions usually related to diabetes mellitus. Associated comorbidities taken into consideration included both systemic and regional conditions likely to impair wound healing or impede interventional measures, as follows: diabetic kidney disease, diabetic retinopathy, and cardio-cerebrovascular disease.

#### Diabetic Kidney Disease

Diabetes is a strong risk factor for chronic kidney disease (CKD), and diabetic kidney disease is an independent risk factor for the development of DFU in this population [70, 155]. Risk factors for foot ulceration are present at all stage, including the earliest stages of nephropathy, for instance microalbuminuria and pedal edema. While foot ulcers are more likely to develop in patients with diabetic kidney disease, they are less likely to heal than are those in diabetic patients without kidney disease [71]. It is well known that impaired renal function is an independent predictor of healing failure and associated albuminuria is reported to increase the likelihood of LEA [9, 16]. Microalbuminuria or proteinuria per se is known to be an independent risk factors for LEA [30, 32, 34, 55, 69].

Association between degree of renal function impairment and LEA has been reported, with patients with moderate (eGFR 30–60 mL/min/1.73m$^2$) and severe CKD (eGFR <30 mL/min/1.73 m$^2$) having a 1.6 and 3.3 times higher risk, respectively, for LEA, compared to patients with mild renal disease [70]. In particular, hemodialysis itself has been proposed as a factor of the utmost importance in the pathogenesis of DFU and amputation. Young et al. reported that the RR for LEA among diabetic patients was the highest among those who started on dialysis [72]. Similarly, Otte et al. [73] reported that major LEA in diabetic patients were more attributed with CKD stage 4–5 (HR 9.5 (95% CI 2.1–43.0, $p = 0.004$) and dialysis treatment (HR 15.0 (95% CI 5.3–71.0, $p = 0.001$).

Diabetic kidney disease and dialysis were found to be predictive factors for LEA in eight studies [17, 49, 60, 72–75, 77, 138]. Interestingly, the increased risk for LEA conferred by renal failure is seen in all ethnic group in the United States [72]. However, some investigators have disputed the predictive role of nephropathy [45, 57, 64, 66]. The risk factors for high LEA rate among DFU patients with kidney disease include history of PAD, peripheral neuropathy, and susceptibility to infection in end stage renal disease (ESRD) [156]. Ndip et al. [156] reported that dialysis patients had a 2–4 times increased odds of having PAD compared to pre-dialysis patients. The majority (64–75%) of dialysis patients have PAD; in contrast, about 12% of patients with diabetes and normal kidney function have PAD. Non-adherence to foot self-care is also an important risk factor for diabetic foot disease in the ESRD population [73]. Dialysis patients lose contact with primary care providers but they typically have several specialists involved in their care. Boersma [157] reported a tenfold increase in the rate of LEA in diabetic with CKD and felt this was able to be reduced through diabetic foot care and prevention program based within the dialysis unit.

#### Diabetic Retinopathy and Visual Impairment

History of retinopathy was assessed in many studies [26–33, 86] and there was a statistically significant association between retinopathy and LEA in each study. Moss et al. [158] in the cohort of retinal vascular changes and 20 year incidence of LEA have concluded that focal retinal arteriolar narrowing may reflect damage to microvasculature, which itself manifest elsewhere in the body as a risk for LEA. In their logistic regression model, each step increase in retinopathy was associated with an OR of 1.15 (95% CI 1.07–1.23; $p < 0.001$). In addition, patients with focal narrowing are at significantly increased risk for a LEA (OR 3.56, 95% CI 1.87–6.76; $p < 0.001$) after controlling for age and sex. Retinopathy may lead to decreased visual acuity, and consequently foot lesions are not observed by the patient at an early stage. Impaired vision in diabetic patients will increase the risk of ulceration and amputation up to one- to sixfold compared to those whose vision is still not disturbed [33, 38, 55]. Retinopathy might reflect the extent of microvascular disease and might also be a proxy for diabetes severity.

#### Macrovascular Complications

The association of amputation with the clinical diagnosis of diabetic complications (coronary artery disease, congestive heart failure, myocardial infarction, or stroke) has previously been described [30, 32, 50, 73]. Many studies have revealed that patients with DFU were more likely to have at least one macrovascular complication. Markowitz et al. [77] using a claim database from private and government health insurance have showed that a high number of comorbid conditions (Carlson comorbidity score ≥4) had an additional influence on the 1.9- to 2.7-fold likelihood of amputation. This fact may suggest that an overall disease burden may affect ulcer healing process and need also to be factored into treatment. Among people with diabetes who developed foot ulcers, those with end-organ disease should receive intensive surveillance and preventive care to decrease or eliminate their risk of amputation.

### 6.2.3 Patient's General Characteristics

#### Patient's Height

Insensate neuropathy is partially determined by peripheral nerve length, which is a function of

height. Since taller diabetic patients are at greater risk of peripheral sensory loss than shorter patients [159], they are also at greater risk of foot ulcer and subsequent LEA. In Taiwan [160], a telephone survey model was used to see if patient's height was associated with the prevalence of LEA. In the sample size of over 93,000, this group reported that every 10 cm increment in height in the population increased the risk of LEA (OR 1.16, 95% CI 1.03–1.32; $p < 0.05$). When height was combined with other known risk factors such as high serum glucose and cholesterol level, the adjusted risk increased to a staggering 79% per 10 cm height and the risk of LEA increased by a factor of 1.8. Height has been previously reported as an independent predictor among patients with diabetes, and Asians in general have a lower body height (1.63 m) and has attenuated the HR for LEA as compared to their blacks, Latinos, and Western counterparts (heights: 1.71, 1.66, and 1.71 m, respectively; $p < 0.001$) [84].

In FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study [83], greater height was an independent predictor of amputation during 5 years of prospective follow-up. The FIELD Study randomized 9795 type 2 diabetes patients aged 50–75 years in double-blind fashion to 200 mg/day of micronized fenofibrate or placebo. Every 10-cm increment in height was independently associated with an adjusted 16% increase in prevalent lower-limb amputation. The explanation for this now-confirmed link between height and amputation risk in diabetic patients is unclear. Of course, height is a non-modifiable risk factor. But the FIELD findings [83] together with the earlier Taiwanese study [160] suggest there may be particular value in aggressively targeting taller diabetic patients for closer monitoring to promote early detection and prompt treatment of foot ulcers in an effort to avoid diabetic amputations.

### Gender

Some studies found that male sex category is a predictor for LEA after foot ulceration, both in type 1 [40] and type 2 diabetes [3, 29, 43, 55]. Male sex has been associated with a 2.8- to 6.5-fold higher risk of amputation in most studies of people with type 2 diabetes. Moura Neto et al. [43] found that men are at increased risk (OR 3.44, 95% CI 1.80–6.56; $p < 001$) of LEA, even after controlling for height and other clinical variables. The reason for male preponderance can be simply that men are more likely to have some of the independent predictors for LEA, such as ulceration, PAD, cigarette use, and peripheral neuropathy. However, it may also reflect that diabetes severity at the time of diagnosis is worse in men than in women, and younger at the time of amputation than women regardless of the level of amputation [161]. Women are known to be more active in preventive foot care whereas men have a more passive attitude, which to some extent may explain the gender differences [162]. Another important aspect is that females may have more efficient wound healing due to the wound healing properties of estrogen receptor beta, whereas androgens are implicated to be detrimental to wound healing [163].

### Older Age

Normal aging process is a predisposition to specific diabetic complications and other comorbid conditions [3]. Older adults with diabetes have the highest rates of major LEA in Western countries. The mean age was just older than 60 years [43, 48, 52, 70, 82, 133] and diabetes duration was over 12–15 years [133]. It occurs in a relatively younger age in developing countries [61, 75]. Two studies that reported on age as a risk factor had contradictory findings. The age group with increased risk was identified at 45–54 years by Chen et al. [164]. Another study that included age was by Trautner et al. [165] who compared diabetic with nondiabetic using surgical departments of seven German hospitals. Using logistic regression to estimate the OR associated with diabetes, age, and sex, they found that amputation risk increases with increasing age and that the OR was higher in younger diabetic women. The FIELD study found that [83], in addition to height, prior skin ulcers, previous amputations, neuropathy, PAD, and older age were important predictors of LEA risk (HR 1.3 per each 5 additional years after 50 years old, $p < 0.001$). Although age appears to be a risk factor for LEA in diabetic patients, it is often compounded by diabetes duration [26, 28].

### Body Mass Index

Increased incidence of foot ulcers in diabetic patients with raised BMI is generally observed [166]. Increase in weight can also affect the foot ulcer by increasing the pressure on the foot [167]. Keeping all other factors constant, overweight diabetic patients having BMI of 24.5–29.5 kg/m$^2$ are at four times higher risk to develop ulcer than diabetic patients with normal BMI (16.5–24.5 kg/m$^2$) [168]. As such, diabetic foot presentation could be changing as individuals gain weight. Each 1 kg/m$^2$ increase in BMI was associated with a 1.20-fold higher change of amputation in patients with DFU (95% CI 1.03–1.41; $p = 0.02$) [58]. Pinzur et al. [169] found strong association between diabetes-associated foot morbidity and morbid obesity; however, a study by Sohn et al. [166] reported paradoxical decrease in LEA risk with BMI > 40 kg/m$^2$ and increased risk with lower BMI. Compared to those with BMI 25–29.9 kg/m$^2$, patients with BMI <20 kg/m$^2$ were 1.9-fold more likely to have any LEA. Similarly, the high rate of amputation in the leanest Europeans (BMI < 21) by Chaturvedi et al. [170] was probably due to subclinical disease resulting in a low BMI and high risk of complications and death. The role of body mass in LEA risk after DFU is complex as this characteristic may also be related to personal nutritional status and functional ability.

### Race/Ethnic Background

Ethnicity might have an effect on amputation. Most reports have identified an increased risk of suffering a diabetes-related LEA in native Indian and African-American populations. American Indians have one of the highest rates of diabetes-related LEA in the world, both in type 1 and type 2 diabetes [29, 85]. There were diabetes duration, poor glucose control, male gender, the presence of microvascular complications (retinopathy and nephropathy), and dyslipidemia, which increased amputation risk of DFU and LEA in this population [26, 29, 69]. In African-American population, Lavery et al. [171] found a 2.4-fold increased risk of LEA, as compared to non-Hispanic Whites patients. A 1.5- to 2-fold higher risk for LEA has been described for Hispanics and blacks as compared to Whites. However, there were studies [72, 87] that suggested that patients with lower socioeconomic status and minorities with diabetes, particularly African-American, are more likely to have DFU and LEA than patients with higher socioeconomic status and non-Hispanic Whites. The idea of an equal access was supported by several authors [30, 36, 99] who found no differences in LEA rates among African-American, Whites, and Hispanics living in the USA and UK, when they had no identified financial barriers, comparable access to healthcare and well educated.

Young et al. [72] compared nearly 430,000 diabetic war veterans in the USA who were stratified into ethnic groups (Asian, Native American, Hispanic, and White). The authors believe all veterans had equal access to healthcare. In this study, Asians were more likely to have toe amputations compared with Whites or other ethnicities, while Native Americans were more likely to have below-knee amputation (RR 1.74, 95% CI 1.31–2.18) followed by African American (RR 1.41, 95% CI 1.34–1.48), then Hispanics (RR 1.28, 95% CI 1.20–1.38) when compared with Whites. Ethnicity was found to be independent of renal disease, COPD, cardiovascular disease, and hypertension in this cohort study.

In a study by Chaturvedi et al. [37], diabeticians were assigned to four ethnic groups based on physical appearance and parental origin (European, South Asian, African-Caribbean, and other/unknown); people of mixed origin were placed in the "other" category. South Asian had poorer glycemic control, but better blood pressure, less neuropathy, less PAD, more smokers, and fewer foot ulcers than Europeans. Of interest, South Asian had a higher rate of dyslipidemia and cardiovascular disease yet still some were protected from LEA than European counterparts. Even among immigrants in the New Zealand [79] or the USA [72, 84], a lower risk of LEA has been reported among patients with Asian origin. A lower rate of LEA among East Asians ethnicity was found to be associated with lower prevalence of PAD, lower body height [160], and better skin microcirculation [172] as compared to their Western counterparts. Ethnicity was not found to

be associated with LEA in Singapore [75] and Malaysian study [44, 88], which also included a multiracial population. Another study that was also conducted in Singapore found that patients of Melayu ethnicity were more likely to have LEA as compared to Chinese or Indian population [78].

### 6.2.4 Socioeconomic, Foot Care Provisions and Environmental Factors

Although poor glycemic control, peripheral neuropathy, PAD, and infection were major components on DFU pathophysiology, there are several other characteristics that may vary among patients which causes the outcome of each DFU case to be different. Differences in unmeasured clinical risk factors, socioeconomic disadvantages, cultural and behavioral factors, including preventive foot care, access to healthcare, poor delivery of care to some ethnic group, genetic predisposition, or other factors are about to be discussed. These variables are likely intermediary in the pathway to LEA and a proxy of other condition that drives the patients to present late for clinical care and modulate the likelihood of being served by endocrinologist and podiatrist so diabetes is often uncontrolled and foot presentation even worse.

#### Healthcare System

The prevalence and outcome of DFU are partly influenced by the quality and availability of healthcare service. It has been suggested that the occurrence of diabetes-related LEA is partially attributed to a failed system of healthcare [173], a problem of many developed countries three decades earlier prior to the advancement of understanding and integrated care in diabetic foot problem. Some literature reports that the risk of amputation is higher in some countries where management of diabetic feet has not been a priority. In addition, the quality of care provided to these high-risk groups is likely to be poorer, the so-called inverse care law [173]. These include the unavailability of a universal coverage insurance system for all residents, in case of developing nations, to obtain health facilities and thus obtain good healthcare [174]. Visit frequency, provider practice, comorbidity, and other discrete measures reflecting parameters of healthcare and health history have been found to be significant protective or risk factors in experimental and analytic studies [175, 176].

#### Foot Care and Prevention Strategies

Harwant et al. [41] in Malaysia reported that risk factors for LEA in their series were low educational level, manual occupation, lower income group, and those with poor foot care practice. Footwear-related trauma may be the most common pivotal event leading to LEA [80]. Unsuitable shoes were associated with an OR for amputation of 5.5 (95% CI 2.91–7.76; $p = 0.001$), showing that the likelihood of amputation in people who did not have proper shoes was higher than in those who had suitable shoes [58]. Non-compliant to follow given prescription regarding medical treatment, to use off-loading equipment, and to attend the clinic are also found as risk factors for LEA [17]. Therefore, the lack of such education guidance of people with diabetes mellitus is a risk factor for the development of ulceration and subsequent amputation of the lower extremity.

Patient education has been proven to be very effective in the prevention of the diabetic foot and may convey a long-term protective effect by altering behaviors such as regular foot inspection. Only one randomized controlled study has been conducted on foot education as the sole intervention. Veterans from a high-risk foot clinic were randomized to "usual education" or a 1-h slide lecture showing ulcers and LEA followed by a simple, one-page instruction sheet to take home. After 2 years, people receiving the educational session had a threefold decrease in ulceration ($p < 0.005$) and amputation rates ($p < 0.0025$) [177].

A low-income African-American population was studied to assess the effectiveness on LEA program by Patout Jr. et al. [81] in Louisiana, USA. Analysis of the data showed a reduction in foot-related ulcer day (−49%; $p < 0.01$), emergency room visit (−81%; $p < 0.01$), hospitalization (−89%; $p < 0.01$), foot operations (−87%; $p < 0.01$), and ultimately LEA (−79%; $p < 0.01$)

using established program compared to standard foot care. The other prospective case-control studies have shown an up to threefold decrease in the incidence of LEA when an intensive educational program was applied [26, 178].

### Socioeconomic Status

The health cost for diabetes management and DFU are high. Included were cost of inpatient and outpatient care, diabetes medications, skilled nursing facilities, home healthcare, timely medical intervention, and all have impact on the probability of a LEA [21]. It is unreasonable to suggest that monetary influences do not deleteriously affect patient with DFU. There are clear indications that poor socioeconomic status, measured by lack of education, low income, or occupation, is a strong predictor of LEA [21, 35, 41]. An association of healthcare coverage with lower rates of microvascular complications has been demonstrated [179]. No health insurance also turns out to be an amputation risk factor when associated with DFU treatment costs. The highest rates of amputation occur in those who do not have health insurance [22]. More attention needs to be paid to addressing DFU especially among patients from lower socioeconomic group, during all contact with healthcare from the early stages of foot ulcers to vascular treatment and rehabilitation [180].

According to a study conducted by Wachtel et al. [181], the central nonclinical risk factors for LEA in age +50 African American, Hispanic American, and others were attributed to family poverty. The influence of poverty, low income, education level, and occupation may have accounted for some of the dietary, self-care, and healthcare differences. A study from Finnish Hospital Discharge Registry [87] reported that low socioeconomic status is associated with a risk of LEA in patients with diabetes and their amputation is more likely to be major, leading to more severe disability. Using logistic regression, HR for highest socioeconomic group was 0.46 ($p < 0.01$) compared to the lowest group, which means that the lowest group have more possible disadvantages associated with resources: less educated, a lack of access to adequate primary care or vascular surgery, delayed PAD diagnosis, as well as cultural distrust.

Selby et al. [30] found no difference in risk due to ethnicity and suggested that previously observed differences may be due to socioeconomic differences or a lack of access to healthcare. In a setting in which 3 million members were enrolled in a prepaid medical care organization, LEA risk was not significantly different by ethnic and racial group [27, 30]. Similarly, in a case-controlled study among veterans having equal access to healthcare, after controlling for the socioeconomic factors, there were no differences in LEA rates among Blacks, Whites, and Latino [84]. It indicates that controlling these extrinsic factors may prevent amputation.

Marital status represents another variable in the set of social factors for amputation risk; the effect of this variable has been exploited in studies notable by three authors [27, 39, 76]. The lack of social connectedness, defined as living alone, no visits from a friend or relative in the past month, no attendance at social or religious gatherings, and personnel life dissatisfaction, was reported by one study to be associated with a 1.9- to 3.8-fold higher risk of amputation. Family support is highlighted in foot care to prevent complications.

### Access to Healthcare Facilities

Treatment for DFU requires access to care. A study of diabetic foot care by Reid et al. [67] in a rural community in Northern Canada showed low rates of foot screening examinations and corresponding to high rates of hospitalization with diabetic foot complications. Residence in rural settings correlated with shorter time from initial clinic visit to major LEA [68]. Gallagher et al. reported that those who require LEA reside at a greater distance (OR per km was 1.01 (95% CI 1.00–1.02)) from the diabetic center than patients who have not [182]. Though not necessarily interpreted as poor access, distance may correspond to a delay in diagnosis and appropriate management and higher amputation rates. The distance from the health center can be a factor for

a person reluctant to seek treatment, causing diabetes and other risk factors to be overlooked, and tend to lose schedule to clinic. Missing clinic appointment has been found to be associated with poor glycemic control [17, 99] and increased risk of LEA due to diabetic foot complications (OR 3.84, 95% CI 1.54–9.52; $p = 0.003$ [90].

## 7 The Use of Established Risk Classification System to Predict Amputation

DFUs are generally evaluated using the following tools of clinical assessment [180]: (1) the Wagner classification; (2) University of Texas (UT) score; (3) S(AD) SAD system; (4) site, ischemia, neuropathy, bacterial infection and depth (SINBAD) system; (5) IDSA-IWGDF classification; (6) depth of the ulcer, extent of bacterial colonization, phase of ulcer and association etiology (DEPA) scoring system; (7) van Acker-Peter classification; (8) diabetic ulcer severity score (DUSS; (9) the Curative Health Service (CHS) system; (10) Margolis et al. classification; (11) the Wound, Ischemia, and Foot Infection (Wifi) classification; and several others. Both retrospective [64] and prospective investigation [59] have shown that some of those classification systems show good accuracy in predicting LEA in DFU patients. The results showed that the prediction of all LEA through DFU classification system had sensitivity values ≥80% and negative likelihood ratios ≤0.5 for the highest risk group each. The area under the curve (AUC) ranged from 0.56 to 0.83 and the positive likelihood ratios from 1.0 to 5.9. Ultimately, wound grade at the initial visit was strongly associated with the likelihood that an individual with DFU will have an LEA [141].

The International Working Group on the Diabetic Foot (IWGDF) has developed a PEDIS classification system that has recently been restructured. These criteria were: the degree of limb ischemia (=Perfusion (P) segment), depth and surface area of the wound (=Extent/size (E) and Ulcer's depth/tissue loss (D) segment), severity of sepsis (=Infection (I) segment), and the presence of peripheral neuropathy (=Sensation (S) segment) [183]. Widatalla et al. [184] conducted a prospective cohort study incorporating this classification system to classify more than 2000 patients with DFU in Sudan, Africa. They found that higher grade of every aspect of the PEDIS assessment may predict risk of subsequent LEA after hospitalization. Critical limb ischemia (Perfusion grade 3) was found to be the most significant risk factors for major LEA (OR 5.08, 95% CI 2.56–10.07; $p < 0.001$). Deep ulcers were significantly associated with minor LEA (OR 3.45, 95% CI 2.23–5.88; $p < 0.05$). Grade 2 sensory neuropathy was found associated with deep ulcer penetrating down to bone and significantly predict minor (OR 2.43, 95% CI 1.32–3.5; $p = 0.002$) and major LEA (OR 2.43, 95% CI 1.08–5.45; $p = 0.027$). Foot risk classification of the IWDGF can predict ulceration and LEA, and can function as a tool to guide prevention of lower extremity complications of diabetes.

## 8 Concluding Remarks

Studies have looked at various risk factors that contribute to LEA in patients with DFU, either in type 1 or type 2 diabetes. These risk factors did not appear to differ from studies to studies, and they can be broadly categorized into metabolic and non-metabolic risk factors (summarized in Table 5). Some risk factors have strong evidence such as male sex, poor glycemic control, diabetes duration, PAD, peripheral neuropathy, severe foot infection, previous foot problems, and diabetic kidney disease, particularly in those ESRD patients who are on dialysis treatment. The other documented risk factors are less consistent such as smoking and body mass. Each risk factor has its own weight on the problem and different contribution to the overall risk of LEA, for example, DFU with PAD have higher risk of LEA compared to neuropathic DFU. The severity of the DFU is the strongest predictor of LEA.

Once one has a DFU, it is perhaps not possible to reverse some of the risk factors such as with

**Table 5** Summary of risk factors for LEA from selected publications

| Metabolic risk factors | Estimated risk | Non-metabolic risk factors<br>Intrinsic (foot-related) factors | Estimated risk |
|---|---|---|---|
| *Diabetes health history* | | Lower extremity arterial disease [26, 27, 31–33, 39, 46, 48, 49, 52, 55–57, 61, 64, 69, 70, 77, 82, 90, 131, 133] | 1.8–55.8 |
| Type of diabetes | | Cutaneus circulation [27] | 7.5–161.0 |
| Type 1 (reference = type 2) [27, 34, 90, 125] | 1.7–3.1 | Medial arterial calcification [26, 69, 132] | 4.8–6.6 |
| Type 2 (reference = type 1) [17, 34, 47] | 1.9–3.5 | Ulcer's size and extension [47, 48, 135, 136, 138] | 1.3–2.6 |
| Long standing diabetes [17, 26, 30, 31, 38, 44, 52, 79, 95, 125, 140] | 1.8–63.1 | Depth of the ulcer [46, 47, 50, 54, 133] | 3.6–14.4 |
| Poor glycemic control [27, 30, 34, 52, 61, 70, 74, 79, 86] | 1.7–20.4 | Ulcer's duration [48, 138] | 1.3–2.6 |
| Use of insulin [29, 39, 42, 86] | 1.2–2.5 | Diabetic foot infection [17, 27, 39, 42, 48, 62, 89, 133, 142] | 1.5–154.5 |
| Non-compliant to diabetes treatment [17, 97] | 1.2–2.1 | Osteomyelitis [50, 54] | 3.7–4.5 |
| | | Peripheral neuropathy [26, 27, 30–33, 39, 56, 131] | 4.0–15.5 |
| *Atherosclerosis risk factors* | | Foot deformity [17, 32, 45] | 1.7–3.6 |
| Hypertension [30, 32, 35, 38, 54, 61, 69, 125] | 1.1–3.6 | Previous foot problem [17, 49, 70, 72, 89, 177] | 3.3–30.3 |
| Any lipid abnormalities [32] | 2.4 | Ulcer's location | |
| Total cholesterol [26, 29, 31, 54] | 1.8–3.7 | Forefoot [59, 64, 152] | 2.2–2.3 |
| LDL-cholesterol [69] | 9.9 | Hindfoot (heel) [17, 60, 149] | 6.1 |
| Low HDL-cholesterol [27, 105] | 1.9–4.9 | Plantar [17] | 4.1 |
| Hypertriglycerides [29, 54, 61] | 2.5–5.5 | Gram negative infection [140] | 1.8 |
| Smoking habit [35, 50, 58, 79] | 1.4–3.4 | Biofilm production [54] | 4.5 |
| Hypoalbuminemia [44, 53, 89] | 1.8–2.5 | | |
| Anemia [41, 50, 53, 60, 66, 71] | 1.8–5.5 | Non-metabolic risk factors<br>Intrinsik (Non-foot-related) | |
| *Acute phase reactans* | | *Diabetes-associated complications* | |
| C-Reactive Protein [50, 63] | 5.2 | Diabetic kidney disease [9, 17, 26, 32, 49, 60, 71, 72, 75, 77, 78, 95, 138] | 2.2–3.1 |
| Erythrocyte sedimentation rate [50, 64, 140] | 1.3–3.8 | Proteinuria [28, 31, 32, 34, 55, 69] | 1.3–13.6 |
| Neutrophil-to-lymphocyte ratio [120] | Not stated | Dialysis [60, 70–72, 74] | 7.8–15.6 |
| Fibrinogen [69, 121, 122] | Not stated | | |
| *Other metabolic factors* | | Diabetic retinopathy [35, 26, 29–31, 34, 45, 55, 86, 140] | 2.1–6.8 |
| Zinc [27] | 5.1 | Visual handicap [29, 33, 38, 55] | 1.8–6.9 |
| Bilirubin [127] | 1.3 | Coronary artery disease [32, 33, 50, 72] | 1.5–6.9 |
| Vitamin C deficiency [27] | 2.1 | Post-myocardial infarction [32, 45, 55] | 2.5–7.5 |
| Uric acid [33] | Not stated | Heart failure [32, 33, 48, 95] | 2.0–4.6 |

| Metabolic risk factors | Estimated risk | Non-metabolic risk factors<br>Intrinsic (foot-related) factors | Estimated risk |
|---|---|---|---|
| Non-metabolic risk factors | | | |
| *Patients' general characteristics* | | *Socioidemographic and environmental factors* | |
| Height [79, 125] | 1.3–1.7 | Distance from diabetic centre or living in rural community [67, 180] | 2.5 |
| Male sex [17, 29, 32, 33, 43, 52, 55, 58, 79, 140] | 1.5–6.5 | Limited access to healthcare facilities [30] | Not stated |
| Older age [43, 48, 52, 70, 82, 89, 133, 165] | 1.7–3.2 | Lower socio-economic status [46, 79] | 1.5 |
| Race/ethnic background (reference = Whites) | | Lack of outpatient education [27, 30, 41] | 3.2 |
| Native Indian American [71, 85] | 1.7–3.8 | Formal education <12 years [35] | 2.1 |
| African American [42, 71, 171] | 1.4–1.7 | Missing clinic appointment [17, 183] | 1.4–2.1 |
| Hispanic [42, 71] | 1.2 | Infrequent HbA1c screening [58] | 13.9 |
| Body mass index | | Marital status as single or widowed [27, 39, 76] | 2.1–3.7 |
| BMI ≥ 24.5 kg/m$^2$ (reference = BMI < 24.5/kg/m$^2$) [58] | 1.2 | Social deprivation [27] | 1.9–3.8 |
| BMI < 20/kg/m$^2$ (reference = BMI 25 kg/m$^2$) [166] | 1.9 | Lack of suitable shoes [39, 58] | 1.8–5.5 |
| | | Poor foot care practice [39, 41] | Not stated |

peripheral neuropathy. Treatment of metabolic risk factors from early is tantamount to overall effort for limb saving, while waiting until neuropathy, PAD, or diabetic foot and ulceration have occurred is very risky. The other non-modifiable risk factors are diabetes duration, older age, male gender, and associated chronic complications. Now, researchers are claiming that one risk factor for amputation may not be modifiable at all: the taller the patient is, the higher the amputation risk. Nonclinical factors such as healthcare system, socioeconomic status, and poor care provision also make a contribution to (1) medical access, (2) clinical risk factors and foot presentation, and (3) health literacy and numeracy. Race or ethnic background may also play an important role, though social status, education, and lack of access to health facilities clearly influence the overall outcomes.

Certain biochemical factors may also be used to identify "at-risk" for LEA in this population: anemia, low albumin, low Zinc and vitamin C level, as well as low bilirubin serum, and high CRP, high neutrophil-to-lymphocyte ratio, and hyperuricemia. Some genetically risk factors such as Lp(a) and hyperhomocysteinemia have also been reported to display contributing factors to poor DFU outcomes. Further research is needed to verify some of these new findings.

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# Factors Maximizing Skin Flaps and Grafts for Diabetic Wound Coverage

Ryan Donegan

## 1 Introduction

Lower extremity wounds are costly and problematic for today's society. Healthcare providers who deal with these problems on a daily basis understand the complex nature of wound care and reconstruction. A broad spectrum of disciplines and vast knowledge in those disciplines are required to best serve this population. This chapter looks at how wound care and limb preservation physicians can effectively treat lower extremity wounds, with special emphasis on diabetic wound closure strategies. The chapter addresses revascularization, infection, and inflammation control of wound beds, and skin flaps and grafts.

It is clear that diabetes mellitus has a global impact. The International Diabetes Federation has estimated that the incidence of diabetes in the world in 2013 was 382 million people, predicting an increase to 592 million people by the year 2035 [1]. In this population, the prevalence of lower extremity ulcers ranges from 4 to 10% with an annual incidence of 2–3%, and a lifetime risk of 15–25% [2]. Although representing only 6% of the population, patients with diabetes account for 46% of the 162,000 hospital admissions for foot ulcers annually. Approximately 15% of diabetic foot ulcers will result in lower extremity amputation, with more than 85% of the lower extremity amputations in diabetic patients precipitated by a foot ulcer. Diabetic patients have a 15–46× greater risk of amputation than nondiabetic patients, and a 5-year. survival rate after amputation of less than 50% [3]. For nontraumatic minor and major amputations of the lower limb, ulceration is the greatest contributor [4]. The cost of treating leg ulceration is staggering. Epidemiologic studies from Sweden estimated annual costs for treatment of lower extremity ulcers at $25 m. In England, the estimated cost of care for patients with leg ulcers in a population of 250,000 was $130,000 annually per patient [5]. Items factored into the equation include physician visits, hospital admissions, home health care, wound care supplies, rehabilitation, time lost from work, and jobs lost. The initial cost of these lower extremity ulcerations is further impacted by the chronic nature of these wounds, high rate of recurrence, and propensity of these ulcerations to become infected; 40–80% develop a superimposed infection, a costly complication. These statistics are staggering, clearly showing that diabetes and ulcerations have a significant impact on the population and healthcare system, and why fast effective closure of these wounds is so critical.

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The pathophysiological mechanisms underlying diabetic foot disease are multifactorial and include neuropathy, infection, immunopathy, and ischemia. In addition, the genesis of diabetic pedal ulcerations has four specific foot-related risk factors: altered biomechanics, limited joint mobility, bony deformity, and severe nail pathology [3, 6–9]. It is therefore not surprising that the management of the diabetic foot is a complex clinical problem requiring a multidisciplinary approach to achieve limb salvage. This triad of vasculopathy, neuropathy, and immunopathy not only leads to pedal ulcerations, but also increases the susceptibility to soft-tissue and osseous infections which can ultimately lead to amputation, loss of limb, and at times loss of life. That is why the restoration of an intact skin barrier is of utmost importance to prevent a portal of entry for infection, ideally in a manner that minimizes wound contraction to maintain function and minimize cosmetic disfigurement.

Knowledge of the clinical picture, pathogenesis, relevant diagnostic tests, and treatment modalities is essential in planning the optimal strategy for approaching diabetic ulcers. An incorrect or a delayed initial diagnosis may increase the risk of serious complications, including permanent disability and amputations. This is why in 2004, Boulton et al. [10] developed a Clinical Practice article for neuropathic diabetic foot ulcers published in *The New England Journal of Medicine*. They concluded: "The failure to reduce the size of an ulcer after 4 weeks of treatment that includes appropriate debridement and pressure reduction should prompt consideration of adjuvant therapy." When determining the proper adjuvant therapy, the reconstructive ladder developed by Attinger should be utilized [11]. This reconstructive ladder drives how wounds and their closure are approached. The success of the reconstructive ladder is dependent on proper preparation of the wound, and this preparation will be much of the focus of the remainder of this chapter.

## 2 General Reconstructive Ladder

The goal of wound healing is to obtain the best closure through the least morbid means. In the surgical treatment of the diabetic foot and ankle, the reconstructive foot and ankle surgeon is tasked with the challenge of repairing a variety of tissue defects. The decision for wound closure method depends on the location of the wound and patient factors (i.e., tissue extensibility and the individual's healing potential). In order of increasing complexity, the clinician should consider the reconstruction algorithm seen in Table 1, as set forth by Attinger and Janis [12]. The reconstruction ladder is built on the concept of utilizing the lowest level that still allows for closure of the wound. The progression is as follows: primary closure, closure by secondary intention including application of various wound care products, application of a negative pressure wound vacuum system, skin grafting, application of dermal matrices, local random flaps, distant flaps, tissue expansion procedures, and when no other options are available free tissue transfers of fasciocutaneous or myofasciocutaneous flaps, island flaps [12–23].

Wound evaluation coupled with the knowledge of various closure techniques and their indications will arm the surgeon with the tools

**Table 1** New reconstructive ladder with modifications from previous model

| Type of closure | Morbidity |
|---|---|
| Free flap | Most morbid |
| Tissue expansion | – |
| Distant flaps | – |
| Local flaps | – |
| Dermal matrices | – |
| Skin graft | – |
| Negative-pressure wound therapy | – |
| Closure by secondary intention | – |
| Primary closure | Least morbid |

From bottom up there is a relationship between ease and least morbid to most difficult with morbidities after previous rungs have been attempted

*Adapted from* Janis JE, Kwon RK, Attinger CE. The new reconstructive ladder: modifications to the traditional model. Plast Reconstr Surg 2011;127(Suppl 1):205S–12S

for a successful closure. This reconstruction ladder is the basis for all wound closure, and should be used for guidance based on the clinical situation to provide the least morbid means of closure, and refrain from jumping to the most morbid treatment available.

## 3 General Principles for Success in Flaps and Grafts

Planning is the most important step in successful wound closure, and should be done before any incision is made, including an excision of the initial ulcer that is paramount. Considerations of general health, blood supply, infection status, and atraumatic technique are critical. Simple closure of these wounds is often difficult because of preexisting bone deformity, tissue inelasticity, location of the defect, and superimposed osteomyelitis. Clinical pathways related to diabetic foot ulcers frequently involve persistent sharp debridement, expensive wound care products, long-term intravenous (IV) antibiotics, total contact casting, use of skin equivalents, electrical stimulation, multiple orthopedic offloading devices, and even amputation. External factors, such as compliance, should be evaluated and never underestimated.

Wounds are often allowed to granulate, contract, and heal by secondary intention. When these wounds occur on the plantar aspect of the foot, they frequently recur since the resulting scar has decreased extensibility and mobility. Attempted primary wound closure of diabetic pedal defects is frequently unsuccessful and may be a sequela of inadequate wound assessment, lack of proper evaluation of comorbidities, and an inadequate treatment plan. Reconstructive surgery has traditionally been performed on select patients with severe deformities that cannot be accommodated by custom footwear (Fig. 1). However, recently some authors have dispelled the unfounded fear of performing surgery on diabetic feet and stressed the importance of proactively addressing underlying bony pathology early in the treatment of diabetic pedal ulcerations (Fig. 2) [24–26]. Reconstructive surgery

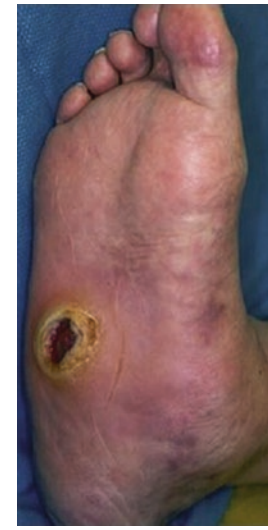

**Fig. 1** Chronic ulcer with underlying osseous deformity

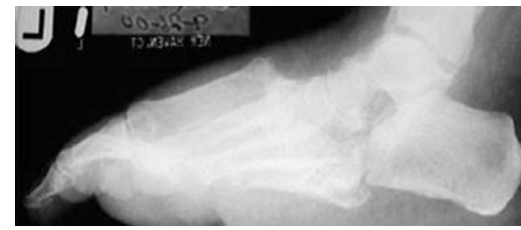

**Fig. 2** Radiographic image of rocker bottom foot with increased propensity to pressure ulcer formation

can range from simple metatarsal head resections to subtotal calcanectomies and Charcot osteoarthropathy reconstructions (Fig. 3). In many cases the planned soft-tissue reconstruction can be planned in concert with osseous reconstruction, eliminating the need for additional incisions often deemed necessary to gain access to a forefoot, midfoot, or rearfoot bony defect.

## 4 Patient Factors

### 4.1 Optimizing Patients

As the number of chronic illnesses increases, the workup and planning phase for wound closure become ever more complicated, making it evermore important to conduct a full history and

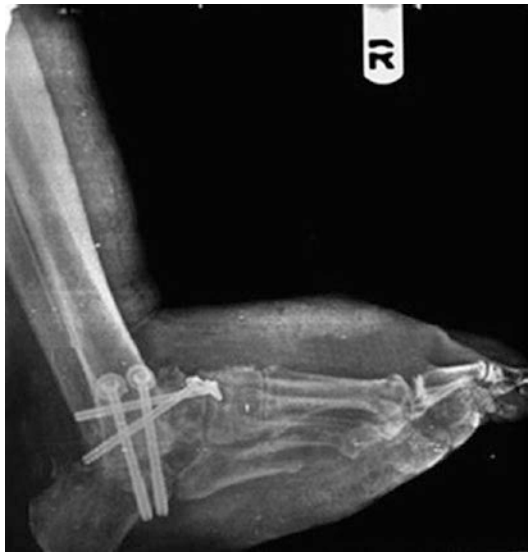


**Fig. 3** Post-reconstruction of Charcot deformity, to eliminate underlying osseous deformity with propensity for ulceration

physical. Lower extremity disease, including peripheral arterial disease, peripheral neuropathy, foot ulceration, or lower extremity amputation, is twice as common in patients with diabetes compared to those without. In addition other comorbidities, such as hypertension and hyperlipidemia; chronic or acute anemia; end-stage renal disease; active infection; history of coagulation abnormalities, including protein S deficiency; age of the patient; musculoskeletal limitations; and use of tobacco, should be addressed preoperatively and optimized [27]. Patients taking medications that affect coagulation, such as aspirin, warfarin, or heparin, must be appropriately managed. Many systemic medications, such as corticosteroids and chemotherapeutic and immunosuppressive drugs, may interfere with wound healing, and must be addressed before surgery [3, 28]. The lower extremity must be assessed for vascular and neuropathic risk factors. Positive findings of vascular insufficiency may require further consultation. The indications for vascular consultation include an ankle brachial index of less than 0.7, toe blood pressure less than 40 mmHg, or transcutaneous oxygen tension (TcPO2) levels less than 30 mmHg; these measures of arterial perfusion are associated with impaired wound healing [3]. If inadequate perfusion is found, a vascular surgery consultation should be sought to determine the need for interventions to increase perfusion. Infections must be eliminated before flap and graft reconstruction. With respect to patient age, one classic analysis of patients undergoing nonsensate free-flap coverage of weight-bearing portions of the foot found good results in 70% of all patients. In contrast, 92% had good or excellent results in patients who were 40 years of age or younger [29]. Finally the patient's occupation and capacity to deal with lost time from work, including personal economic situation, need consideration. The patient's expectation regarding the surgical outcome, possibility of additional surgeries, and risks such as amputation should be discussed in order to fully inform patient to allow a comprehensive decision process concerning limb salvage. In the following sections we focus on the more common and important factors to address prior to grafting and flapping in order to maximize successful closure, including biomechanics, immune system, and vascular disease.

## 4.2 Biomechanics

Altered biomechanics and limited joint mobility are some of the mechanical foot-related risk factors that have been identified in the genesis of pedal ulcerations [30]. In the majority of diabetic ulcerations, neuropathy plays a major role. Neuropathy affects the foot along two pathways, somatic and autonomic nerve fibers. Autonomic involvement affects hydration and nutrient delivery to skin making it more susceptible to breakdown, and that is why a regimen of moisturizing is required to protect the skin after grafting closure [31]. Motor neuropathy is associated with depression of the metatarsal heads, digital contractures, and cocked-up toes; equinus deformities of the ankle; or a varus hindfoot [32]. Atrophy of the small muscles within the foot results in nonfunctioning intrinsic foot muscles referred to as an "intrinsic minus foot" leading to dorsal contractures developing at the MTPJs with development of hammer toe syndrome. This results in elevated plantar pressures that increase the risk of skin breakdown and ulceration due to shearing forces [33]. These elevated pressures need to be

addressed prior to grafting or flapping to prevent continuation of the underlying pathology causing the focal breakdown of skin. Determination needs to be made if abnormal structures can be treated with accommodative shoe gear and inserts, or surgical intervention is required. Surgical intervention can range from simple exostectomy to complete reconstruction of an unstable Charcot osteoarthropathy foot.

### 4.3 Immune System

Patients with diabetes appear to be more prone to infections than their nondiabetic counterparts. Several factors increase the risk of development of diabetic foot infections including diabetic neuropathy, peripheral arterial disease, and immunologic impairment. Several defects in immunologic response relate to increased infection risk in diabetics. Diabetic patients demonstrate a decrease in function of polymorphonuclear leukocytes that can manifest as a decrease in migration, phagocytosis, and decreased intracellular activity. Evidence suggests that impaired cellular immune response as well as abnormalities in complement function occur [34, 35]. This impaired immune response accounts for clean neuropathic foot ulcers often rapidly converting to acute infections with abscess and/or cellulitis [36]. Diabetic foot infections can be classified into those that are nonthreatening and those that are life or limb threatening. In contrast to nondiabetic individuals, complex foot infections in diabetic patients usually involve multiple organisms. Studies report an average of five to eight different species per specimen [37–39]. The quickness that infections can develop and progress highlight both the importance of vigilantly treating infections and obtaining wound closure in order to prevent portals for bacterial entry.

## 5 Vascular Disease and Reperfusion

One of the major factors affecting diabetic foot disease is the development of lower extremity arterial disease. Peripheral arterial disease is estimated to be two to four times more common in people with diabetes than in nondiabetics [40, 41]. Limb salvage requires a combination of infection management, wound closure, and surgical reconstruction which are all dependent upon the perfusion of the lower extremity; to allow adequate antibiotic delivery, and osseous and skin healing. Once formed, the blood supply necessary to allow healing of an ulcer is greater than that needed to maintain intact skin. This leads to chronic ulcer development unless the blood supply is restored. The inadequate blood supply is commonly a result of peripheral artery disease (PAD). PAD, a progressive disease process, results from plaque accumulating in the arterial walls, with the vessels of the lower extremity being some of the most affected.

Diagnosis of PAD is generally noninvasive and can be performed in physician offices or outpatient hospital settings. The ankle-brachial index (ABI) measurement is considered the most accurate noninvasive diagnostic method when evaluating PAD [42]. The value of the ABI can provide an assessment of the severity of the disease [43]. Mean ABI in PAD is 0.64 compared with 1.08 in controls [44]. When evaluating symptomatic patients, Khan et al. [45] found that the most useful clinical findings are the presence of cool skin (LR 5.90), the presence of at least one bruit (LR 5.60), or any palpable pulse abnormality (LR 4.70). There are other techniques utilized for evaluation of lower extremity vasculature, including computed tomography (CT) and magnetic resonance angiography (MRA). Standard angiography is the method against which all other imaging procedures are compared for accuracy. Angiography can provide a definitive diagnosis of PAD by showing a road map of the arteries, and depicting the exact location and length of the stenosis or occlusion. It is helpful to understand that noninvasive ABI testing provides evaluation of the volume of blood flow, while invasive angiography provides the road map of that blood flow and helps determine the feasibility and approach to arterial revascularization [46].

When it comes to treating peripheral arterial disease, there are specific patterns of diseased vessels that require open surgical bypass, but greater and greater pathology can now

successfully be treated with percutaneous endovascular interventions. Balloon angioplasty and stenting are first-line endovascular techniques, with drug-eluting stents and drug-coated balloons offering low rates of needing repeat revascularization. Hybrid surgical techniques such as iliac stenting and common femoral endarterectomy are commonly used to reduce operative risk. Below-the-knee, angiosome-directed angioplasty may lead to greater wound healing in the foot. Combined antegrade and retrograde approaches can also increase success in limbs with long total occlusions.

Endovascular techniques, such as percutaneous transluminal angioplasty (PTA) and cryoplasty, play an important role in patients considered poor or noncandidates for surgical revascularization secondary to comorbidities such as coronary artery disease, uncontrolled hypertension, diabetes mellitus, or inadequate target vessel. Percutaneous transluminal angioplasty, especially in the arteries below the knee, has made significant progress in recent years. There are some new appliances being used in the PTA of peripheral arteries in treating diabetic foot, such as intravascular ultrasound ablation, cutting balloons, drug-eluting balloons, and other special micro balloons and stents.

Basco et al. [47] reported on 126 lesion treated in 88 patients who underwent lower extremity revascularization utilizing cryoplasty. Limb salvage rates were 75% and 63% for patients with critical limb ischemia after 1 and 3 years, respectively. Siracuse et al. [48] reported their results in treating 221 patients with below-knee popliteal artery lesions. Treatment included percutaneous transluminal angioplasty with or without a stent, atherectomy with or without percutaneous transluminal angioplasty/stent, and stenting with percutaneous transluminal angioplasty and atherectomy. They concluded that diabetic patients benefit most from atherectomy with percutaneous transluminal angioplasty, and statin use is protective against restenosis and mortality, and should be the standard of care in peripheral endovascular interventions. Wu et al. [49] performed a meta-analysis investigating percutaneous transluminal angioplasty versus primary stenting, to determine which procedure is more beneficial for treating infrapopliteal arterial disease. From the prospective randomized trials included, they found that 1-year outcomes did not show any significant differences between the percutaneous transluminal angioplasty and primary stenting groups, both having the same 1-year benefit. They concluded that there was insufficient evidence to support the superiority of either method. Jens et al. [50] conducted a randomized controlled trial comparing either balloon angioplasty or drug-eluting balloon with optional bailout stenting, or primary stenting using a bare stent or drug-eluting stent to one another in critical limb ischemia patients with below-the-knee arterial lesions. They concluded that balloon angioplasty with optional bailout stenting using bare stent should remain the preferred strategy in treating below-the-knee arterial lesions.

Newer alternatives to bypass include processed lipoaspirate cell autologous transplantation, lipoprostaglandin E1, granulocyte colony-stimulating factor, De Marco formula, low-dose urokinase, and heparin-induced extracorporeal low-density lipoprotein precipitation, which directly removes fibrinogen levels from the cardiovascular system and improves microvascular circulation [51]. Pedal access is a relatively recent innovation in vascular interventions. Retrograde intervention has allowed revascularization of distal lesions in the presence of widespread multilevel long and complex occlusive lesions, in which the conventional way of crossing the tibial lesion has failed in the presence of pedal/tibial vessel disease. This new concept of endovascular intervention approaches lesions from the opposite direction of traditional anterograde endovascular treatments. It is important for the treatment of critical limb ischemia patients with tibial disease when the regular antegrade approach for crossing the occlusion is not possible [52]. Antegrade access can be used in combination to treat any lesion that requires a stent placement, after the retrograde wire snares antegrade wire and brings the antegrade guide wire distal to the lesion.

Brazan et al. [53] analyzed the outcomes in patients with chronic limb ischemia who were not surgical candidates for a tibial bypass and had

undergone an unsuccessful attempt at revascularization through an antegrade access for a popliteal or tibial lesion. In the 13 patients who underwent retrograde pedal access, diabetes was present in 77% and chronic renal insufficiency in 69%. Technical success rate was 69%, for popliteal and tibial vessels treated with angioplasty or angioplasty/stent placement through a retrograde approach. At a mean follow-up of 17.1 months, the limb salvage rate was 77%. Brazan et al. concluded that retrograde pedal access for limb salvage in high-risk patients who have failed an antegrade intervention and are poor candidates for a tibial bypass is feasible and safe, with acceptable limb salvage rates at intermediate follow-up.

## 6 Physiologic Considerations in Flap Perfusion

Ian Taylor [54] pioneered describing the entire body in territories of 3D blocks of tissue and blood supply called angiosomes. Angiosomes are 3-dimensional units of tissue, fed by a source artery, totaling six in the foot and ankle region. An angiosome is a composite of tissue composed of integument and underlying deep structures that is supplied or drained by a source, segmenting, or distributing artery. Nomenclature of an area refers to its specific named arterial supply. The angiosome concept allows for evaluation of a source artery for healing of a particular area of tissue when planning for a flap or graft.

A random flap and wound bed receive blood from a perforator artery from the dermis to the subdermis plexus. This is in contrast to an axial flap, which has a direct cutaneous artery, vein, and plexus already associated with it. Both of these rely on the principles Ian Taylor developed in order to understand the perfusion to the flap/graft you are performing, which then has to be incorporated into the treatment of PAD to ensure that perfusion is present in the area of the wound. The foot and ankle have main arteries with numerous arterial-arterial connections that allow alternative routes of blood flow to develop if the direct route is compromised called choke vessels. Choke vessels link neighboring angiosomes, allowing a given angiosome to provide blood flow to an adjacent angiosome if the latter's source artery is damaged. So if one angiosome has succumbed to poor circulation, another angiosome may aid in perfusion of said area by use of choke vessels. This can be exploited when treating wounds, whereby a surgeon will raise a flap in the donor area by opening up choke vessels to the flap beyond its direct neighboring skin, referred to as the delayed procedure [55–58]. Using Doppler to determine direction of flow, by systematically occluding source vessels, provides additional information to that obtained by routine angiography. The Doppler exam along with the concept of source arteries and choke vessels is used to stage incisions and flaps to maximize healing. In those cases, if one of the three major vessels of the foot receives endovascular therapy, then wound healing is likely to be achieved [57–59].

When planning random flaps, it is important to ensure open perforators at the base of a random flap. If the perforator is not open, antegrade flow toward the base is at least needed; in cases of retrograde flow, distal dissection can interrupt flow. Doppler should always be used to determine blood flow and perfusion for random/pedicle flaps. This is where angiosome concepts need to be incorporated when planning incisions, flaps, and amputations. A palpable pulse or triphasic Doppler over the source artery to the angiosome indicates adequate blood flow. When there is good flow to adjacent angiosomes, the safest incision is along the border between the angiosomes. As choke vessels take 4–10 days to become patent for collateral circulation, staging of flaps for closure is required. When the source artery to an angiosome with a wound is not patent, then the incision should be placed in the center of the inadequately perfused angiosome, as far away as possible from the patent artery that is supplying the adjacent angiosome in order to preserve the patent source artery supplying perfusion and maximizing tissue perfusion. When no source artery is patent and collateral circulation to adjacent angiosomes is absent, vascular intervention should be attempted prior to surgical reconstruction.

Caution must be used in patients with mild PAD, as progression of disease may compromise the collateral circulation supplying perfusion that was previously relied on. The take-away point is that any incision should be planned in order to prevent the disruption of arterial sources of angiosome perfusion, from either source artery or collateral circulation. In the regular population, the practitioner should be cognizant of the angiosome concept; but it is not as critical as it is for the diabetic population, for random flaps and incision placement as arterial-arterial connectors are established and patent, and collateral circulation is present or available, as long as the skin bridge is not within the same angiosome for the nondiabetic population.

Taking advantage of the angiosome concept with direct angiosome-based revascularization strategy for in-line vessel of the angiosome containing wound is associated with shorter healing times and lower rates of amputation and other major adverse limb events when compared to indirect revascularization. When attempting limb salvage in the presence of distal disease, it is important to remember the concept of angiosomes. These anatomic regions of tissue, fed by feeder vessels, are used when utilizing flaps for closure of distal amputations and ulcerations. Zheng et al. [60] evaluated the clinical significance of the involvement of collateral vessels in interventional therapy based on the angiosome concept for infrapopliteal critical limb ischemia (CLI). 486 patients with unilateral infrapopliteal CLI were categorized into three groups: the direct revascularization (DR) group, the indirect revascularization through collaterals (IR-tc) group, and the indirect revascularization without collaterals (IR-wc) group. Zheng concluded that following the angiosome model of perfusion for endovascular therapy, directly revascularizing the feeding artery and indirectly achieving revascularization through collaterals can effectively prompt the healing of ulcers and decrease the amputation rate in patients with infrapopliteal CLI.

Ultimately a thorough Doppler exam utilizing the angiosome principles should be utilized to determine viability of limb salvage, and make the decision between a long process of saving a limb and quick higher level amputation based on accurate assessment of perfusion and capability of healing. Readers should always remember four important factors when choosing the location of an incision. The incision should provide adequate exposure for planned procedure, adequate blood supply to either side of the incision to optimize healing, and spare sensory and motor nerves, and be placed parallel to a joint to minimize scar contracture and reduce joint mobility. Overall the direct angiosome-based revascularization strategy of an in-line vessel of the angiosome containing a wound is associated with shorter healing times and lower rates of amputation and other major adverse limb events when compared to indirect revascularization [61].

## 7 Intraoperative Care and Flap Technique

During surgery, atraumatic technique, including skin hooks, use of bipolar cautery, and sharp dissection, must be utilized. Incision lines for the flap should be parallel to the lines of relaxed skin tension, to allow for minimal transverse force [62]. However, if concomitant bone surgery is performed, relaxed skin tension lines (RSTL) may be partially or entirely ignored. To raise a flap, one undermines below the subdermal plexus of the vessels in the subcutaneous plane and thus releases the tethering effect of this tissue [63]. Undermining of wound edges may help reduce tension of the flap; however, excessive undermining may endanger blood flow to the flap [13]. Also, meticulous hemostasis must be achieved before suturing. Finally, after planning, performing, and suturing, the flap should be evaluated for excessive tension and adequate vascularity. If a flap cannot be set with a 4.0 or suture of lesser strength, then most likely there is too great of tension on the flap and delaying procedure may be sensible. A variety of methods can be applied to evaluate vascularity. These include assessment of color, capillary refill timing, ultrasound Doppler examination, and even bleeding from stab wounds [64]. Options also include laser-assisted indocyanine green dye angiography. Adequate time spent in the OR evaluating a flap has been shown to be a better predictor of skin flap

necrosis versus clinical judgment and fluorescein dye angiography [65, 66]. Taking these steps into consideration will aid in planning, creation, and success of a flap procedure.

## 8 Flaps

A brief discussion of a few choices of advancement flaps, rotational flaps, transpositional flaps, and axial flaps is undertaken to demonstrate their diversity for closure (Table 2). For many years, flap designs were based on the concept that blood supply was from deep to superficial. However, Hidalgo and Shaw [67] proved that local plantar flaps could be designed to include sensation and blood supply without subfascial dissection.

**Table 2** List of skin flaps that can be used in foot and ankle surgery

| Advancement flaps | Rotation flaps | Transposition flaps |
|---|---|---|
| Single or double | Single or double | Single, bi-, or modified-lobed |
| M<br>Modified M-plasty | Satterfield-Jolly | Z-plasty |
| T | Classic | Double-Z rhomboid |
| V-to-Y | Catanzariti-Wehman | Double-opposing Z-plasty |
| Modified V-Y | | |
| Double reverse V-Y | | |
| Double V-to-Y | | Four-flap Z-plasty |
| Crescentic advancement | | |
| Oblique sigmoid island flap | | Double-opposing semicircles |
| Y-V plasty | | W-plasty rhomboid of Limberg |
| V-Y-S plasty, single V-Y island flap | | Flap of Dufourmentel |
| Extended V-Y island flap | | 30 Transposition flap (Webster flap) |
| | | Double or triple rhomboid |
| | | Note flap |

Listed are the different types of skin flaps that can be used to obtain closure in diabetic foot and ankle wounds. They are listed according to the motion needed to perform each flap

Milton [15] wrote an important article that showed that the artery supply at the base of a flap determined its success and not the length-width ratio. The concepts of wound bed preparation that will be covered in the section on maximizing split-thickness skin grafts are also just as important when performing flaps.

Flaps are classified by blood supply, anatomy within flap, and donor site location. Anatomical classification indicates the depth of tissue included in the flap; for example fasciocutaneous flaps contain both skin/subcutaneous tissue and deep fascia. Blood supply of a flap is based on the vascular plexus supplying the flap; for fasciocutaneous flaps perfusion comes from the deep fascia but does not include adjacent muscle. However a musculocutaneous flap includes skin/subcutaneous tissue, deep fascia, and muscle, and is supplied by a dominant vascular pedicle. Donor site refers to the area where donated tissue for transfer is located. Axial and free are other categories of flaps, but necessary to cover in the scope of this chapter. We briefly review random flap design, which is characterized by a lack of specific isolated blood supply, and rely on Ian Taylor's angiosomes of the foot/lower leg. Some of the most commonly used random flaps are as follows.

### 8.1 Advancement Flaps

Advancement flaps are mobile in one direction without laterality or rotation. They include single- and double-advancement flaps, M-plasty, T-plasty, V-to-Y, double V-to-Y, crescentic advancement flaps, and oblique sigmoid island flaps (Fig. 4). These flaps advance into the defect, and are best used in a location with adequate tissue laxity and elasticity. Advancement flaps enable closure of the donor and defect site simultaneously. Advancement flaps rely on direct cutaneous perforators. Care should be utilized to avoid excessive undermining, which may lead to flap necrosis. Because advancement flaps do not redistribute tension, dog-ears or resulting cutaneous defects may occur. Advancement flaps should be placed to benefit from the elasticity of the surrounding skin while incorporating the angiosome blood supply. Plan them perpendicular to the resting

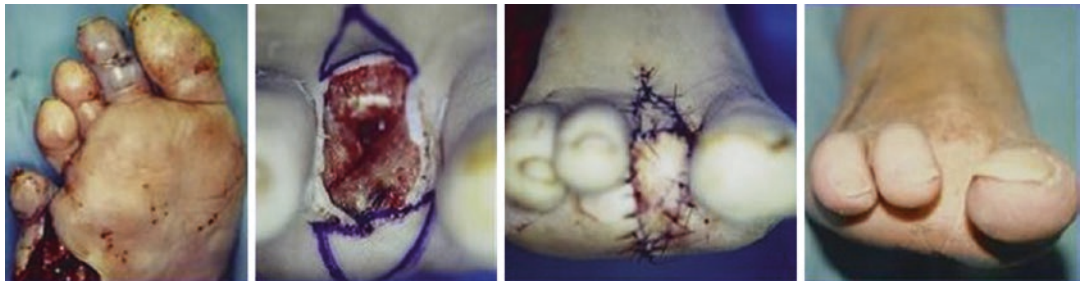

**Fig. 4** (**a**–**d**) Double V-Y advancement flap to close deficit after digital amputation

skin tension lines with advancement parallel to the lines of maximal extensibility (LME). Although advancement flaps allow for closure of the donor and defect simultaneously, their use can be limited due to mobility restrictions and need for exposure of underlying osseous pathology.

## 8.2 Rotation Flaps

Rotation flaps are those that pivot about a point and move in arc motion. These flaps include single-rotation flaps, double-rotation flaps, and Satterfield-Jolly. Rotation flaps provide redistribution and redirection of tension from the primary defect to the donor site [68]. They are frequently used in areas with convex surfaces, or where tension lines are curved [68]. Rotation flaps can be subfascial or suprafascial as described by Hidalgo and Shaw [67]. They can also be fasciocutaneous, myocutaneous, or a combination of both. Rotation flaps can be axial or random, depending on the level of dissection and angiosome involved. Rotation flaps can be elevated and mobilized from the non-weight-bearing arch to areas of pathologic weight-bearing surfaces. They can be used to correct defects on the plantar aspect of the heel, by including the heel pad (Fig. 5) [69]. They can also be used to cover large defects in the foot; however, they often require a skin grafting component for closure of the donor site. Rotation flaps are an excellent adjunct in diabetic foot soft-tissue coverage [70]. Their inherent design yields a wide-based exposure to the osseous structures. It must be remembered if donor-site skin grafting is required, strict elevation and bed rest are recommended and must be strictly enforced for 5–7 days.

The rotation flap used to close a circular defect is usually a combination of both primary and secondary movements. The primary movement is the rotation and advancement of the flap itself over the defect, and the line of the greatest tension extent from the pivot point toward the defect site. This distal tension point is the area of greatest vascular compromise. The secondary movement is the movement of the adjacent or surrounding skin in the opposite direction of the flap movement. The skin from the side of the defect opposite the flap moves over the defect more than the flap itself moves over the defect. This requires less rotation of the flap and creates less puckering at the flap pedicle. An arch-shaped flap is then designed so that the leading tip of the flap will rotate around the circumference of the circle on which the defect lies. To enable primary closure of the donor site, the flap should have a circumference five to eight times the width of the defect [71] or an area of three to four times the area of the defect [68].

## 8.3 Transposition Flaps

Transposition flaps move over adjacent intact skin to close a defect, and combine the use of both rotation and advancement. These flaps include the single-lobe flap, the bilobed flap, Z-plasty, double-Z rhomboid, double-opposing Z-plasty, 5-flap Z-plasty, 4-flapZ-plasty, double-opposing semicircles, W-plasty, rhomboid or Limberg flap, flap of Dufourmentel, and double- and triple-rhomboid flaps. As with rotation flaps, these flaps redistribute and redirect tension from the primary defect to the donor site [72]. It is critical that the flap extend beyond the defect, thereby ensuring adequate length after

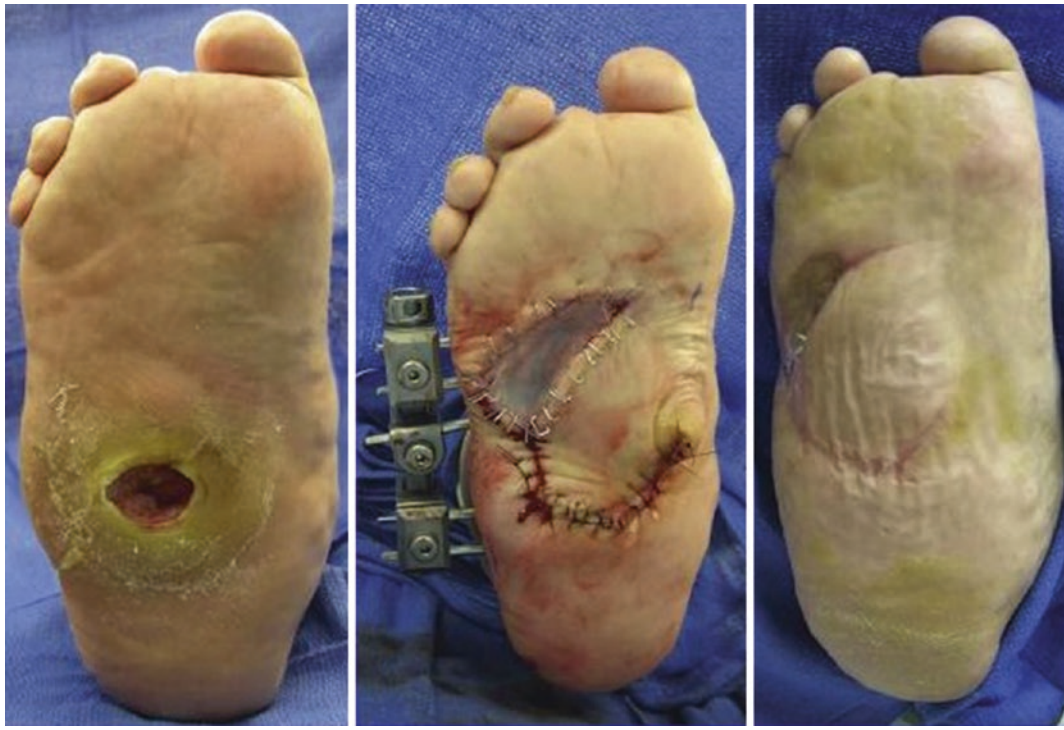

**Fig. 5** (**a**–**c**) Direct rotation flap for a plantar wound, as a single-stage reconstruction in a diabetic neuropathic patient

its transposition [73]. If additional length is required, a back cut away from, or into, the base can be made; the latter option poses a risk of reducing the blood supply into the flap [71]. Transpositional flaps, like the Z-, rhomboid, and bilobed flaps, depend on the pliability of adjacent skin [72–74]. Primary closure of the donor site is possible if the adjacent skin is elastic enough, but because of pedal skin, another skin flap or graft may be used to obtain closure.

## 8.4 Maximizing Split-Thickness Skin Grafting

The majority of this chapter's focus is on skin grafting, as it plays a diverse role in reconstructive surgery of the foot and ankle. A graft is defined as any free tissue that is transplanted, unlike flaps that remain connected to their vascular source [75]. A skin graft is the separation of all or a portion of the skin from its donor site and local blood supply, followed by transplantation to a recipient site [76]. The transplanted skin subsequently relies entirely on the recipient site's blood supply for survival. Skin grafts incorporate all of the epidermis and varying depths of the dermis. A full-thickness skin graft consists of the epidermis and the entire dermis, whereas a split-thickness skin graft consists of the epidermis and a variable portion of the dermis. According to the thickness of the dermis, split-thickness skin grafts are described as thin, intermediate, or thick. A thin split-thickness skin graft consists of the epidermis and approximately 0.008 to 0.012 in. of dermis. An intermediate split-thickness skin graft consists of epidermis and approximately 0.012 to 0.016 in. of dermis, whereas a thick split-thickness skin graft consists of epidermis and 0.016 to 0.020 in. of dermis [76, 77]. Split-thickness skin grafts (STSG) currently represent the most rapid, effective method of reconstructing large skin defects [78, 79] such as granulating tissue beds, and tissue loss across joints in areas where contraction will cause deformity, and where epithelialization alone will produce an unstable wound cover [77, 80]. To maximize STSG success requires a few

conditions: red granulation tissue dominating the wound bed, no visible tendon or bone, no discernible sloughing or exudate in wound, no residual necrotic tissue, no local signs of soft-tissue infection, no systemic signs of infection, and no severe peripheral arterial disease (ankle-brachial index >0.9 or distal pulses present)*n* [81].

Given the focus of the chapter being wounds in diabetic patients, some specifics about STSG in diabetic patients need to be covered. In the diabetic population without comorbidities, when compared to nondiabetic patients, Driver et al. [82] showed no significant difference in healing times for STSG; however compared to diabetics with comorbidities there was significant difference. Looking at the diabetic population as a whole, healing time is 1.99 weeks longer and there is a 5.15 times higher risk of postoperative complications after STSG compared to patients without diabetes. These complications include wound dehiscence, infection, and need for revisional surgery. Diabetic patients without comorbidities fair much the same as nondiabetics, and both have lower risk for complications and delayed healing than diabetics with comorbidities. For diabetic patients, the presence of any preexisting comorbidity, history of amputation, or trauma is negatively associated with the successful outcome of STSG. Furthermore, duration of diabetes, hemoglobin A1c level, chronic kidney disease, blood urea nitrogen level, and creatinine concentration represent modifiable characteristics that need to be addressed when selecting patients for STSG of diabetic foot wounds. Therefore a comprehensive medical and surgical approach is imperative to maximize STSG success rate in diabetic patients [83–85].

## 9 Wound Bed Preparation: Infection, Inflammation, Perfusion

### 9.1 Wound Bed Preparation

Recipient bed preparation is a vital component of skin grafting. The bacterial count should be less than $10^5$ organisms per gram of granulation tissue [86–89]. A recipient wound bed ready for skin grafting should have a red hearty granular base with increased skin lines and neoepithelialization at the wound edges [90]. Periwound erythema should be absent. Wound conversion from chronic to acute is paramount to a successful STSG take [11, 86, 87, 91, 92]. Wound debridement is accomplished with the following techniques: topical treatment, serial debridement, and use of biologic wound dressings [18, 93]. Topical treatment with silver sulfadiazine three times a day after serial debridements will often decrease the bacterial count. Silver sulfadiazine may actually stimulate epithelialization as well [94–97]. Other agents, such as 3% hydrogen peroxide, 1% povidone-iodine, 0.25% acetic acid, and 0.5% hypochlorite, are acceptable as a one-time use to decrease bacterial load. These agents can be used daily, but care is needed because of their cytotoxic effects on epithelial migration at higher concentrations [98]. Less potent concentrations of these agents are not toxic and may render them non-antibacterial [99]. The recipient bed is pre-wounded, which allows for conversion to the proliferative phase, which may decrease time to graft take when the permanent graft is applied [18, 77]. Successful incorporation of STSG requires vascularized granulation tissue. Given the high prevalence of peripheral vascular disease in the diabetic population, it is important to identify the need for comanagement of vascular surgeons. Peripheral neuropathy plays a role in the etiology of over 80% of diabetic foot lesions [31, 100], but inadequate perfusion always results in nonhealing wounds [101, 102]. Lower extremity ischemia secondary to peripheral vascular disease reduces the pedal supply of oxygen, nutrients, and soluble mediators that are involved in the repair process [103]. It is important to realize that palpable pedal pulses do not guarantee the absence of limb-threatening ischemia. Upon wound bed debridement and preparation, there should be prompt signs of healing, including the development of wound granulation within several days; otherwise a low threshold for noninvasive vascular studies and arteriography should be undertaken for these patients.

## 9.2 Tissue Management: Biofilm, Bacteria, Inflammation

A prepared wound bed needs to be free of necrotic tissues, to allow for successful assessment of the wound bed as well as removal of potential sources of bacterial growth. Bacterial colonies present several challenges. First they can produce unwanted metalloproteinases that negatively affect extracellular matrix components, which can shift a wound to chronic nonhealing. In addition bacterial colonies also form biofilm in the wound beds. Biofilm is bacterial colonization of the wound surface, many times polymicrobial, which is highly resistant to antibiotic treatments including standard treatments such as systemic antibiotics [104–108]. Resistance also stems from attaching to surfaces of wounds and forming protective exopolysaccharide matrix microenvironments which makes them highly resistant to removal and eradication [104, 109–114]. All of this leads to biofilms' increased resistance to antimicrobial, immunological, predatory, and chemical attacks (Fig. 6) [115–117].

James et al. [117] reported the presence of biofilms in 60% of chronic wounds, defined as open for 30 days, versus 6% of acute wounds [118]. Unlike an infection, mature biofilm develops within 10 h and persists indefinitely while the wound remains open [119]. Once matured beyond this (48 h), biofilm becomes increasingly resistant to antibiotics [114]. Seven features have been used to indicate the presence of bacterial biofilm in human chronic wounds [103]. Clinically these include indicators such as a pale wound bed, friable granulation tissue, increased serous exudate, a yellow discharge, necrotic tissue, a clear slime, a putrid smell, wound bed color change, and pain at wound site. At present, unless the wound is heavily populated, tissue biopsies or swabs are required combined with microscopic identification techniques to confirm the presence of a wound biofilm [120].

Wound beds range from contamination, colonization, and critical colonization to infection [121]. Usually critical colonization and infection stages impede wound healing. The impact of bacteria in a wound depends on three factors: bacterial load, bacterial strain virulence, and capability of host to mount resistance. In diabetic patients, the effect of bacterial loads can be observed even at a lower count or even with the normal skin flora due to a weak immune system and impaired leukocyte function. Infections in diabetic foot ulcers are commonly polymicrobial and contain both aerobic and anaerobic bacteria (Fig. 7) [122]. The most common bacteria observed in chronic wound infections are *Staphylococcus aureus* (93.5% of ulcers), *Enterococcus faecalis* (71.7%), *Pseudomonas aeruginosa* (52.2%), coagulase-negative Staphylococci (45.7%), *Acinetobacter baumannii* (13%), and *Klebsiella pneumoniae* (6.5%) [117, 123]. Many studies have reported the evidence of antibiotic-resistant isolates in biofilms,

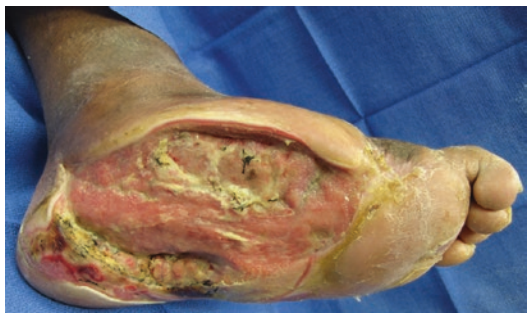

**Fig. 6** Biofilm present on wound bed

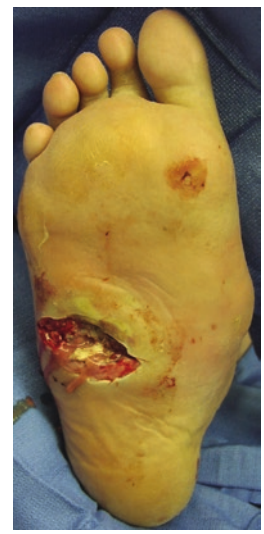

**Fig. 7** Infected diabetic foot wound, polymicrobial

in particular methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant Enterococcus, and multidrug-resistant *Acinetobacter baumannii* [124, 125].

When applying to practice, the practical question to answer is does a wound bed need to be sterile for successful skin grafting. Not all wounds can be cleared of bacteria, despite prolonged antibiotic administration and sustained wound bed preparation [77]. In an analysis by Bosman et al. [126], wound swabs taken immediately before grafting showed that approximately half the wound beds (53%) had been contaminated, the other half (47%) being sterile. Methicillin-resistant *Staphylococcus aureus* was detected in five cases, and either *Pseudomonas aeruginosa* or *Staphylococcus aureus* was detected in 23% of wounds. Contaminated wounds did not display a lower mean graft take percentage than near-sterile wounds (87% vs. 90%, respectively). Wounds containing either *Pseudomonas aeruginosa* or *Staphylococcus aureus* did have inferior outcome (mean take percentage 78.9% vs. 91.3%), whereas diabetes also appeared to be a deteriorating factor (mean take percentage 83.0% vs. 90.7%) [127–135]. They found that although wound cultures showed that positive swab cultures did not impede good graft take, the presence of specific strains, such as *Pseudomonas aeruginosa* or *Staphylococcus aureus*, infers suboptimal outcome. They concluded that qualitative instead of quantitative analysis of the wound swab, whereby specific strains of bacteria are identified, is recommended. Wolcott and Rhoads [129] observed that the chronic wounds treated by specifically targeting biofilms transformed nonhealable wounds into healable wounds. When combined with antibiofilm compounds, the use of antibiotics declined 25% during the 4-year study period.

This persistent colonization by bacteria in the form of biofilms is likely the reason acute wounds progress through stages of healing, while chronic wounds appear to stall in the inflammatory stage with abnormal cytokine and matrix metalloproteinase levels [136–138]. Unwanted metalloproteinases negatively affect extracellular matrix components, and can shift a wound to chronic nonhealing. Chronic wounds often exhibit a highly persistent inflammatory phenotype, epitomized by the influx of polymorphonuclear leukocytes (PMNLs) to the wound site, elevated matrix metalloproteinases (MMPs), and an imbalance of several cytokines [139]. Bacteria in the wound further exacerbate the situation by causing additional infiltration by PMNLs, together with MMP production [126]. Diabetic patients exhibit further dysregulated inflammatory and immune responses that predispose them to chronic wound infections. In diabetic chronic wounds, there is a disruption of the balance between extracellular matrix (ECM) synthesis and degradation [140].

MMPs regulate extracellular structural proteins and consequent tissue remodeling [141]. Nonhealing ulcers have shown higher presence of MMPs than those carried out on healing wounds, and this excessive MMP expression results in excessive matrix degradation, preventing the normal matrix formation and remodeling leading to formation of chronic wounds [131, 133, 142–145]. The inflammatory stage is extended in nonhealing wounds, and this is reflected by the continued presence of neutrophils and elevated MMP [140]. This chronic inflammatory condition results in the continual infiltration by poly- and mononuclear cells that include neutrophils, PMNs, macrophages, and foreign-body giant cells at the site of injury resulting in a continuous secretion of potent proteases, such as collagenases, gelatinases, and neutrophil elastase into the wound. Nguyen et al. [146] have also shown that diabetic biofilm-containing wounds had significantly less TLR 2, TLR 4, interleukin-1β, and tumor necrosis factor-α expression than wild-type wounds with biofilm. However both groups had similar bacterial burden and neutrophil infiltration after development of biofilms at 3 days post-wounding.

## 9.3 Wound Bed Debridement

Biofilms are the reason why thorough aggressive wound bed debridement is paramount for successful STSG. Studies have shown that many commercial topical agents and wound dressings are ineffective against biofilm infections [147]. Debridement helps to reduce the bacterial burden

within the wound, controls ongoing inflammation, and encourages formation of granulation tissue [120]. Instead thorough debridement and systemic antibiotics, where antibiotic treatment is tailored specifically to each wound infection together with a rotating topical antiseptic for the extremely recalcitrant wounds, are required [148].

The molecular and cellular environment of chronic wounds should be converted to resemble that of acute wounds to allow rapid healing, and for this to occur nonhealing wounds may require repeated debridement [149]. There is debate over the depth of tissue debridement necessary to remove biofilm, and even after debridement repopulation of biofilm within 24 h can occur [150]. Therefore, maintenance debridement in a clinic setting may be required to decrease the biofilm load after the initial operative debridement.

There are multiple techniques that can be used for the debridement of necrotic, sloughy, fibrous, and unhealthy tissue ranging from various mechanical and surgical methods. A wound bed may also be prepared by various nonsurgical debridement techniques: autolytic debridement facilitated by interactive dressings, larval therapy using sterile maggots, and enzymatic debridement with ointments containing papain, urea, or collagenase mixtures. Autolytic debridement can be slow and take a long time to be effective [151]. Negative-pressure therapy can also be a useful adjunct to biofilm reduction. Morykwas et al. [152] and Timmers et al. [153] suggest that negative-pressure therapy expedites wound healing through the evacuation of drainage, promotion of angiogenesis, granulation tissue formation, and biofilm reduction. Gabriel et al. [154] demonstrated fewer days of treatment, more rapid wound closure, and fewer hospital days with the use of negative-pressure therapy and antimicrobial solution to soak the wound bed.

## 9.4 Topical Therapy

Antimicrobial treatment guided by superficial wound culture has been challenged repeatedly in the literature [155–158]. Slater et al. [159] reported that only 62% of microorganisms identified through swab cultures correlated with deep-tissue cultures and different microorganisms were found in the swab cultures as compared with the deep cultures. Also relevant, superficial wound culture might not accurately reflect the diversity of the bacteria present in the chronic wound enveloped by biofilm. The use of polymerase chain reaction microbial speciation and quantification is increasingly recognized as a more efficient and accurate method of guiding topical biofilm treatment in the chronic diabetic wound [160–163].

There is a growing consensus that systemic antibiotics may be ineffectual in the treatment of biofilm and/or mild infections associated with chronic diabetic wounds, and aid in the development of antibiotic resistance [164–167]. Topical antimicrobials (ointments, creams, and gels) have long been utilized for the prevention and treatment of localized, mild-to-moderate, soft-tissue wound infection. Triple antibiotic, gentamicin, iodine based, and silver based are some examples of commonly used topical antimicrobials. Silver-impregnated dressing materials are commonly used because of their purported antimicrobial properties identified through in vitro studies [163–170]. Beele et al. [171] reported in a small prospective randomized trial that the use of a silver-impregnated dressing decreases the likelihood of conversion from colonized wound to a clinically infected wound as compared with a non-silver-impregnated dressing. In one study, it was observed that 90% of all sessile bacteria within the biofilm progressively died within 24 h in the presence of silver-containing wound dressings [172]. Thorn et al. [173] investigated the antimicrobial effectiveness of silver- and iodine-containing wound dressings on preformed biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* grown in biofilm model. It was found that the iodine dressing was more efficacious than the silver dressing on biofilms. In a recent retrospective single-center study, Wolcott and Rhoads [128] evaluated the frequency of complete healing in subjects with a chronic wound in a limb with critical limb ischemia when managed using biofilm-based wound care. In total 77% of the wounds healed completely and 23% were classified as nonhealing, highlighting the importance of biofilm management.

## 9.5 Biologic Debridement

Enzymatic debridement finds use when other techniques are not feasible during the initial management of a chronic wound [126]. Over the last decade, biologic debridement using maggots has become increasingly popular. The maggots are highly selective and rapid, but often need to be combined with other forms of debridement after initial larval application [174, 175]. The larvae of the green butterfly *Lucilia sericata* [176] or *Lucilia cuprina* [177] can be used for biological debridement to digest the necrotic tissue, and they also secrete bactericidal enzymes. This approach is effective in wounds with methicillin-resistant *Staphylococcus aureus* and beta-hemolytic streptococcus.

## 9.6 Surgical Debridement

Mechanical (wet-to-dry) debridement damages healthy granulation tissue [178–180]. Surgical debridement, the current gold standard against which other forms of therapy are measured, is quick and effective, although expensive as it requires the need of an operating room and many times hospital admission [181]. Surgical debridement is the fastest means of debridement, allowing surgeons to accurately assess the severity and extent of the wound. The drawbacks of sharp surgical debridement are the nonselective nature of the method, and thus normal healthy tissue is at risk of removal at the same time [120]. In the presence of ischemic ulcers, it is critical that management should aim toward restoring tissue perfusion prior to aggressive wound debridement or aggressive surgery to ensure wound healing and prevent the "dieback" phenomenon.

Hydrosurgery combines both physical and surgical debridement techniques allowing for precise, controlled, and expedited debridement (Fig. 8) [182]. Hydrosurgery allows for select removal of necrotic tissue while decreasing debridement times by 39% [181, 183]. Caputo et al. [181] published a random controlled trial comparing hydrosurgery debridement to conventional surgical debridement in patients with diabetic and venous leg ulcers [105]. On average,

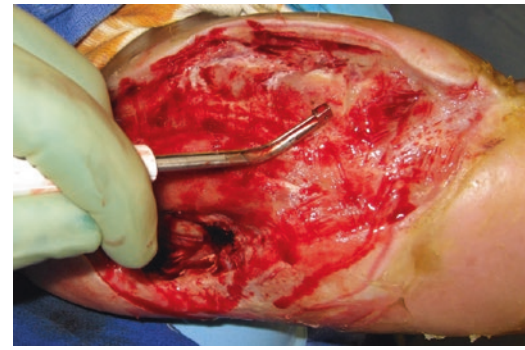

**Fig. 8** Beefy granular base free of biofilm after hydrosurgery debridement, ready for STSG

hydrosurgery debridement was quicker by about 7 min per procedure, and required significantly less instruments and sterile saline. The median time for wound closure was similar in both groups. Mosti et al. [184] compared the use of hydrosurgery debridement to moist dressings in patients with vascular leg ulcers. The mean time to debride the wound was 5–8 min, and average time to obtain a clean wound was reduced by nearly 5 days compared to wet-to-dry dressings. Hydrosurgery also minimizes the amount of normal tissue that is accidentally removed by surgery, and in most cases the wound bed is ready for immediate skin grafting [184]. Vanwijck et al. [180] reported on 167 wounds treated by hydrosurgery. Of all the debrided wounds, 95% were immediately covered with an autologous split-thickness meshed graft. Hydrosurgery left a smooth wound surface, which allowed immediate skin grafting in the majority of patients. For all but eight patients, the engraftment was total. Studies have demonstrated that hydrosurgery efficiently reduces the bacterial load of the wound and prevents the diffusion of microbial contamination deeper into the wound [185].

## 9.7 Ultrasonic Debridement

The use of acoustic energy continues to grow in popularity as a method of biofilm debridement. The effects of ultrasound on tissue can be classified into mainly two categories: thermal and nonthermal [186]. High-intensity ultrasound debrides necrotic tissue likely as a result of

cavitation [187]. Low-intensity ultrasound is thought to promote wound healing predominantly by acoustic streaming effects such as increased protein synthesis and production of growth factors [188]. In addition, low-frequency ultrasound has been reported to have antibacterial effects [189, 190], and enhance fibrinolysis in vitro [191, 192]. Ennis et al. [191] compared low-frequency (40 kHz) noncontact ultrasound to placebo in 55 diabetic patients with recalcitrant foot ulcers in a randomized, multicenter, double-blinded study. At 12 weeks, they reported significantly higher healing rates in the treatment group. Kavros et al. [139] published another study on 163 patients with chronic lower extremity wounds. In the retrospective study, they reported significantly higher percentage of wounds healed with low-intensity and low-frequency ultrasound compared to standard care alone. A recent meta-analysis reported significantly improved complete healing rates with low-frequency and high-intensity ultrasound (20–30 kHz, 50–60 W/cm$^2$) compared to sharp debridement at 3 and 5 months [140].

### 9.8 Hyperbaric Oxygen

Reported benefit of hyperbaric oxygen therapy (HBOT) is its detrimental effect on bacteria via the production of oxygen free radicals and enhancement of leukocyte activity [192]. The antimicrobial effects result from targeting anaerobic bacteria by increasing oxygen concentrations in deeper tissues. The MMP–tissue inhibitor of metalloproteinase (TIMP) balance is critical for regulating cell-matrix composition. Many studies have documented the important role of this balance in the pathophysiology of chronic wounds [135, 192]. Hyperbaric oxygen therapy has been shown to significantly decrease MMP protein levels in ischemic wound tissue, while the level of TIMP significantly increases [134]. Chen et al. [193] reported increased limb salvage rates (78.3%) for infected diabetic wound patients who underwent greater than ten HBOT treatments. However, literature on evaluating the effect of HBOT on biofilm reduction or eradication is rare.

## 10 Bioengineered Alternative Tissues

Bioengineered alternative tissues (BAT) are products derived from human, animal, and synthetic tissues that have been manufactured, cleaned, or otherwise altered [194–196]. BAT can be categorized as dermoinductive or dermoconductive. Dermoinductive products contain viable cells, including fibroblasts and keratinocytes, which are delivered to the nonhealing wound site with the goal of activating senescent cells in the chronic diabetic wound by releasing cytokines and growth factors that are produced in the grafted cells. Dermoinductive products should be reserved for more superficial wounds. This includes products such as Apligraf (Organogenesis, Canton, MA) and Dermagraft (Advanced Biohealing, San Diego, CA), which have both demonstrated clinical efficacy [197, 198]. In contrast, dermoconductive products provide an organized scaffold to facilitate cell migration of fibroblasts and serve as a template for the formation of neodermis, which is histologically similar in appearance and structure to normal dermis. This provides a durable dermal layer necessary for granulation tissue formation, allowing a skin graft to be placed over the neodermis for definitive wound closure. Examples of this type of tissue include Integra Bilayered tissue (Integra Life Sciences, Plainsboro, NJ) and hMatrix (Bacterin, Belgrade, MT). Thorough debridement must be performed before application of dermoconductive products, to remove biofilm and necrotic tissue. This category of products should be reserved for deeper wounds with exposed fascia, tendon, or bone [199]. The failure rate of skin grafting on neodermis is 7%, which is lower than that of direct skin grafting on tendons with or without granulation tissue [200]. As the neodermis matures, it appears golden yellow, which indicates its readiness for skin grafting, usually occurring between 16 and 28 days after artificial dermis placement. Shores et al. [200] placed Integra Bilayer Matrix Wound Dressing directly over exposed tendons with a subsequent STSG several weeks later in 42 patients. STSG was applied after generation of highly vascularized neodermis, on average 35.3 days after the initial placement of Integra. The size of the tissue

defect including the area of tendon exposure ranged from 4 to 336 $cm^2$ with an average of 65.1 $cm^2$. Average STSG thickness was 0.0011 in.. There was 92.5% take in all skin grafts, with all patients exhibiting durable skin coverage at the end of their follow-up period. With physical and occupational therapy, patients were able to attain an average range of motion in their skin-grafted joints of the lower extremity that was 90.6% compared to their contralateral side. Yeong et al. [201] reported on 23 patients with 33 wounds, in which artificial dermis was used to prepare tendon-exposed wounds for STSG, 11 of which were chronic ulcers. Thirty-nine percent of the patients had underlying diabetes mellitus, and 55% of the wounds were found in the lower extremities. The mean area of artificial dermis implantation was 67 $cm^2$, with mean duration from artificial dermis implantation to STSG of 21 days. Overall success rate was 82%, with 63% in the chronic ulcer group. Silverstein [202] also described the use of this technique to close tissue defects on five diabetic feet. All (100%) wounds healed with complete coverage of exposed bone, tendon, cartilage, and fascia.

## 11 Exposed Bone, Ligament, Tendon in Wound Bed

Pedal wounds with exposed bone, ligament, and tendon pose additional challenges as direct placement of STSG has high rates of failure as these structures do not provide adequate vascular wound bed to allow take and nourishment of skin grafts. The other complication of STSG over these structures is adhesions, limiting function and resulting in breakdown [203]. Inducing granulation tissue over such structures requires additional wound bed preparation. Successful options include bioengineered alternative tissues, allografts, and wound VAC therapy (Fig. 9). Since

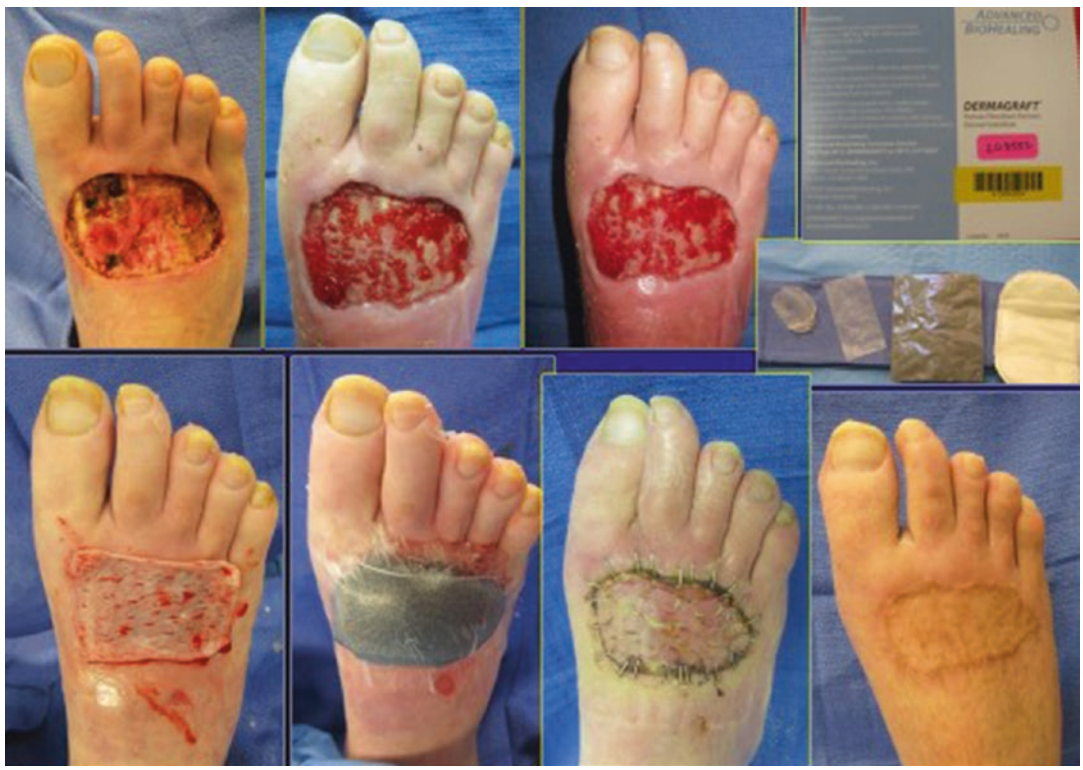


**Fig. 9** Multiple adjunctive therapies may be required to achieve a good outcome from a chronic diabetic foot wound. (Top) Preoperative. (Bottom) Postoperative

chronic ulcers often lack healthy, vascularized tissue to support artificial dermis, recipient bed preparation for dermis implantation via wound VAC therapy or allografting may be necessary. Wound VAC therapy promotes granulation growth, while allografting stimulates angiogenesis. In a series of 20 patients, Helgeson et al. [204] applied wound VAC therapy to the wound bed prior to grafting. The vacuum was set to a continuous negative pressure of 125 mmHg, with dressings changed on alternate days. Adequate granulation tissue formation was achieved, allowing for direct application of meshed split-thickness skin grafts. Some clinicians have also suggested the use of wound VAC therapy on meshed Integra to speed rates and success of revascularization. Much like STSG, hematoma and shearing forces can interrupt healing and lead to loss of artificial dermis, which can be actively managed with wound VAC therapy by immobilizing the Integra Bilayer Matrix Wound Dressing for 5 days [205].

## 12 Physiologic Consideration in Grafting

An understanding of physiologic processes of successful incorporation of STSG is required to understand the clinical process and maximize physician input and minimize complications. Let us focus on the actual grafting now that the wound bed has been prepared.

### 12.1 Skin Graft Adherence and Healing

Skin grafts are a form of tissue transplantation requiring adherence and formation of a new blood supply from the recipient bed. It is believed that split-thickness skin grafts can reestablish circulation faster and more efficiently than full-thickness skin grafts because the harvest is more superficial in the dermis [91, 205]. In split-thickness skin grafts, the number of transected vessels increases, and the number of portals available for recipient bed penetration is increased; thus revascularization of the skin graft occurs sooner [76]. The three phases to skin graft healing are (1) the phase of serum imbibition (plasmatic circulation); (2) the phase of revascularization, a combination of neovascularization and inosculation; and (3) the phase of organization [76, 126, 206–208].

The phase of serum imbibition is also known as plasmatic circulation because no true circulation exists between graft and recipient bed [76, 78, 90, 91, 97, 209–211], and is dependent on recipient bed fluid for nutrients [14, 76, 98, 209, 212]. This influx of fluid into the graft causes edema over several days until venous and lymphatic circulation develops at about day 9 after graft application, which must be managed [14, 76, 78, 209]. The second phase of skin graft healing is the phase of revascularization, which is divided into neovascularization and inosculation [76]. Neovascularization is the outgrowth of blood vessels from the recipient bed into the graft dermis at a rate of 2 mm/day. The new vessels grow into the graft from the host bed with simultaneous degeneration of the old vessels in the graft [14, 76, 78, 209, 212]. Blood enters the graft during this phase, and the graft will gain a pink hue caused by fine extravasations on the undersurface of the graft. This signifies that anastomoses are being formed. Inosculation occurs where circulation is restored in the original graft vessels through anastomoses with the recipient bed vessels [14, 76, 98, 212].

The phase of organization begins when the graft has fully adhered to the recipient bed [213]. This phase begins with adherence of the graft to the host, whereas the recipient bed produces exudate containing plasma, erythrocytes, and leukocytes [14, 98, 212]. The fibrinogen in the exudate precipitates into fibrin that allows for graft adherence to the host bed. The succeeding fibrinogen-free serum penetrates the fibrin layer and enters the graft dermis to provide nourishment and assists in maintaining a moist environment until a more thorough blood supply can be established [91]. Leukocytes enter the dermis and eventually focus on degenerating appendages and epidermis [14, 91, 98, 212]. Leukocytes will remain until reestablishment of circulation is complete. The

graft now functions in a similar manner to native peripheral skin. The graft match and durability is a function of the percentage of dermis transplanted.

Throughout the first 25 to 30 days after graft application, the graft nerves degenerate [14, 87, 214]. At approximately 2 months, reinnervation begins, with pain sensation returning first, followed by touch, hot, and cold distinction, and finally the ability to perspire [25, 87, 215]. This process can occur for as long as 2 years. Graft reinnervation is most successful in full-thickness skin grafts and less in thin split-thickness skin grafts; however, the rate at which sensation returns is quickest in thin split-thickness skin grafts and slowest in full-thickness skin grafts [87]. In all types of sensation, the graft tends to assume the sensory pattern of the recipient bed [87, 215]. Grafts placed over periosteum or muscle develop poor sensation [87].

## 12.2 Skin Contraction in Skin Grafts

Two types of skin contraction occur with skin grafting. Primary contraction occurs at the graft site, where the graft is removed from the donor site. Contraction occurs greater in full-thickness skin grafts than in split-thickness skin grafts, likely from the elastin fibers in the dermis [76, 91, 92, 216, 217]. Full-thickness skin grafts retract approximately 44%, intermediate.

Split-thickness skin grafts retract approximately 22%, and thin split-thickness skin grafts retract approximately 9% [56, 57, 98]. Tension applied to the graft when it is applied to the recipient bed reestablishes graft length and width. Secondary contraction occurs once the graft is fixed to the recipient bed [217]. Secondary contraction results from the host bed myofibroblasts within the wound pulling the skin graft. Full-thickness skin grafts are more resistant to secondary contraction than split-thickness skin grafts [90]. The greater the percentage of dermis in the graft, the less the skin graft contracts secondarily. Therefore, the greater the relative thickness of graft dermis, the speedier the myofibroblast life cycle [217]. Thus, full-thickness skin grafts resist secondary contraction better than thin split-thickness skin grafts [14, 91, 92, 98, 212, 218].

## 12.3 Performing Split-Thickness Skin Grafting

### 12.3.1 Instrumentation

The donor site should be chosen and measured preoperatively, allowing the patient to be properly positioned for the harvest. The donor region is shaved preoperatively and prepped in the usual aseptic manner. Combination of general, spinal, and local anesthesia with or without epinephrine can be used [96, 219]. If povidone-iodine is used to prep the donor site, it should be washed off before harvesting, because it can cause the dermatome to stick to the skin and "skip." It is recommended to use sterile saline while harvesting the skin graft to increase the life of the instruments. The dermatome should be set to 0.015 in., which can be checked with the thickness of a no. 15 scalpel blade [91, 92, 212]. The graft harvesting requires the dermatome to enter the skin at a 30° angle with power running, advances parallel across the skin with constant pressure, and quickly exits the skin at 30° after harvesting the desired length of graft. It is useful to visualize the dermatome as an airplane landing on an aircraft carrier, moving along the runway, and taking off again (Fig. 10). Having an assistant apply pressure to the skin in front of the dermatome with fingers can ease the process by creating uniform tension during removal, resulting in a more accurate harvest. Another assistant can then grasp the skin, with atraumatic pickups, to allow the graft thickness to be judged. Thicker grafts are opaque and thinner grafts are translucent. Once harvested, the graft is placed on a sterile saline-soaked sponge until use. If donor-site bleeding is uncontrolled; the site may be sprayed with topical thrombin or 1:200,000 dilute epinephrine. Intraoperatively, the harvest site may be dressed with adaptic or a sheet of Xeroform (Sherwood Medical Industries, St. Paul, MN).

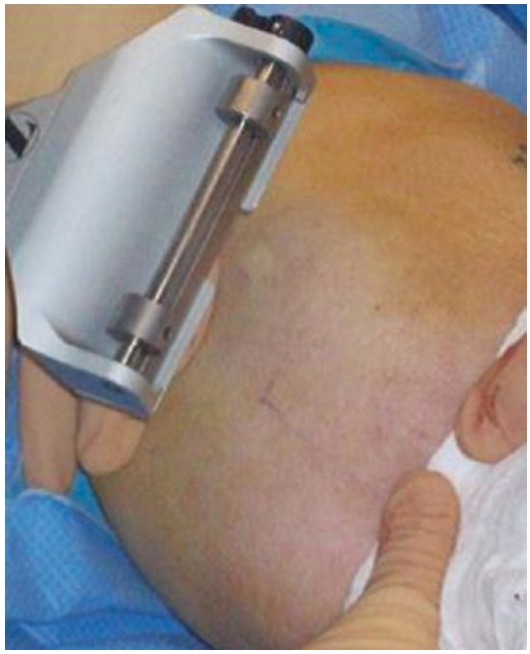

**Fig. 10** Harvesting of STSG with dermatome

### 12.3.2 Meshing and Pie-Crusting

The majority of the time split-thickness skin grafts are meshed or pie-crusted prior to application to the wound bed [220]. Pie-crusting is the practice of placing the split-thickness onto a hard surface, dermis side up, and using a scalpel to create a variable number of small cuts or slits into the graft [221, 222]. Pie-crusting, like meshing, allows for drainage, decreasing the likelihood of hematoma or seroma formation beneath the graft. Pie-crusting does not have the crisscross pattern in the healed skin like meshed skin grafts, but has the disadvantage of not allowing the same degree of hematoma or seroma drainage or expansion. The technique of meshing involves placing the graft on a plastic carrier and hand-cranking the tissue through the meshing machine [16, 221, 223, 224]. Meshed grafts should not exceed a ratio of 1.5 to 1.0. A ratio greater than 1.5 to 1.0 leaves a crisscross pattern on the skin after healing because of the inability of the larger spaces to fill in by epithelial migration from the surrounding graft. Overexpansion by larger ratios also leads to greater contraction of the graft during healing. Meshing has three distinct advantages. First, it allows for expansion of the skin graft to cover larger areas. Second, it adheres better to irregular surfaces. Third, it allows excessive fluid to drain from the recipient bed and thereby reduces the risk of hematoma/seroma formation below the graft [91, 92, 212].

## 12.4 Application of the Split-Thickness Skin Graft

Split-thickness skin grafts, especially if meshed, have a tendency to fold on themselves. Patience and a steady hand are required for insetting the graft, which can be transported on the meshing plate, saline-soaked gauze, or Telfa pad (Kendall Co, Boston, MA) to the recipient bed. There are four ways to fixate the graft to the recipient site: (1) skin staples; (2) simple interrupted suture technique with 4.0 or 5.0 monofilament nylon; (3) running 5.0 polyglycolic acid, polyglactin, chromic, or plain gut suture; and (4) no fixation at all (Fig. 11) [176]. It is not advisable to stretch the graft or to let it fold back on itself. The surgeon may spray the recipient bed with topical thrombin to aid in graft adherence. Finally, once in place, trimming of the excess skin graft is completed for appropriate fit [176].

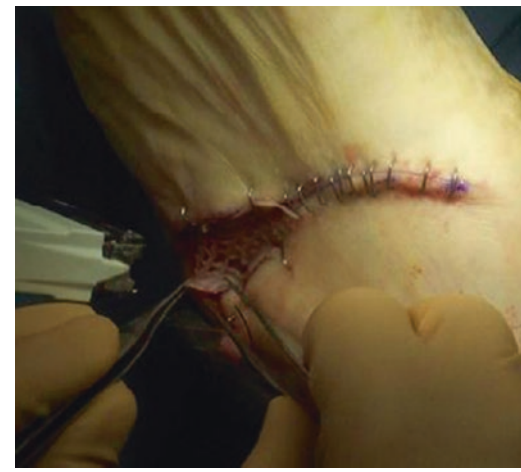

**Fig. 11** STSG fixated to wound bed

### 12.5 STSG Management

The most common complication leading to graft failure is hematoma or seroma formation within the serum imbibition period. After harvesting the STSG, to ensure success, seroma/hematoma formation must be managed beneath the graft. Managing mechanical shear forces that would disrupt the plasmotic phase and angiogenesis phase, and adhering the graft over irregular surfaces while the STSG is being incorporated on to the wound bed, is necessary. To achieve all of the above requirements, a uniform pressure over the entire grafted area through a non-adherent, semi-occlusive, absorbent dressing material is required. Negative-pressure wound therapy (NPWT) finds a role both before split-thickness skin grafting by decreasing bacterial load and assisting with wound bed preparation and after grafting by fixating graft and reducing/eliminating seroma/hematoma [206]. Wound vacuum-assisted closure has greatly improved skin graft take, and lessened seroma and hematoma formation [14, 212]. The vacuum will reduce shearing forces and eliminate most fluid buildup between the graft and wound bed to allow for serum imbibition [208, 209]. Graft take and complication rates have significantly improved because of the preferential use of meshed grafts and the introduction of NPWT [78, 141, 210]. Blume et al. [210] compared conventional therapy (CT) dressing, cotton bolster/sterile compressive/stainless steel gauze dressing that is used for at least 5 days, to negative-pressure wound therapy using reticulated open cell foam (NPWT/ROCF). Mean graft take at the first follow-up was 95% for NPWT/ROCF compared to 86% for CT, with maximum graft take of 96% for NPWT/ROCF compared to 83% for CT. There were significantly fewer repeated STSGs required in the NPWT/ROCF group (3.5%) compared to the CT group (16%). There were fewer complications (seroma/hematoma/infection) leading to graft failure in the NPWT/ROCF group compared to the CT group. The reticulated open cell foam dressing conformed to the wound geometry with negative pressure, promoting skin graft adherence while removing exudates and edema from surrounding tissues (Fig. 12).

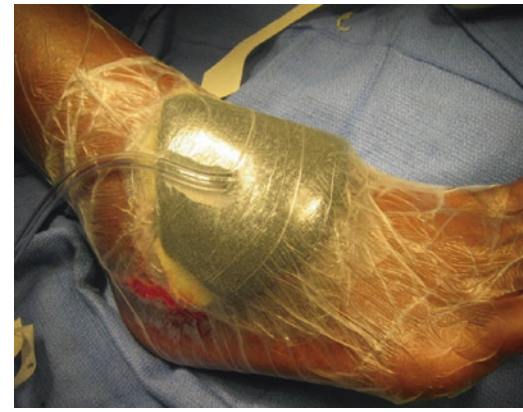

**Fig. 12** Negative-pressure wound therapy is very effective in reducing shear forces on fresh skin grafts on the wound bed, and also aids in removal of seroma and hematoma from under the graft

## 13 Postoperative

Postoperative care of the limb is critical for the prevention and mitigation of potential complications. Postoperatively the extremity is placed on strict elevation, non-weight-bearing status, and continuous use of wound VAC therapy for 5 days to allow development of graft-host circulation, and decrease graft edema from dependency. During this period of time, the wound vacuum is not changed unless complications arise. After 5 days is complete, the vacuum is removed. A sequential dangling regimen is undertaken consisting of 5 min per hour on day 7, 10 min per hour on day 8, and 15 min per hour on day 9, which allows the skin graft to adjust to venous gravitational force without increased edema [90, 91, 97, 211]. Physical therapy begins near the third postoperative week to increase range of motion. By 4–6 weeks postoperatively, the patient may progress to guarded weight bearing with an appropriate assistive device. The foot should remain wrapped with an ACE bandage whenever it is in a dependent position. By 6–8 weeks postoperatively, the patient may progress to full weight bearing with decreasing assistance as tolerated. Preoperative physical therapy and gait training are often beneficial. In a small study, Hegelson et al. [211] demonstrated that with appropriate pre-wounding and STSG placement

associated with wound vacuum therapy, closure was obtained in more than 90% of their patients (Fig. 13). A Cochrane database review agrees that loss of skin graft is less in NPWT–STSG groups [212].

## 14 Skin Graft and Skin Flap Complications

Many factors may cause skin graft and flap failure. Complications include infection, mechanical shearing forces, inadequate vascularity, seroma and hematoma formation, and technical/surgical error [14, 90, 213]. Skin graft failure is often a result of inappropriate preparation of the recipient bed; therefore, careful attention is placed on debridement techniques. Intraoperative preparation of the recipient bed begins with excision of the wound edges and curettage of the granulation tissue from the base of the wound to create a clean, healthy bed for skin graft application. Thorough preparation of the granulation tissue is critical as it contains many crevices that allow for bacterial colonization [87]. Infection is another cause of graft failure during reconstruction closure attempts. Organisms including *Staphylococcus aureus*, Pseudomonas, and beta-hemolytic Streptococcus have been identified in graft failure more than other organisms [214, 215]. To demonstrate this, a study from 1998 revealed both a 35% and 25% decrease in graft take in grafts infected with *S. aureus* and Pseudomonas, respectively [25]. Inadequate vascularity can be avoided by proper patient selection through a thorough history and physical, and noninvasive vascular examinations as needed [56, 216, 217]. If the patient has peripheral vascular disease, then working as a team with a vascular surgeon is crucial [57]. Intrinsically, vascular failure can result from flap design and geometric parameters, or extrinsically due to venous and arterial issues as mentioned previously. Many times, patients with decreased perfusion will need a reperfusion intervention before proceeding with grafting intervention [57, 218]. Skin grafts should be avoided on weight-bearing surfaces [219–222]. Consider that skin grafting normally occurs in patients who do not have "normal" feet and will therefore unlikely have normal plantar pressure distribution. Many patients have had previous foot reconstruction, creating mechanical and structural instability, or have markedly deformed feet [16, 220]. Long-term mechanical and pressure distribution issues should be addressed before surgery, by addressing underlying osseous deformities. Intraoperatively, the use of traumatic techniques can doom sensitive surgery. As mentioned previously, excessive undermining and lack of attention to details, such as not making an incision perpendicular to skin, will decrease the chances of success. The inexperienced surgeon may and can do harm without careful planning and execution of said plan. After surgery, there are other factors that can lead to surgical site failure. External healing confounders, such as cigarette smoking, uncontrolled diabetes mellitus, unrecognized malnutrition, or use of medications that result in vitamin deficiencies, also increase the risk of failure [98, 223–225].

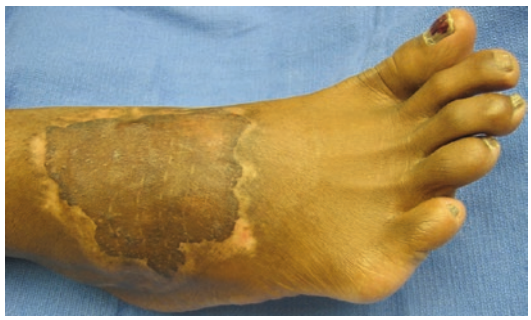

**Fig. 13** Fully incorporated and healed STSG

The most common complication of skin grafts is hematoma formation. It prevents graft recipient bed contact, increasing the ischemic period and decreasing the chance of graft survival [97, 225]. If there is 0.5 mm of fluid between the graft and the recipient bed, then revascularization is delayed by 12 h. If the amount of fluid is increased to 5.0 mm, then revascularization is delayed by 5 days if the graft survives at all [97]. The best way to prevent hematoma formation from the beginning is to achieve hemostasis. If excessive bleeding is noted, the team may want

to delay graft placement by 24 h. Seroma occurs where lymphatic channels meet [90, 91]. In split-thickness skin grafts, elevation by seroma may not permit epithelialization on the underside of the graft, thereby inhibiting revascularization that can result in sloughing of the graft and/or graft failure.

Be open and be vigilant with patient care after flap and graft surgery because pain, infection, and delayed healing may be encountered and can be difficult to resolve. Patient noncompliance, poor dressing technique, and irregular follow-up can also complicate the postoperative course of these surgeries. Healing is often a long course that involves many ups and downs.

## Conclusions

Treatment of pedal soft-tissue deficits in the diabetic patient population continues to be a medical and surgical challenge, extending the length of their disability and significantly increasing the cost of medical care. Despite all interventions, only two-thirds of ulcers eventually heal, with the remainder resulting in some form of amputation. Worldwide over one million lower extremity amputations are performed annually on people suffering from diabetes, and the majority of these amputations are preceded by ulcers. It is estimated that more than 3.2 million people will be living with limb loss by 2050.

Accurate diagnosis of the underlying cause of lower extremity ulceration is essential for successful treatment. The etiology of most leg ulcers can be ascertained quite accurately by careful, problem-focused history taking and physical examination. Diagnostic and laboratory studies are occasionally necessary to establish the diagnosis, but are more often performed to guide treatment strategy. Patients with ulcers due to venous insufficiency usually complain of aching and swelling of the legs. They may recount a history of recurrent cellulitis, previous deep-vein thrombosis, or previous superficial venous surgery. Symptoms are often worse at the end of the day, exacerbated when the leg is dependent, and relieved by leg elevation. Arterial insufficiency is suggested by a history of underlying cardiac or cerebrovascular disease, complaints of leg pain brought on with activity and relieved with rest (intermittent claudication), or pain in the forefoot aggravated by elevation and relieved by dependency (rest pain). The presence of an extremity ulcer is an easily recognized but late sign of peripheral vascular insufficiency. Patients with lower extremity ulcers resulting from atherosclerotic disease usually have a risk factor profile that includes older age, male sex, smoking, diabetes mellitus, hypertension, hypercholesterolemia, and obesity [23, 52]. Up to one-third of patients with diabetes mellitus can have significant atherosclerotic disease, without specific symptoms. Most common cause of ulcers in diabetic patients is neuropathic disease, which includes history of numbness, paresthesias, and burning pain in the lower extremities. Patients often report previous episodes of foot ulcers and chronic skin infections.

Although there are many options, the reconstructive ladder should be utilized. STSG offers fast effective closure of wounds. STSG maximization is predicated on several factors: graft failure in the presence of infection, highlighting the importance of biofilm management and eradication; strong initial take or incorporation occurring by diffusion of nutrition from the recipient site, termed “plasmatic imbibition”; and immobilization of the graft to prevent shearing, seroma, and hematoma formation beneath the graft. STSGs must be placed on a well-vascularized bed with low bacterial counts. Skin grafts generally will not take on poorly vascularized wound bed, such as bare tendons, cortical bone without periosteum, heavily irradiated areas, or infected wounds. However, virtually any tissue type with a vascular granulating bed is acceptable for grafting. NPWT has been shown to provide many aspects of STSG success, by promoting granulation tissue, lowering bacterial counts, and removing accumulated fluid such as hematoma/seroma, both of which reduce the chronic inflammatory process that occurs in chronic wounds such as elevated MMPs.

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# Diabetic Foot Infections

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and Michael Casteel

## 1 Introduction/Epidemiology

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, impaired insulin production (type 1), defective insulin utilization (type 2), or a combination thereof [1]. Type 1 diabetes involves the selective destruction of insulin-producing pancreatic β-cells while type 2 involves downregulation of peripheral insulin receptors and decreased insulin utilization. Of the two, type 1 diabetes represents roughly 10% of the world's cases, while its counterpart represents 90% of cases [2]. The incidence of diabetes increases year by year and was the seventh leading cause of death worldwide in 2010 [3]. It is estimated that the worldwide prevalence of diabetes will rise from 415 million in 2015 to 642 million in 2040 [3].

There are many clinical consequences of diabetes including autonomic dysfunction, retinopathy, and nephropathy. However, diabetic foot ulcers (DFUs) are one of the most common and serious complications of diabetes, affecting nearly 15% of all diabetic patients. Of patients with diabetic foot ulceration, 20% will have inadequate blood flow, 50% will have neuropathy, and approximately 80% will have both conditions [3]. In addition, the rate of lower extremity amputation is 15-fold greater in diabetics compared to nondiabetics [1]. It is therefore integral that specialists of different disciplines work hand in hand to tackle all aspects of this debilitating disease.

## 2 Pathophysiology

Hyperglycemia is at the center of the physiologically negative effects of diabetes. In the human body, the polyol pathway is responsible for the metabolism of excess glucose into sorbitol and, eventually, fructose. Glucose is first degraded by aldose reductase into sorbitol, followed by the conversion of sorbitol into fructose by sorbitol dehydrogenase. With hyperglycemia, large amounts of sorbitol and fructose are created by this pathway, resulting in oxidative stress, endothelial dysfunction, inhibition of nitric oxide production, and formation of nonenzymatic advanced glycation end products (AGEs) [4, 5]. In addition, fructose is a ten times more potent glycation agent than glucose [6].

AGEs are produced when glucose (or fructose) binds with cellular proteins, nucleic acids, and lipids, resulting in the formation of a product known as a Schiff base. This product then rearranges itself into a different form known as an

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Amadori product; it is the Amadori product that is the direct precursor to AGEs. Once formed, AGEs interact with cellular surface receptors (RAGEs) to convert those molecules into pro-oxidant, procoagulant, and pro-inflammatory agents [1, 4, 7]. AGEs also produce a biochemical alteration of joint and muscular tissue by increasing collagen cross-links. This leads to mechanical alteration of the tissues with a resultant loss of elasticity and tensile strength. [8].

Within the vascular endothelium, AGE accumulation leads to oxidative damage, basement membrane thickening, and a propensity to develop atherosclerotic plaques. AGEs also reduce the bioavailability and activity of endothelium-derived nitric oxide, decreasing vessel's vasodilatory potential [7]. Oxidative stress by AGEs is further compounded by the depletion of NADPH in the polyol pathway, decreasing the NADPH needed for production of key antioxidants such as glutathione. The end result of these physiologic changes is micro- and macrovascular compromise leading to retinopathy, nephropathy, and neuropathy [4, 9].

The pathogenesis of the diabetic foot ulcer is a multifactorial combination of vascular disease, neuropathy, and autonomic dysfunction. In regard to vascular disease, diabetic patients can develop calcifications of the endothelial tunica media leading in a loss of vessel elasticity. This calcification, known as Mönckeberg's sclerosis, is secondary to the differentiation of vascular smooth muscle cells into chondrocyte-like cells, capable of expressing and releasing proteins regulating calcification [10]. As a result of this calcification, it is easier for atherosclerotic plaques to develop along the intimal lining, damaging the vessels in the process and putting the patient at risk for ischemia [11].

Diabetic peripheral neuropathy has three main components and how it impacts the diabetic patient.

## 2.1 Loss of Protective Sensation

The neuropathic manifestations of diabetes include the loss of protective sensation, proprioception, temperature recognition, decreased sweating, and decreased muscle tone (specifically the intrinsic muscles of the foot). Nerve damage stems from the accumulation of reactive oxygen species secondary to the polyol pathway, as well as a loss of nerve blood flow from nutrient arteries known as the vasa nervorum [6]. With a loss of protective sensation, the diabetic foot is more apt to mechanical and thermal injury. Often, patients do not recognize cutaneous damage to their feet until they start noticing other manifestations, such as drainage and malodor coming from the wound.

## 2.2 Autonomic Diabetic Peripheral Neuropathy

Damage to the autonomic nervous system causes the opening of cutaneous arteriovenous shunts and malfunction of the precapillary sphincter, resulting in decreased blood flow and dry skin [12].

## 2.3 Motor Diabetic Neuropathy

Intrinsic pedal musculature also loses its tone and mechanical strength, resulting in extrinsic muscles from the leg gaining mechanical advantage. Glycosylation of muscle and tendon structures also leads to stiffness and a loss of joint range of motion, specifically the gastroc-soleus aponeurosis [8]. The sum of these changes leads to the formation of biomechanical pathology (i.e., hammer toes, equinus), abnormal pressure distribution, and cutaneous ulceration [4].

Diabetic patients have a decreased ability to combat infection. Hyperglycemia has been shown to inhibit the chemotactic, phagocytic, and antimicrobial activities of neutrophils and promote the nonenzymatic glycosylation (and eventual damage) of immunoglobins [13, 14]. Studies have also shown a decrease in the proliferative function of CD4 lymphocytes in diabetic patients [13]. As a result, diabetics are not only more susceptible to infection but also have a harder time mounting an adequate immune response.

**Case 1** (Fig. 1)
This is a diabetic with an ischemic third digit with a deep-space infection. Wound is foul smelling, fluctuant with drainage. The patient

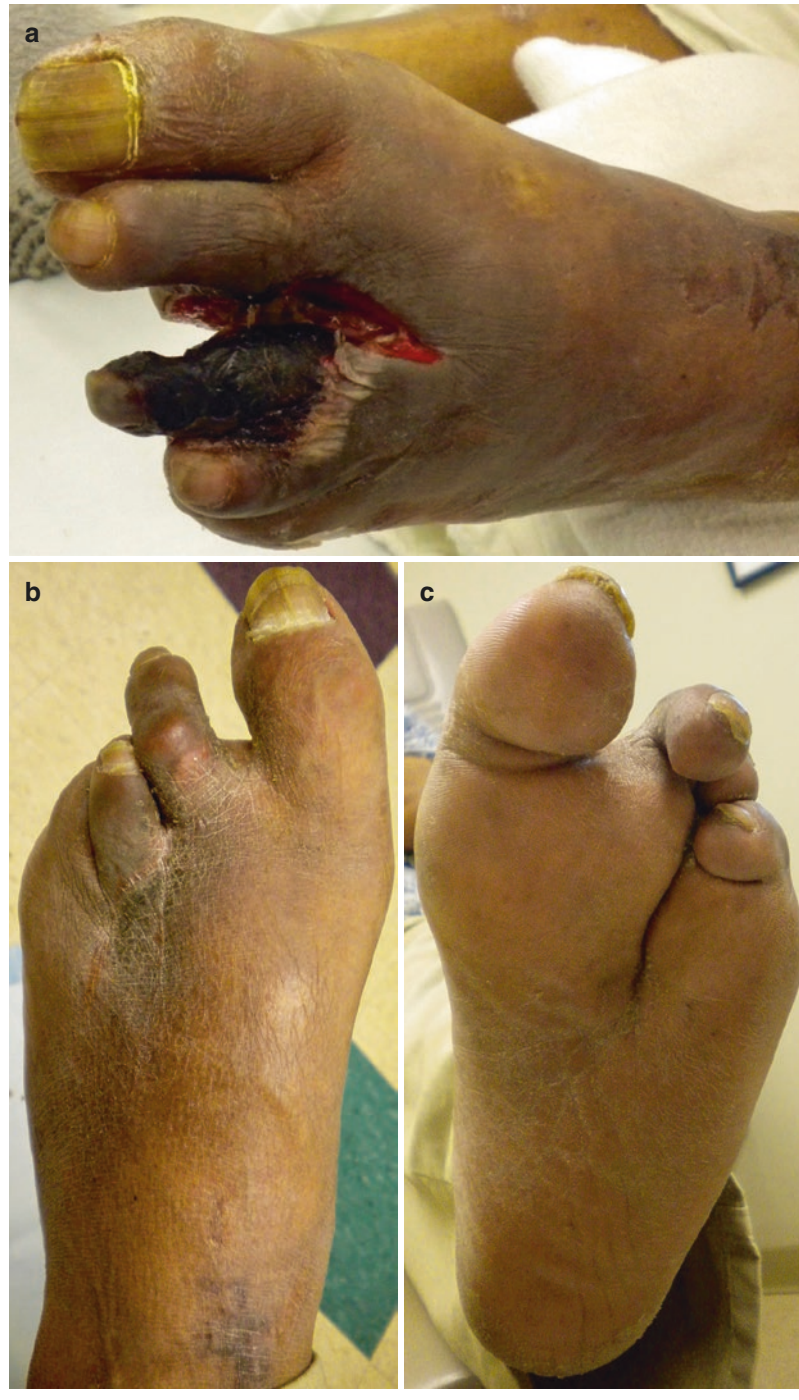


**Fig. 1** (**a**) Preoperative. (**b**, **c**) After deep-bone and soft-tissue resection and the use of negative-pressure therapy and hyperbaric oxygen treatment, the wounds healed well

presented with an elevated white blood cell count, inflammatory markers, and a clinical presentation of an urgent need to go to surgery for an aggressive incision and drainage, bone and soft-tissue debridement, and a bone biopsy with irrigation. There was an aggressive bone and deep soft-tissue resection, followed by the use of negative-pressure therapy and hyperbaric oxygen treatment. The wounds are well healed and the patient now wears custom-made diabetic shoes and is completely independent and functional.

## 3 Risk Factors

Several risk factors have been identified to reduce the risk associated with ulcers, infection, and amputation. The most important of these are a history of previous ulceration, neuropathy, foot deformity, and peripheral vascular disease [15]. A study of 1300 type 2 diabetics recognized the above risk factors, as well as an elevated hemoglobin A1C (>7), as the best predictors of risk for amputation [15]. These risk factors have been utilized to develop risk classification systems to aid providers in categorizing patients. One of the most widely used systems is the International Working Group on the Diabetic Foot [16]. They categorize patients as follows:

Group 0—no neuropathy
Group 1—neuropathy with no deformity or PVD
Group 2—neuropathy with deformity or PVD
Group 3—history of ulceration or amputation

In a prospective study of 225 diabetic patients, stratification using this system was found to be predictive of amputation and ulceration, with only patients classified in groups 2 and 3 undergoing an amputation. This study underscores that those patients with these specific risk factors are at greatest risk. Diabetic patients that develop any or all of these attributes require close monitoring [17].

Unfortunately, shortcomings by physicians have been identified in several studies regarding these risk factors being identified or monitored. A survey of over 1400 clinicians regarding their adherence to the recommendations of routine foot care by the American Diabetes Association showed only a 50% compliance rate with semiannual neurologic and foot exams [18]. Additionally, a retrospective review of a major California health maintenance organization identified 14,539 diabetic patients, only 6% of which had a documented diabetic foot exam within the last 12 calendar months [19].

**Case 2** (Fig. 2)
This is a young diabetic male who was previously treated at an outside institution and presented with earlier great toe amputation and an attempt to salvage the dorsal soft tissues. The patient now presents with a severe diabetic foot infection with an elevated white count, inflammatory markers, and clinical signs of severe infection. Following aggressive debridement and resection of all necrotic issue and after the use of negative-pressure therapy, the wound bed is prepared for a split-thickness skin graft for coverage of the wound. A split-thickness skin graft harvested from the thigh is applied to the dorm of the foot.

## 4 Workup/Diagnosis

In addition to a thorough history, the physical exam performed by the provider/surgeon is the most vital step in identifying diabetic foot infections. The goal of the exam should be to determine the extent and severity of infection, identifying underlying factors that predispose to and promote infection, and assessing the microbial etiology. Initial examination begins with assessment of the patient's vital signs, temperature, heart rate, respiration rate, and blood pressure. The core measurements can instantly provide feedback to the provider of the severity of the patient. One should perform a brief general physical exam to eliminate other possible sources of infection or systemic distress. Lower extremity assessment should be next, and should cover the five major systems consisting of dermatological, musculoskeletal, orthopedic, neurological, and vascular. Identification of the cause and source of the infection, likely from a wound, is critical. Full assessment of the wound should include measurements, depth, tracking, tunneling, exposure of bone, purulence, fluctuant, or crepitus. Debridement may need to be performed in this initial stage in order to obtain an accurate culture. Global or isolated foot deformities contributing to the cause of the infection should be identified at this stage. Extent of swelling, edema, cellulitis, lymphangitis, and palpable lymph nodes should all be noted. Consultation of other medical or surgical services should be determined by your physical exam. Several studies have reported improved outcomes with a multidisciplinary approach to diabetic foot infections. This includes involvement of specialists in wound care, infectious

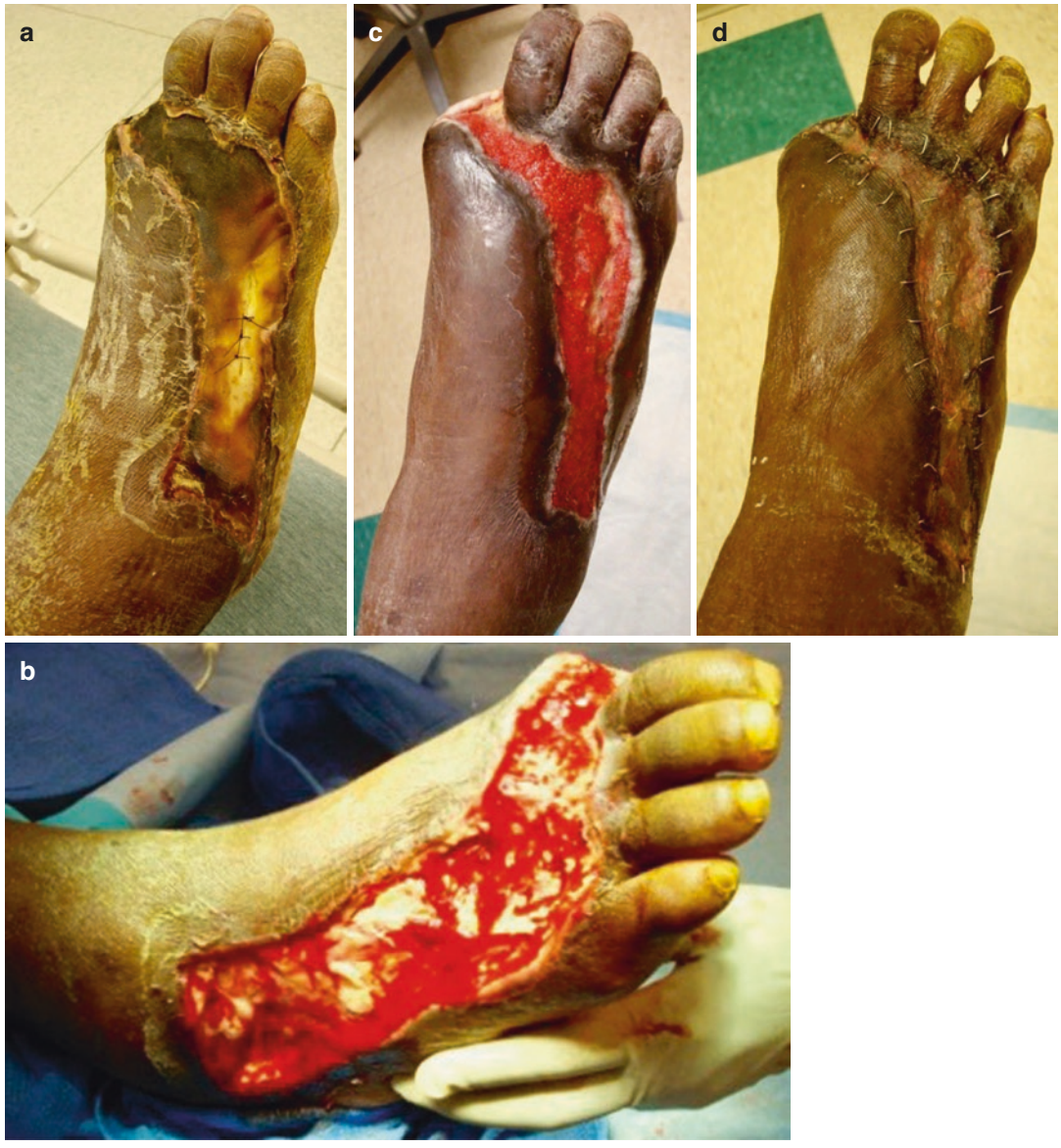


**Fig. 2** (**a**) Preoperative. (**b**) Following aggressive debridement and resection of all necrotic issue. (**c**) Following negative-pressure therapy, the wound is prepared for a split-thickness graft. (**d**) Following split-thickness skin graft

diseases, endocrinology, and surgery [20–22]. It has been the author's experience that the diabetic patient appears to respond best when a foot and ankle specialist is involved. The foot and ankle specialist is often on the "front lines" in treating these patients. The quicker and more aggressively this patient population is treated the more likely limb salvage is successful. Additionally, there is typically an underling cause of the initiating wound from failed biomechanics. Once the infection is stabilized a qualified foot and ankle surgeon should attempt to balance the foot and ankle through soft-tissue and/or bony reconstruction to eliminate future problems when appropriate.

Laboratory testing and advanced imaging are the next critical steps after physical exam by the provider. When treating the diabetic patient with a limb-threatening infection, laboratory values can provide information in determining the patient's

medical status globally, as well as the severity of the infection. It is common practice for the diabetic infected patient to undergo CBC, CMP, ESR, CRP, renal and hepatic testing, pan culturing, X-rays, and noninvasive vascular studies. Although these values can guide treatment, they should not be relied on solely. As already established, this patient population is immunocompromised and lab values can be grossly skewed or underestimated [13, 14]. Hepatic and renal function testing not only can guide or aid in antibiotic selection, but can also give the provider a gauge of the patient's immunocompromised status. ESR and CRP, while nonspecific, are used as indicators of systemic inflammation. Specifically, they can be indicators of bone infection when elevated, and when trended over an extended period can be indicators of therapeutic success. It is also common practice for these patients to receive lactic acid and procalcitonin lab monitoring in cases of severe sepsis [23]. But these values have yet to be universally utilized by foot and ankle surgeons since they are not always readily available. Recent literature has shown that procalcitonin can be an effective biomarker for diabetic foot infection and its therapeutic response [24].

Culturing of the diabetic foot infection should involve deep tissue, and depending on the situation bone as well. A meta-analysis showed that superficial swabs have low predictive value of 49% sensitivity and 62% specificity. Additionally, after deeper tissue cultures were performed, antibiotic therapy was changed 56% of the time [25]. Cultures should be taken prior to the administration of antibiotic therapy, and in the most sterile setting whenever possible. In cases of severe septicemia and extreme limb salvage situations provider should not delay appropriate therapy to obtain a higher yield culture. Blood cultures should also routinely be taken in the moderate-to-severe diabetic foot infections as these patients are prone to bacteremia and septicemia. Bone cultures should be taken through uninfected tissue whenever possible, and the provider should consider multiple specimens as the situation dictates. Bone should also be sent to pathology for evaluation.

Noninvasive vascular studies are grossly underutilized in the treatment of diabetic foot infection patients. As previously described in this chapter, the pathophysiology of diabetes leaves these individuals prone to vascular disease. Diabetics with PAD have a threefold increased risk for amputation [26]. It is estimated that 20–30% of diabetic patients have PAD, and 40% of those that present with infections [27]. Accurate and rapid identification of this can ultimately determine the outcome for these patients. Adequate perfusion to the area of infection is paramount for antibiotic delivery, and tissue oxygenation for healing and recovery [28]. Even patients with palpable pulse baseline levels should be established in the setting of limb-threatening infection. ABIs have been shown to underestimate PAD in up to 40% of patients, due to calcification of vessels [29]. Several studies have showed that an absolute toe pressure >30 mmHg is favorable for wound healing although toe pressures >45 to 55 mmHg may be required for healing in patients with diabetes. Because the digital vessels are spared from calcifications, toe pressures are useful to define perfusion at the level of the foot, especially in patients with incompressible vessels [30–32].

Advanced imaging may also be warranted, although surgical intervention of emergent limb-threatening infections should not be delayed. When physical exam eludes to possible deep infection, or in cases where infection is caused by a foreign body/puncture wound, MRI can be of high yield to the surgeon to identify deep or tracking abscesses. In cases of osteomyelitis, MRI can be of beneficial use given the lag of X-ray bone changes that are typically indicative of concern. Additionally, bone scans can be of limited use in patients when trying to discern osteomyelitis from Charcot neuroarthropathy. Despite the advances in imaging in regard to these predicaments, surgical soft-tissue and bone debridement, biopsy, and culture remain the gold standard as shown by Senneville et al. [33] as the most successful outcome predictor.

**Case 3** (Fig. 3)
This is a diabetic foot infection that began from a long-standing diabetic foot ulcer from the plantar aspect of the first metatarsal. The infection became deep and tracked proximally both dorsally and

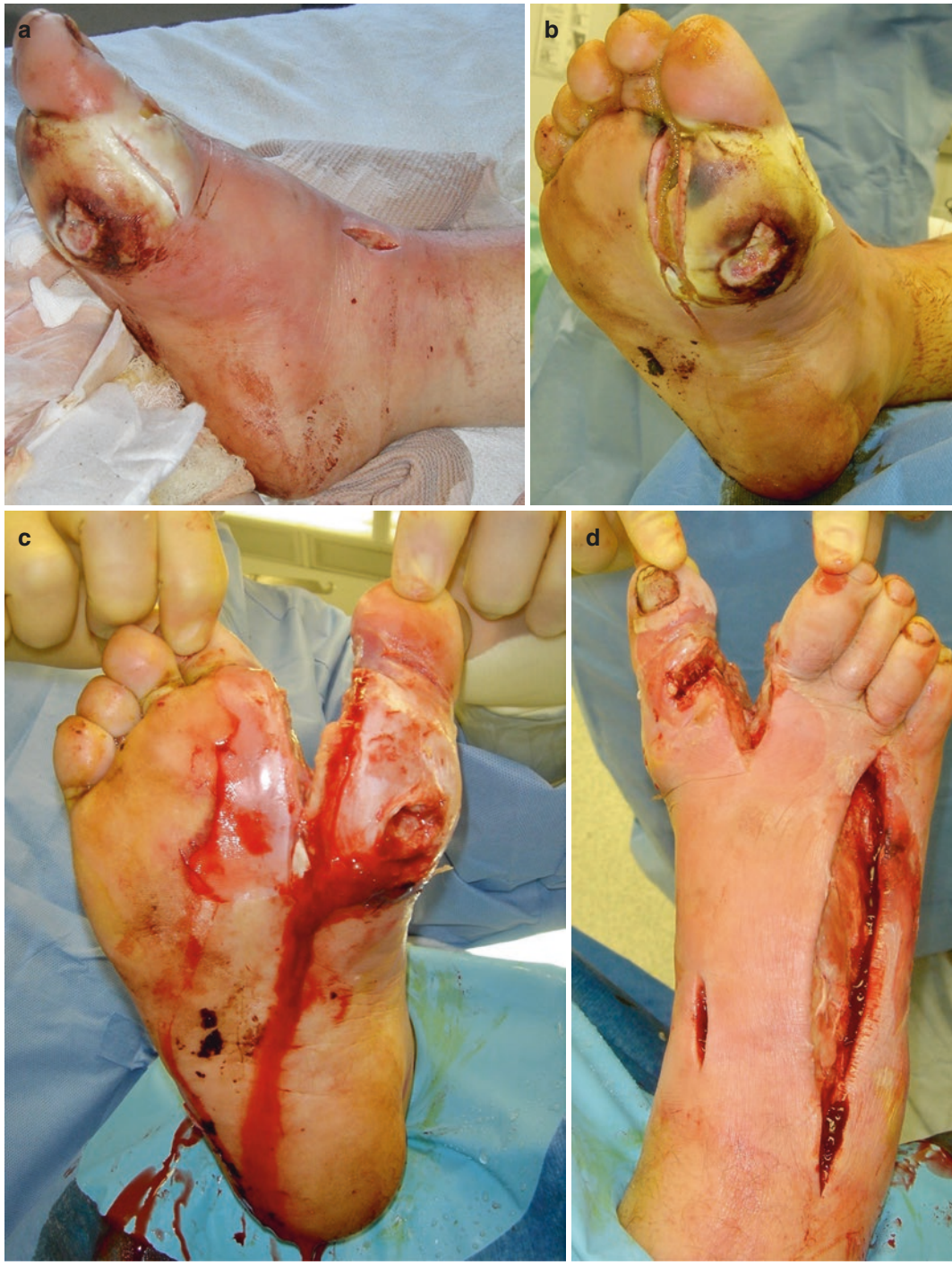


**Fig. 3** (**a**, **b**) A diabetic foot infection that began from a long-standing diabetic foot ulcer from the plantar aspect of the first metatarsal. The infection became deep and tracked proximally both dorsally and plantarly along the tendon sheaths creating a severe emergent diabetic foot infection. (**c**, **d**) Following multiple bone and soft-tissue debridements along with negative-pressure wound care, the wounds appear to be much healthier, with improved tissue and color along with a reduction with edema. (**e**) The diabetic foot infection utilizing negative-pressure therapy. (**f**) The diabetic foot infection is healing and maintaining a stable, plantigrade foot

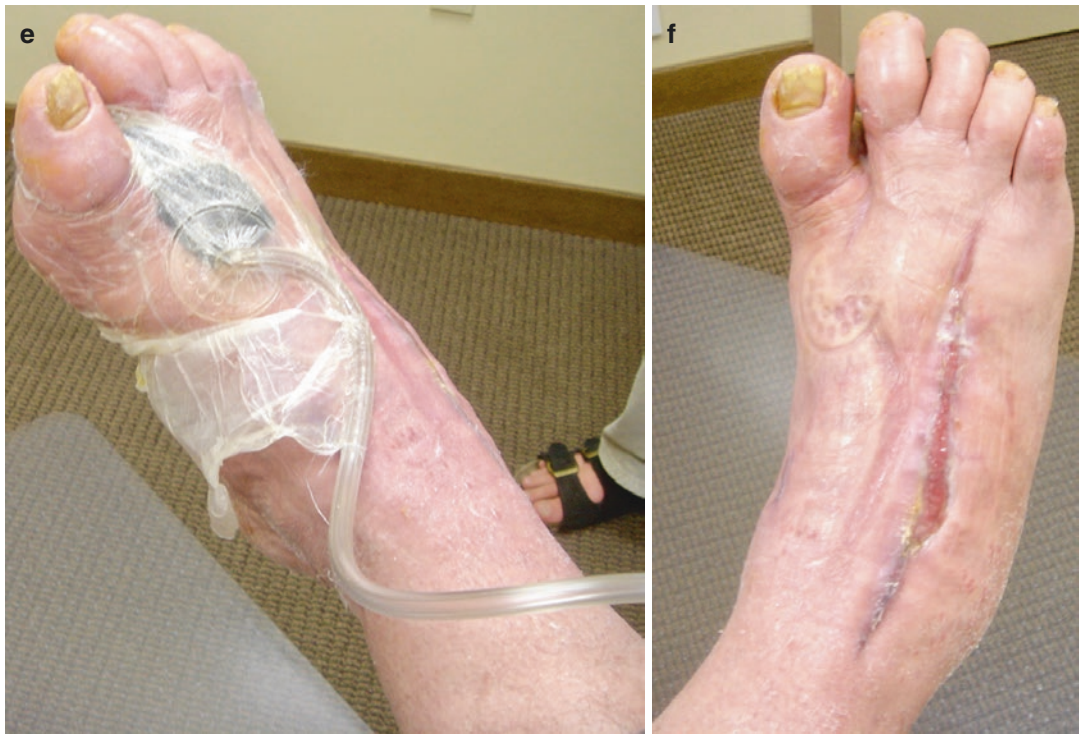


**Fig. 3** (continued)

plantarly along the tendon sheaths creating a severe emergent diabetic foot infection. Following multiple bone and soft-tissue debridements along with negative-pressure wound care, the wounds appear to be much healthier, with improved tissue and color along with a reduction with edema. There is maintenance of a stable, plantigrade foot with wounds that are successfully healing.

## 5 Treatment and Surgical Management

Treating the infected diabetic foot presents its challenges to providers. The IDSA Practice Guidelines for the Diagnosis and Treatment of Diabetic Foot Infections have been evaluated as a useful tool in grading, and accurately treating these patients [21, 22]. The system gives the provider a tool in predicting the likely causative organism, and guidance in selecting appropriate empiric therapy or whether to pursue hospitalization. Under these guidelines infections are graded as mild, moderate, or severe.

Mild infections are classified as showing two cardinal signs of infection and a host response. There is generally cellulitis localized to the area and not extending greater than 2 cm in any plane. Pus may be present. There is no ascending cellulitis or lymphangitis; vital signs are within normal limits. WBC count and blood glucose levels should be within the patient's baseline. These patients can be treated with oral antibiotic therapy directed toward gram-positive organisms. It has been shown that the majority of these infections are caused by Staph aureus/Group B Strep, and broader spectrum therapy is no longer warranted in these lower grade infections [21]. In patients with a history of CA-MRSA, hospitalization, or residence in long-term care facility, more aggressive oral therapy may be warranted based on patients' history and clinical indications.

Moderate infections are classified as showing greater than two cardinal signs of infection, and with cellulitis extending greater than 2 cm. There is extension of the infection beneath the superficial fascia into muscle or bone. The patient is

systemically well, and vitally stable, but with an elevated WBC count and elevated blood glucose abnormal to their respective baseline. The transition to severe infection has the same clinical indicators yet these patients are septic. They are vitally and/or metabolically unstable. Patients identified to have severe arterial insufficiency also fall into this category. These infections, contrary to mild infections, tend to be polymicrobial. Additionally, these infections are of greater risk for limb and life loss due to the infection. Staph aureus and Group B Strep continue to be the predominant organisms. Antibiotic coverage against other organisms is continually up for debate, as increasing evidence has shown that these organisms are not "infectious" [21]. Additionally the IDSA Guidelines also give providers an algorithm to help decision-making processes for surgical intervention.

A. When to Consider a Trial of Nonsurgical Treatment
   1. No persisting sepsis (after 48–72 h if on treatment)
   2. Patient can receive and tolerate appropriate antibiotic therapy
   3. Degree of bony destruction has not caused irretrievable compromise to mechanics of foot (bearing in mind potential for bony reconstitution)
   4. Patient prefers to avoid surgery
   5. Patient comorbidities confer high risk to surgery
   6. No contraindications to prolonged antibiotic therapy (e.g., high risk for *C. difficile* infection)
   7. Surgery not otherwise required to deal with adjacent soft-tissue infection or necrosis
B. When to Consider Surgical Intervention/Bone Resection
   1. Persistent sepsis syndrome with no other explanation
   2. Inability to deliver or patient to tolerate appropriate antibiotic therapy
   3. Progressive bony deterioration despite appropriate therapy
   4. Degree of bony destruction irretrievably compromises mechanics of foot
   5. Patient prefers to avoid prolonged antibiotics or to hasten wound healing
   6. To achieve a manageable soft-tissue wound or primary closure
   7. Prolonged antibiotic therapy is relatively contraindicated or is not likely to be effective (e.g., presence of renal failure)

Excisional and surgical debridement is pivotal and one of the most powerful modalities in the treatment of moderate-to-severe foot infections [34]. The removal of nonviable, contaminated, or infected material decreases the overall bioburden. This tissue is no longer "biologic," and is a harbinger to bacteria. With its removal, restoring a completely biologic environment reactivates the area increasing the capacity for healing [34, 35]. In cases of severe or necrotizing infections, rapid and aggressive debridement directly impacted salvage outcomes [36]. Sudarsky et al. [37] showed that patients who underwent surgical debridement more than 12 h after presentation had a higher amputation and mortality rate than those debrided sooner. The utility of early surgical debridement was illustrated in a retrospective review of 112 diabetic patients with severe foot infections. Those patients who underwent surgical intervention at the time of presentation had a significantly lower rate of above-ankle amputation than those who received debridement after 3 days of intravenous antimicrobial therapy prior to surgery. Irrigation has also been shown to decrease the overall bacterial load. While much debate has revolved around specific methods and products, low-pressure lavage with large volumes has been widely accepted [35].

Other modalities are available for surgeon usage including ultrasonic debridement devices and pulse lavage systems, and these should be used according to surgeon judgment. Negative-pressure therapy is another modality widely used in the diabetic foot infection setting. Negative-pressure therapy aids in exudate management, decreases the bacterial bioburden thru serial debridements with vac changes, and stimulates angiogenesis to the area. Newer systems even include timed irrigation of the wound sites to further decrease the bacterial load. In a randomized trial evaluating vacuum-assisted wound closure including 342 patients with diabetic foot

ulcers, complete ulcer closure was achieved more often among those who used vacuum-assisted closure than those who did not (43% vs. 29%, respectively) [38]. This should be considered on a case-by-case basis.

Many surgical debridements of diabetic foot infections require multiple-staged procedures. It is during these follow-up procedures that one should consider adjunctive procedures to correct the structural or biomechanical abnormality that contributed to the development of the infection. As previously noted, these patients' tissues undergo glycosylation and lose their elasticity [8]. Therefore, the surgeon should consider soft-tissue contractures, as well as skeletal structural abnormalities. Without addressing these issues, the patient will be left in a compromised position and odds of successful limb salvage in jeopardy. Specifically, a gastroc or TAL has been shown to reduce forefoot pressures by 27%, thus reducing the risk of further ulceration [39]. Also falling into this category are those patients with Charcot deformity. Although this topic is too broad to cover in the scope of this chapter, these deformities should also be addressed whether surgically or with bracing to assure long-term success.

**Case 4** (Fig. 4)
This patient presents with a severe diabetic foot infection with an ischemic third toe, ascending cellulitis to the ankle and lower leg. This is a medical emergency as the patient is septic and the infection is progressing proximally. An aggressive incision and drainage of the foot were performed, halting the infectious process, and resection of the third toe was done. A split-thickness skin graft, harvested from the right thigh, was applied to the former infected site following multiple debridements associated with adjunctive care to prepare the wound bed for skin grafting. This patient had continued local wound care until the all wounds were completely remodeled.

## 6 Osteomyelitis

Osteomyelitis is of great concern as these patients are at higher risk for limb loss. Certain clinical findings can support the diagnosis of osteomyelitis. In two systematic reviews that evaluated the diagnostic accuracy of exam findings in the setting of diabetic foot ulcers, the following factors increase the likelihood of osteomyelitis: grossly visible bone or ability to probe to bone, ulcer size larger than 2 $cm^2$, ulcer duration longer than 1–2 weeks, and erythrocyte sedimentation rate (ESR) >70 mm/h [40, 41]. If the radiograph is indeterminate or normal and the diagnosis remains uncertain, such patients should undergo magnetic resonance imaging (MRI), which is highly sensitive and specific for osteomyelitis and superior to radiographs, three-phase bone scans, and white blood cell scans [40–43]. Biopsies and cultures of the bone in question remain the gold standard at guiding empirical therapy, and possible surgical debridement. In one retrospective study of diabetic patients with osteomyelitis of the toe or metatarsal head, remission (absence of signs of infection and no need for surgery after 1 year) was more likely in the 22 patients treated with regimens guided by bone biopsy data compared with the 28 treated based on swab culture data (82% vs. 50%) [33].

**Case 5** (Fig. 5)
This is a patient who came into the emergency room with a limb-threatening infection. The patient was septic and the infection involved the soft tissue and bone of the plantar right foot. Deep tissue and bone cultures and biopsies were performed. The patient was treated with long-term intravenous antibiotics and a multilevel external fixator was applied for stability and to maintain anatomical alignment. The patient underwent serial debridements in order to prepare the wound bed for skin grafting. Amputation of the fifth digit and ray was performed. A split-thickness skin graft was harvested from the thigh and applied to the foot. Once the wounds healed, the external fixator was removed and the patient was placed into an AFO and a pair of accommodative custom-made diabetic shoes to assist with his gait and function and provided continued independence.

**Case 6** (Fig. 6)
This is a patient who was seen in the ICU of the hospital with an extremely elevated white blood cell count. The patient was septic and in a diabetic

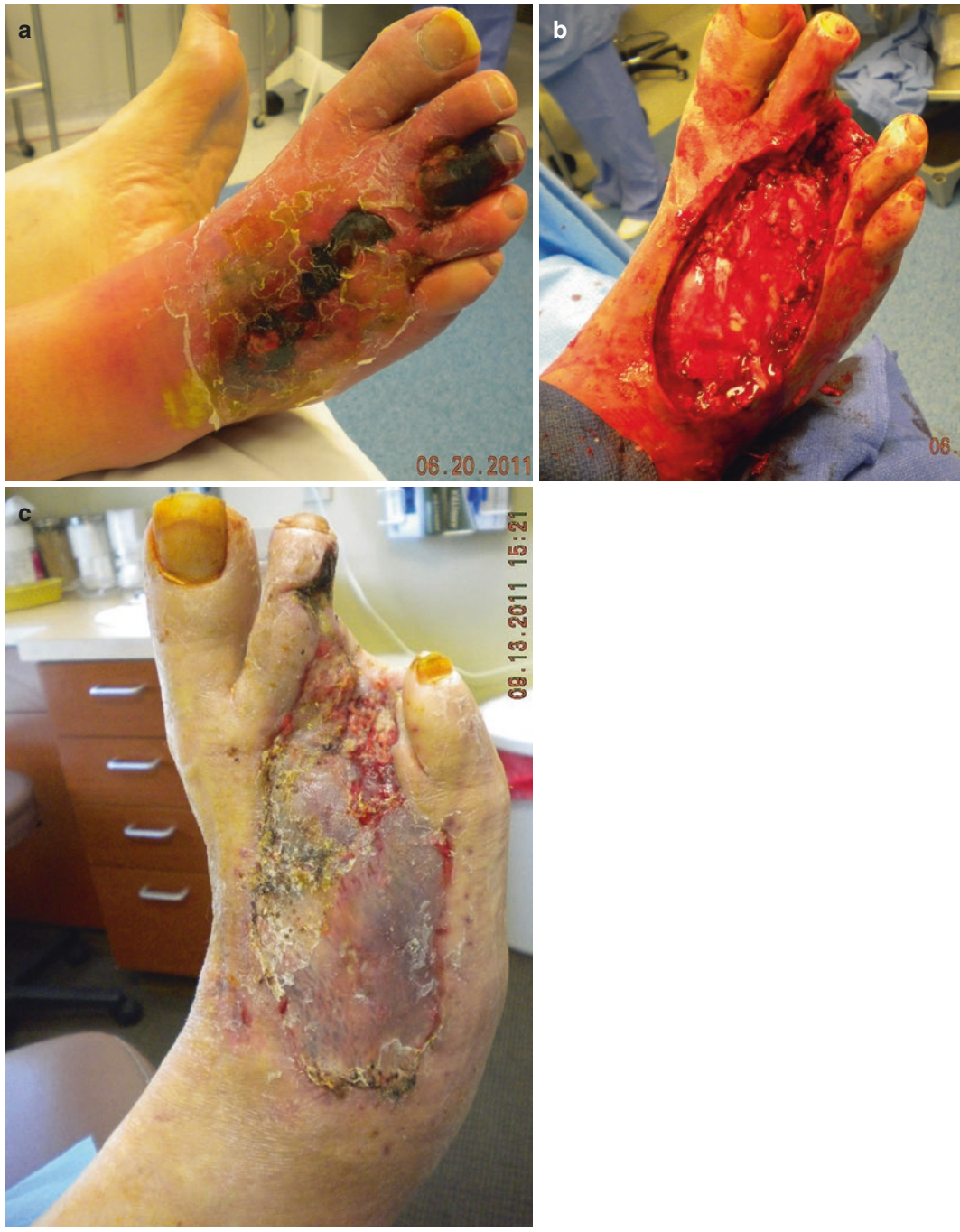


**Fig. 4** (**a**) Patient with severe diabetic foot infection. (**b**) Aggressive incision and drainage of the foot. (**c**) Following split-thickness skin graft

coma. He presented with red, hot, swollen ankle joint that was very fluctuant. There was valgus deformity of the ankle that caused a diabetic ulcer to the medial ankle leading to the diabetic ulcer of the medal ankle and a portal to the ankle joint. The talus was dislocated from the tibial talar joint

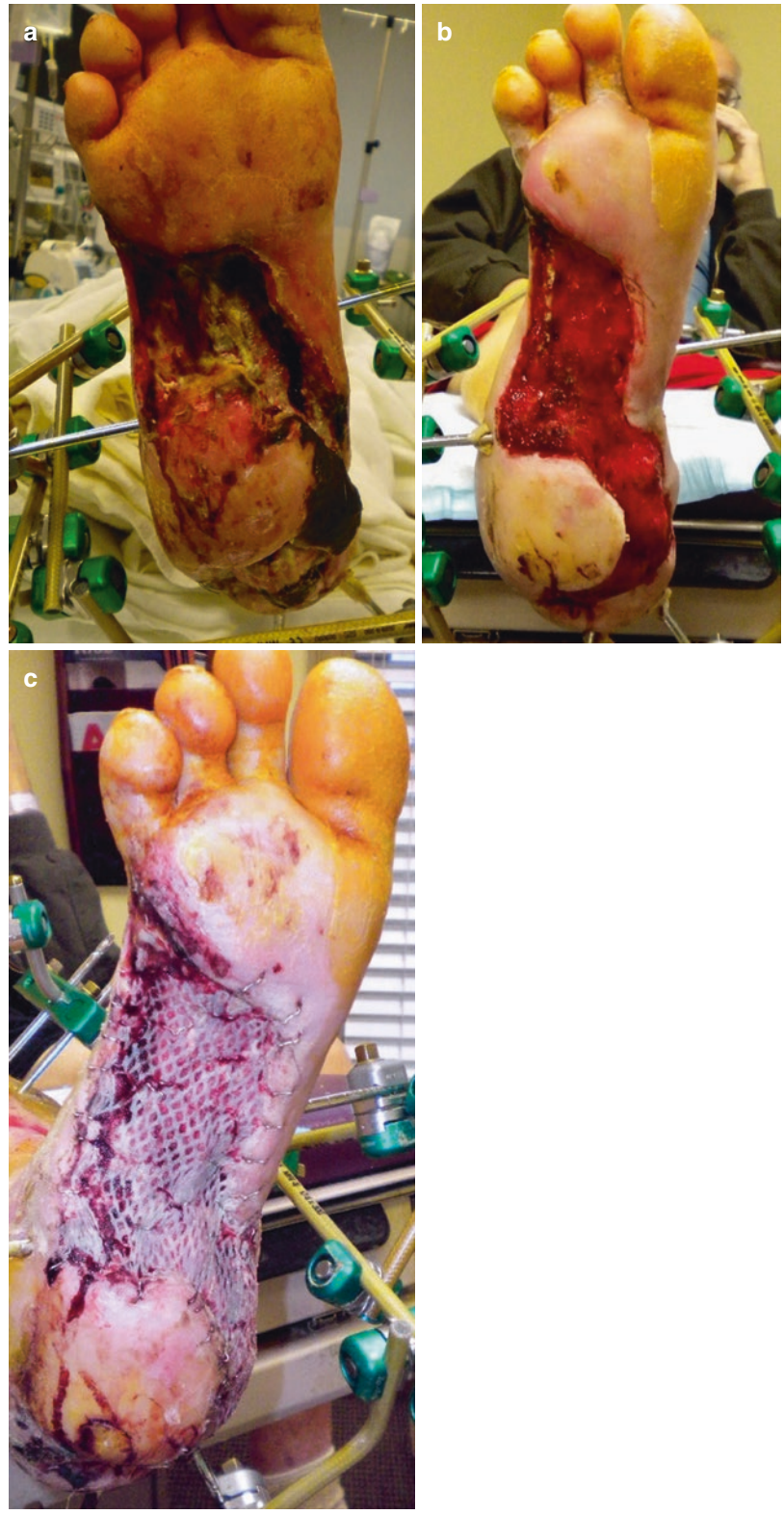


**Fig. 5** (**a**) Preoperative for the second debridement following an initial debridement and application of external fixations for gross instability of the mid foot and hind foot. The patient was admitted for sepsis stemming from a Charcot foot and ankle deformity. (**b**) Following multiple serial debridements, amputation of the fifth digit and ray demonstrating good granulation tissue and coverage over the osseous and soft-tissue defects. Note that the external fixation provides excellent stability. (**c**) Following a split-thickness skin graft that was harvested from the thigh and applied to the foot after multiple serial soft-tissue and bone debridements. Once the wounds healed, the external fixator was removed and the patient was placed into an AFO and a pair of accommodative custom-made diabetic shoes to assist with his gait and function and provided continued independence

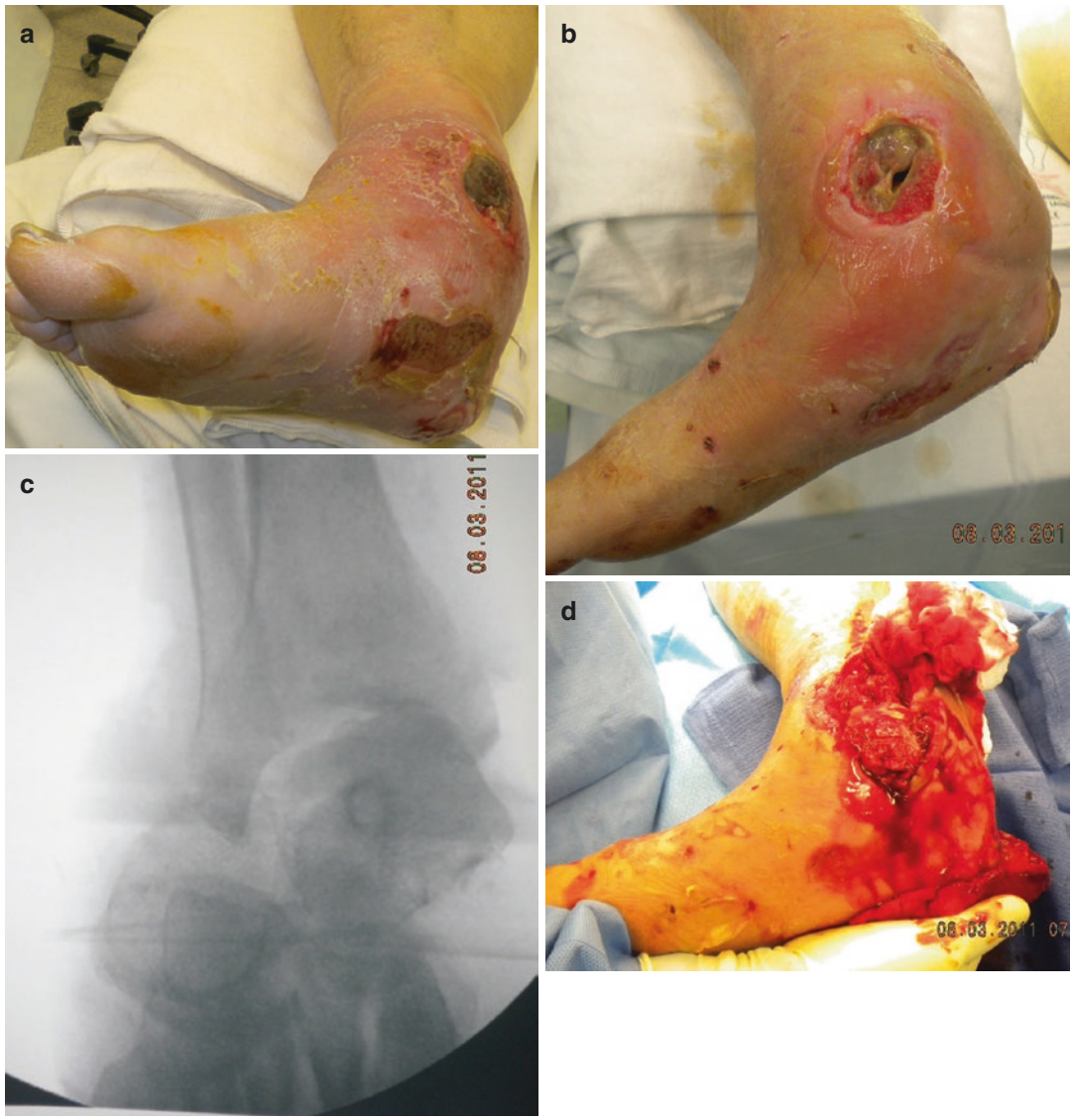


**Fig. 6** (**a**, **b**) This is a patient who was seen in the ICU of the hospital with an extremely elevated white blood cell count. The patient was septic and in a diabetic coma. He presented with red, hot, swollen ankle joint that was very fluctuant. Note the valgus deformity of the ankle that caused a diabetic ulcer to the medial ankle and a portal to the ankle joint. (**c**) The talus dislocated from the tibial talar joint as well as the subtalar joint secondary to severe infectious process of the ankle and subtalar joint. (**d**) An incision drainage with an aggressive resection of bone and soft tissue of the right ankle. (**e**) The talus was resected from the ankle joint. (**f**) An antibiotic-impregnated bone cement (polymethyl methacrylate) shaped similarly to the talus to fill the void and the dead space following the talectomy. The antibiotic spacer will provide and elude high doses of local antibiotics in combination with intravenous antibiotics to treat the osteomyelitis of the foot and ankle. (**g**) An external fixator was applied for stability and to maintain anatomic alignment. (**h**, **i**) Following multiple soft-tissue and bony debridements and long-term IV antibiotics, all inflammatory markers were stabilized and negative cultures were maintained. A reconstructive tibial calcaneal arthrodesis was performed providing excellent anatomic alignment, stability, and plantigrade foot and ankle allowing the patient to maintain function and independence

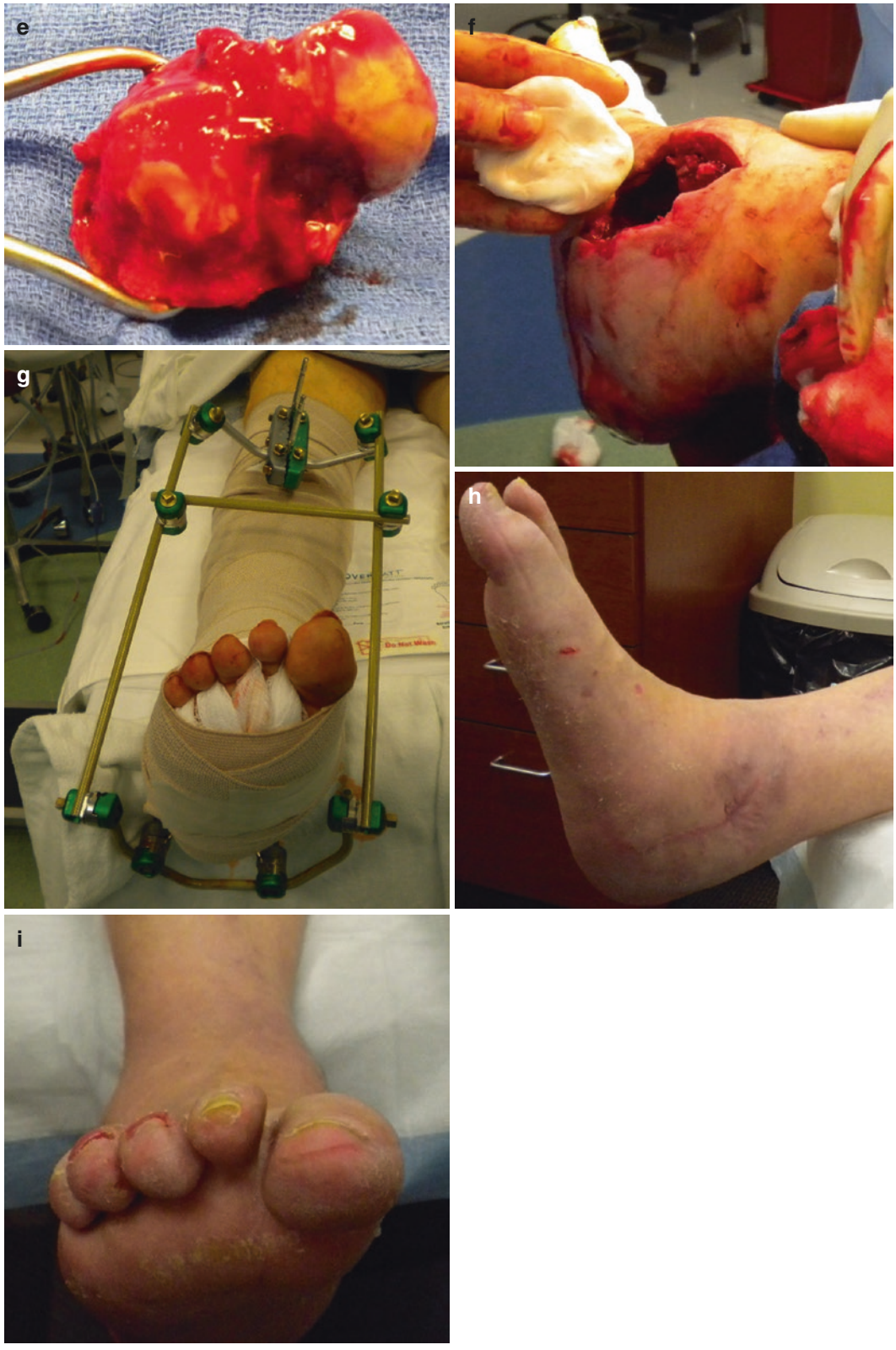


**Fig. 6** (continued)

as well as the subtalar joint secondary to severe infectious process of the ankle and subtalar joint. An incision and drainage were performed with aggressive resection of bone and soft tissue of the right ankle. The talus was resected from the ankle joint and an antibiotic-impregnated bone cement (polymethyl methacrylate) was shaped similar to the talus to fill the void and the dead space following the talectomy. The antibiotic spacer will provide and elude high doses of local antibiotics in combination with intravenous antibiotics to treat the osteomyelitis of the foot and ankle. An external fixator was applied for stability and to maintain anatomic alignment. Once all inflammatory markers were stabilized and negative cultures were maintained a reconstructive tibial calcaneal arthrodesis was performed providing excellent anatomic alignment, stability, and plantigrade foot and ankle allowing the patient to maintain function and independence.

## 7 Postsurgical/Long-Term Care

Maintenance of these patients is of utmost importance. One prospective study found a 70% 5-year recurrence rate among diabetics who primarily healed a foot ulcer [44]. Close monitoring, daily foot checks, and extreme diligence help prevent recurrence and early recognition of potentially hazardous complications. Good local wound care, off-loading, and accommodative shoe gear help reduce the risk of infection and need for possible amputation. A nonhealing ulcer precedes 85% of lower extremity amputations in diabetics [25, 26, 44]. Regular assessment for changes in vascular status should also be monitored. Noninvasive vascular studies should be considered on a yearly basis, or if a wound has not progressed by 50% with 4 weeks of standard local wound care. These patients quite often require custom bracing to achieve proper off-loading or accommodation for amputations. This should be handled by a qualified pedorthotist, and the patient should be checked routinely in case alterations or adjustments are needed.

Studies over the past two decades have established that the majority of diabetic foot ulcers take at least 20 weeks to heal [16, 17, 31]. Given these statistics, it is clear why aggressive wound care is necessary to facilitate closure and reduce the risk of infection and amputation. The longer the wound remains open, the greater the risk. Creation of an environment conducive to healing will remain the foundation of good foot care in diabetic patients.

### Conclusions

Limb salvage in a diabetic patient who is suspected of having a deep-space infection should be treated as early and aggressively as possible. This patient population can change abruptly for the worst given the circumstances if not treated appropriately. If in question, the physician should utilize all diagnostic modalities as needed as well as his/her clinical skills to make the diagnosis and error on the side of being aggressive with a surgical intervention. It has been the authors' experience that those patients who have been mistreated/undertreated continue to be at risk for limb and sometimes life-threatening scenarios. In the event that the patient has a component of peripheral vascular disease in the face of an infection, it is necessary for the foot and ankle physician to halt the infection and stabilize the patient and then consult vascular surgery for possible vascular reconstruction. The goal is to save a life and then a limb. If a patient has an infected extremity, the vascular surgeon cannot perform a vascular reconstruction in the event of a limb-threatening infection; therefore it is priority for the foot and ankle surgeon to halt the infection and stabilize the patient. Once the infection is halted and the wounds heal, reconstructive foot and ankle surgery can be performed in order to provide a stabile, plantigrade foot/ankle to allow the patient independence and function.

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# Low-Level Laser Therapy (LLLT) in Diabetes Mellitus for Wound Healing: Surgical Wound, Diabetic Ulcer and Burns

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and Thayrine Rosa Damasceno

## 1 Introduction

### 1.1 Diabetes

Diabetes mellitus (DM) is an ancient illness with symptoms described more than 3 millennia ago by the Egyptians. Constant thirst (polydipsia) and frequent urination (polyuria due to great urine volume) aroused the interest of several populations, including the Indian, that observed the sweetened characteristic of the urine and blood of the diabetic individuals. The final denomination of this pathology consolidated years later with the union of both findings throughout history regarding the signals and symptoms. Aretaeus of Cappadocia (81–133 AD) used the Greek word "diabetes" (siphon), while British Thomas Willis in 1675 inserted the word "mellitus" (sweet honey) [1].

Insipidus diabetes (tasteless), less frequent and less known, is also an illness presenting polyuria. However, its pathogenesis is related to alterations in production, secretion and/or antidiuretic hormone action (ADH), and its complications happen mainly from dehydration. While in DM, these disturbs originate from insulin, in which there is an insufficient regulation of blood sugar with repercussions in various organs, being abnormal wound healing an important aggravation [2].

The two main types of diabetes are classified according with their connection to insulin. When pancreas beta cells do not produce this hormone or the production is insufficient, it is called type 1 diabetes or insulin dependent. Moreover, there are cases in which this hormone is present in the organism; however there is a decrease of tissue sensibility to its action, and the body is unable to use it efficiently, being classified as type 2 diabetes, which is the most prevalent of the two [3].

Insulin is a hormone produced in the pancreas that acts on the carbohydrate metabolism and that is also related to protein and lipid metabolism. One of its main functions is regulating glucose in the blood, so as that it may be used inside cells for energy regeneration in the form of ATP (adenosine triphosphate), necessary for the development of its respective functions. The synthesis of many genes is modelled by insulin; for this reason the body metabolism can suffer alterations when the insulin action is not efficient [3, 4].

When not treated, DM may adversely affect the functioning of several systems, such as the visual, renal, nervous and cardiovascular system. According to the World Health Organization

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(WHO), hyperglycaemia-related deaths were 3.7 million in 2012, taking into account that 43% of the early deaths in these cases occurred before 70 years of age. Furthermore, the global prevalence of diabetes among adults went beyond 400 million registered in 2014, corresponding to a 8.5% of the entire population, data which practically doubled in correlation with the year of 1980, with 4.7% [5].

DM is therefore considered a public health problem. Repercussions are extensive and affect the lives of diabetics physically, psychologically and socioeconomically. Abnormal wound healing, characteristic of the illness, is frequently associated with pain and infection that demand intensive care, potentially leading to severe complications such as lower limb amputation. Constant hospitalizations are related to these aggravations or associated diseases. These factors, including depressing stages, are among the ones that alter the daily routine and decrease functionality and, consequently, life quality. The public system financial burden is high, and the injury management is a great challenge for health professionals, requiring a great multidisciplinary action [5, 6].

In this context, many trials have been realized aiming at improving the treatment and provide support for the patients and professionals, as to reduce the impact generated by the illness. Among the tools that have gained repercussions throughout the years, there is the photostimulation therapy using the low-intensity laser or low-level laser therapy (LLLT), which has been shown very promising in diabetic wound healing through in vitro and in vivo studies. It is a non-invasive intervention approach, employed in wound healing of various aetiologies aiming mainly at its acceleration and pain reduction. However, as with other therapeutic modalities available on the market, it is necessary that it be used according to the specificities of each situation of application. Deepening the knowledge regarding this technique provides base for a successful treatment [7–9].

## 2 Normal Wound Healing

The organs of the human body act together through systems and are connected for the perfect development of its functions. Among them, the skin is considered the most extensive. This organ is a physical barrier that is part of the first immunological defence line, known as natural immunity. Among other goals, its main function is to protect the organism against harmful agents of the environment. When an injury to the skin occurs, the body becomes vulnerable to the development of pathologies. For this reason wound cicatrization represents an important process for health integrity [10].

The loss of continuity and tissue functionality induces the onset of epithelial repair through three main stages which are interconnected: inflammation, proliferation and remodelling. After the injury, haemostasis starts the inflammation. Vascular responses act to control blood loss and reduce the injured area, inducing coagulation. The platelets adhere to the injured area and contribute for its tamponade with clot formation. An extracellular matrix (ECM) is established for receiving the other components. These are drawn by cytokines and growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β) produced by blood platelets. Cytokines and growth factors are substances (mostly proteins) that possess various origins and functions. They are launched during all stages, modelling cellular migration, proliferation and differentiation.

With the intervention of chemoattractant factors, neutrophils are the cells that mainly infiltrate the wound, followed by the action of macrophages, which are differentiated monocytes. These phagocytic cells have an important role in defending the metabolism, because they participate in cellular residue degradation and antigen combat. Furthermore, macrophages regulate this stage of the repair process and produce growth factors, which stimulate cellular reproduction and initiate the formation of granulation tissue [11].

In the proliferative phase, the epithelial tissue begins to be restored with the initial activity of fibroblasts and keratinocytes regulated through chemical mediators. The final stage of the proliferative phase shows a great blood supply for the formation of new vessels and a significant quantity of cells, including fibroblasts, phagocytes, granulocytes and collagen, being for this reason denominated granulation tissue.

ECM is rich in proteins, and its formation is necessary for acting as support, coordinating the activity of various cells until its final organization. In this context, fibroblasts are essential for producing substances that comprise this matrix such as, for example, collagen. Upon completing this stage, they will differentiate in myofibroblasts, which will act in the next phase.

The closure of wound healing or remodelling starts at approximately 21 days and may extend to 1 year. The local metabolism is progressively reduced, including the blood flow. Finally, the apoptosis (programmed death) of the cells involved in the previous phases concludes the formation of the granulation tissue. In this stage, there is the occurrence of some alterations in the extracellular matrix with the goal of wound maturation and enhancement of its tensile strength, such as collagen type III substitution for type I and wound contraction through action of myofibroblasts [12].

## 3 Diabetic Wound Healing

The metabolic chronic disturbance that characterizes DM interferes in the cicatrization cycle from the primary responses, but although the featured irregularities are attributed to a set of factors, the common origin of the majority of them relies on the inefficient action of insulin.

Concisely, some aspects may be highlighted. At the cellular level, the hormonal failure affects the obtaining of energy in the form of ATP and, consequently, its functional performance. Moreover, in diabetics there is a great prevalence of micro- and macroangiopathy. As a consequence of hyperglycaemia, there are endothelial damages and a haemoglobin glycosylation that hinders the delivery of oxygen to tissues. Vascular alterations affect perfusion, which may trigger the onset of hypoxia and reduce inputs of nutrients.

As a result, the leukocyte migration to the wound location is subject to a decline. In insufficient quantity and with an inadequate phagocytic capacity, neutrophils and macrophagocytes will have their performance affected, and the immune system therefore decreases its defensive ability. This deficit along with the excess of blood glucose increases infection vulnerability. Similarly, fibroblasts become dysfunctional and collagen production is directly affected. Quantity and quality decrease and the deposition is disorganized. There is the occurrence of a compensatory enhancement of inflammatory cytokines in response to the leukocyte deficit. The anti-inflammatory effect is suppressed and it reduces cellular proliferation. Thus, the initial phase of the repair process is prolonged. Physiologically, neutrophils produce proteases and proteins that remove cellular waste and help cellular migration. However, as the inflammatory stage extends in virtue of the elevated glycaemic index, deregulated protease action time contributes to the onset of oedema and the destruction of the new extracellular matrix. The deficit in the growth factor synthesis for various cellular groups also contributes significantly in delaying wound closure. If the first cicatrization phase is delayed, the following will be altered [13–15].

The formation of granulation tissue during the proliferation stage is an important step so that the whole cycle be effective, especially by means of neoangiogenesis and neocollagenesis. However, the metabolic activity and the great concentration of cells in this period need to be reduced progressively to enable epithelial restitution. If there were no apoptosis, these cells could remain in proliferation and affect the entire process. Normally, programmed death is higher in modelling phase, corresponding to when scars

are being formed, but it occurs since the inflammatory response with the finalization of neutrophilic functions. By contrast, in diabetics this happens frequently before time, favouring matrix destruction [16].

The advanced glycation end products (AGEs) are also involved in the genesis and in the progress of various DM-related pathologies, including abnormal wound healing. These compounds generated due to the electrolytic reduction of sugar come in a great variety of molecules which may be acquired as external sources as food and smoking or being produced by the organism mainly during ageing. In diabetics, AGEs are produced in excessive amount as a result of hyperglycaemia, causing, among other harmful effects, vascular damages. Through cross-links they are capable to induce structural and functional alterations in proteins and lipids. They may also contribute to enhancing oxidative stress by binding to receptors in the cell membrane and affect cellular functions [17].

The union of all of these factors makes the cure much slower than normal or even blocks it, a condition in which the wound becomes chronic. In situations in which wound closure is successful, the new resulting skin is generally fragile when compared to a normoglycaemic person. Once less resistant, the reoccurrence of a wound in this area is more likely to verify [16, 17].

## 4 Low-Level Laser Therapy (LLLT)

Research about Light Amplification by the Stimulated Emission of Radiation or LASER has as one of the main milestones in literature the study of Maiman in 1960 [18] with the inquiry on light irradiation through high-powered flash lamp on a Ruby Crystal, based on the principles of the quantum theory developed by Albert Einstein in 1917. Throughout the years, animal models and cell cultures were inserted in various studies with the aim of verifying the biological effects [18–20].

In 1971, Mester et al. [20] evaluated the low-energy laser irradiation in wound healing of mechanical wounds and third-degree burns induced in mice with 1, 4 and 5 J/cm$^2$ doses. Histological and photographic analyses showed acceleration in the repair, being the best result obtained with 1 J/cm$^2$. However the stimulating effect aroused doubts regarding possible late damages such as neoplasia development, encouraging research continuity.

In the 1980s clarification regarding the mechanisms of monochromatic light effect to the cellular level progressed significantly. Passarella et al. (1984) [21] through the helium and neon laser (He-Ne) and 5 J/cm$^2$ dose in vitro identified an increase in mitochondrial membrane potential and in protein synthesis, which rose to 70% with regard to the control. At the end of the research, they suggested that light absorption occurs in sensible components in the mitochondrial compartments.

The device of coherent light initially available on the market was the He-Ne laser with wavelength of 632.8 nm. This explains its extensive use since the first recorded experiments. Karu (1987) [22] was one of the pioneers in this research line which also made use of this wavelength. In her studies she asserted that the biological effects of the laser vary according to its wavelength and that primary and secondary mechanisms happen during and after the irradiation, respectively. She suggested that molecules in the respiratory chain such as flavoproteins (e.g. NADH-dehydrogenase) and terminal oxidase (e.g. cytochrome c oxidase) mitochondrials acted as photoacceptors, absorbing the incident light according with the wavelength. These electronically excited chromophores will provide a temporary temperature elevation.

Among the possible responses that occur from the light incidence are mainly changes in the redox properties, electron transfer acceleration and biochemical alterations. Subsequently, the amplification of these responses as secondary reactions, upon ceasing light stimulation, will affect the electrophysiological characteristics and the membrane potential. As a result, an ATP, DNA synthesis increase and cellular proliferation (Fig. 1) are obtained. Moreover according to the author, deregulated cells such as the ones that are

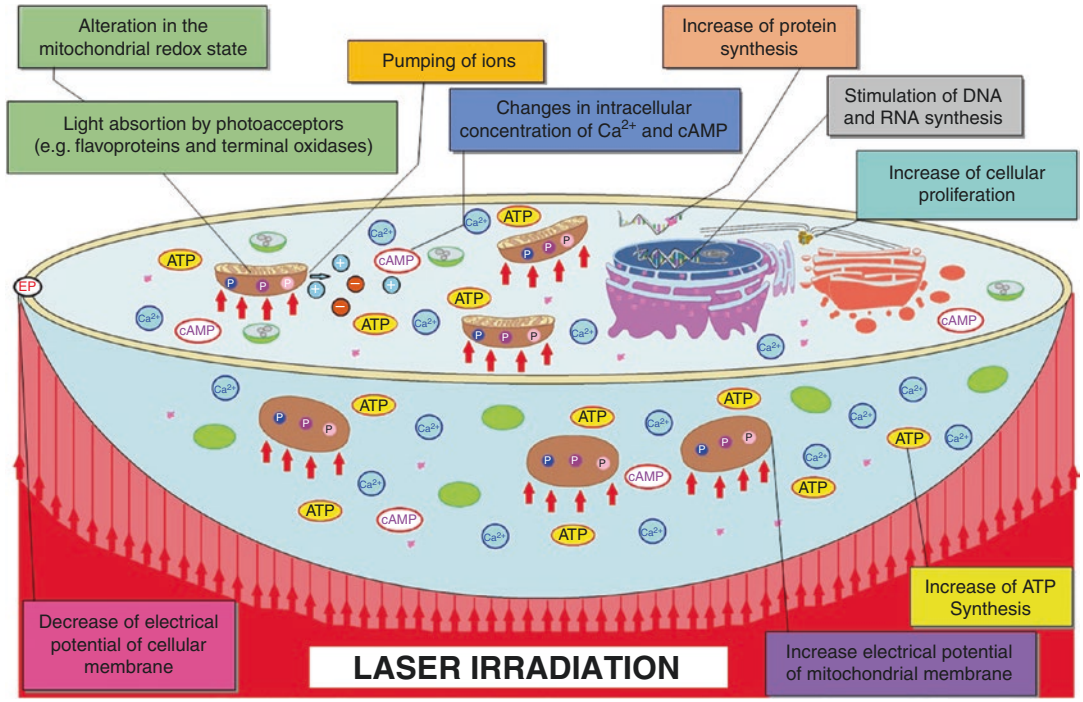


**Fig. 1** LLLT irradiation generated effects to cellular level. *ATP* adenosine triphosphate, *$Ca^{2+}$* calcium ion, *EP* electrical potential, *cAMP* cyclic adenosine monophosphate, *P* photoacceptors. Adapted from [23]

to be found in chronic wounds tend to better respond; in other words, the laser effects will probably be more evident once healthy cells, due to their regular functioning, would not need many adjustments or stimulations. Although the same wavelength is used, dosage is a very important factor, because according to its variation it may cause stimulating, inhibiting or even deleterious effects. Low doses characterized by short time tend to show better results for being closer to natural physiology. It is also important to consider that there are many cellular types and the responses of each one may vary according to its nature [22–24].

Research extended to human beings and entered in the clinical practice of countless pathologies while different laser types were originated. Laser therapy has as main characteristics monochromatic, coherent and collimated light, emitted from a gas, liquid or solid medium or through free electrons. Medical lasers available on the market may be classified in two categories: surgical lasers and therapeutic lasers, differentiated due to operating power. Unlike surgical lasers (e.g. laser de $CO_2$ and Alexandrite) which are capable of realizing tissue ablation through the use of high power and generated thermal effects, therapeutic lasers, also denominated low power or low-intensity lasers, operate through cellular biomodulation mechanisms, acting on pain control, inflammation and healing acceleration in injuries of different aetiologies [18–25].

Some implemented devices are of helium and neon (He-Ne), gallium-aluminium (GaAl), gallium-aluminium-arsenide (GaAlAs), aluminium-gallium-indium-phosphide (AlGaInP) and gallium-arsenide (GaAs). The emitted wavelength is what determines visibility, as well as its penetration capacity, which is directly proportional. Protection glasses are necessary during laser therapy to avoid sight injuries coming from potential radiation damages while operating in the visible aspect (e.g. He-Ne) as well as when working with invisible and infrared light (e.g. GaAs and GaAlAs). Experiments with

minor wavelengths realized in vitro tend to show good results, but it is important to consider that in those in vivo the existence of tissue layers may require infrared lasers for their reaching deeper structures. Regarding the form of application, waves may be used in a continuous or pulsed mode. Thus, all of these parameters, including energy density, power density and application time, need to be insightfully selected according to the particularities of each individual case [25–27].

The remarkable results obtained in tissue repair acceleration provided evidence that sustained the insertion of low-intensity lasers among the therapeutic modalities directed to diabetic patients. In these situations in which wounds are generally difficult to treat, laser irradiation helps regulating the inflammation period and the functionality of many cells during wound healing, including the ones of the immune system, fibroblasts, endothelial cells and keratinocytes, as well as reducing pain. Precocious wound closure is one of the most important contributions of laser therapy, because it minimizes the likelihood of complications. It is a non-invasive form of treatment that, in addition to being effective and with minimal risks, is economically viable [7, 8, 28–30].

## 5 Laser Therapy In Vitro

Research conducted on cell cultures has provided good clarifications regarding the LLLT action mechanisms and the hyperglycaemic condition simulations contributing so that the use in diabetic patients be more conscious and effective (Table 1). Among the selected parameters for wound irradiation, energy density or dosimetry plays a fundamental role for a treatment with positive results. In the Houreld and Abrahamse's (2007) [29] in vitro study, it happened that in diabetic wounded fibroblasts cells, a 5 J/cm$^2$ dose with 632.8 nm favoured cicatrization, while interleukin level 6 (IL-6), proliferation and cellular migration were superior with regard to the irradiated diabetic cells without injure. Moreover, migration was not observed in diabetic wounded cells that did not receive treatment.

According to the authors, these findings demonstrated that the beneficial effects of the laser may be much more evident in dysfunctional cells, since the light stimulus favours the regularization of its activities, while healthy cells have their functions preserved and do not show the same deficiencies than the previous ones, situation in which the laser influence may be insignificant. A 16 J/cm$^2$ dosimetry was also tested in this experiment: divergently, it showed inhibitory effects. Both irradiated groups (diabetic and diabetic wounded fibroblasts cells) were damaged in the analyses and did not show the benefits obtained with the lower dose [29].

Subsequently, in a study with similar sample, Houreld et al. (2010) [31] reaffirmed the modulation capability of laser therapy on pro-inflammatory cytokines in hyperglycaemic people. With 830 nm and a 5 J/cm$^2$ dose, interleukin-6 (IL-6) did not suffer any statistical alteration, but the interleukin levels1 beta (IL-1b) and tumour necrosis factor-alpha (TNF-α) were reduced. After 1 day from irradiation, the proliferation of diabetic fibroblasts and apoptosis reduction were significant and possible to be verified. In chronic wounds and in diabetic people, pro-inflammatory cytokines such as IL-1b e TNF-αgenerally are to be found in elevated levels. This alteration is important for having the capacity of generating a constant inflammatory cycle, affecting injury closure.

Using fibroblasts of the human skin with the same energy density and 660 nm, Ayuk et al., 2012 [7] also obtained excellent results, including chemotaxis stimulation, proliferation and collagen synthesis. However, they proved that despite the stimulating effect in irradiated wounded diabetic cells and the superior gains concerning the nonirradiated diabetic wounds, the collagen values produced were always lower when compared to normal cells that had not received the laser.

Based on their clinical practice with diabetic patients, Khoo et al. (2014) [27] selected the 810 nm laser considering this applicability more favourable due to its deeper penetration, aiming to verify how the fibroblasts of diabetic and

**Table 1** Laser therapy in diabetes mellitus (revision of the last 10 years)

| Author(s)/Year | Total sample | Treated diabetic group | Laser/Emission form | Energy density/Application time | Power density | Wavelength (nm) | Treatment schedule |
|---|---|---|---|---|---|---|---|
| *In vitro* | | | | | | | |
| Houreld and Abrahamse, 2007 | Cell culture | $N = 16$ | He-Ne/Continuous | 5 J/cm²/37 min; 16 J/cm²/2h | 2.206 mW/cm² | 632.8 | Single dose |
| Houreld et al., 2010 | Cell culture | $N = 6$ | Diode/DNE | 5 J/cm²/18 min 56 s | 4.4 mW/cm² | 830 | Single dose |
| Ayuk et al., 2012 | Cell culture | $N = 4$ | Diode/Continuous | 5 J/cm²/8 min 9 s | 10.22 mW/cm² | 660 | Single dose |
| Khoo et al., 2014 | Cell culture | DNE | GaAlAs/ Continuous | 1 J/cm²/1 min 40 s | 10 mW/cm² | 810 | Single dose |
| Esmaeelinejad et al., 2014 | Cell culture | $N = 54$ | He-Ne/DNE | 0.5 J/cm²/757 s; 1 J/cm²/1512 s; 2 J/cm²/3024 s | 0.66 mW/cm² | 632.8 | Daily irradiation for 3 days (first irradiation after 24 h) |
| *Surgical wounds* | | | | | | | |
| Dancáková et al., 2014 | 21 male Sprague–Dawley rats | $N = 7$ | Diode/Continuous | 0.9 J/cm²/wound/day 30 s/wound/day | 30 mW/cm² | 810 | Daily irradiation for 7 days (first irradiation immediately after surgery) |
| Güngörmüs and Akyol, 2009 | 36 female Wistar rats | $N = 9$ | GaAlAs/ Continuous | 10 J/cm²/DNE | 0.1 W/cm² | 808 | Irradiation with 2 days interval totalizing 5 sessions (first irradiation immediately after surgery) |
| Sharifian et al., 2014 | 48 male Wistar rats | $N = 24$ | Diode/Pulsed | 0.2 J/cm²/200 s/point (total of 18 points) | 1.08 mW/cm² | 890 | Irradiation 6 days per week (first irradiation immediately after surgery) |
| Tatmatsu-Rocha et al., 2016 | 20 male Swiss mice | $N = 5$ | GaAs/Pulsed | 18.28 J/cm²/60 s | 304.8 mW/cm² | 904 | Daily irradiation for 5 days |
| Lima et al., 2017 | 120 volunteers | $N = 11$ | GaAlInP/DNE | 6 J/cm²/60 s/point (total of 8 points) | 0.1 W/cm² | 660 | Irradiation with 2 days interval totalizing 5 sessions (first irradiation immediately after surgery) |
| *Ulcers* | | | | | | | |
| Kilík et al., 2014 | 48 male Sprague-Dawley rats | $N = 24$ | GaAlAs/DNE | 5 J/cm²/83 min 20 s | 1 mW/cm² | 635 | Daily irradiation for 6 days maximum |
| | | | | 5 J/cm²/16 min 40 s | 5 mW/cm² | | |
| | | | | 5 J/cm²/5 min 33 s | 15 mW/cm² | | |
| Hegde et al., 2011 | 105 male Swiss Albino mice | $N = 77$ | He-Ne/Continuous | 1 to 5 J/cm²/4 min 15 s to 21 min 17 s | 4.02 mW/cm² | 632.8 | Single dose immediately, after 24 h or after 48 h from wound induction |

(continued)

**Table 1** (continued)

| Author(s)/Year | Total sample | Treated diabetic group | Laser/Emission form | Energy density/Application time | Power density | Wavelength (nm) | Treatment schedule |
|---|---|---|---|---|---|---|---|
| Maiya et al., 2009 | 192 male Albino Wistar rats | *N* = 168 | He-Ne/Continuous | 3 to 9 J/cm²/3 to 27 min | DNE | 632.8 | Irradiation 5 days per week until the wounds healed completely |
| Eissa and Salih, 2017 | 14 Wistar rats (6 males and 8 females) | *N* = 7 | He-Ne/Continuous | DNE/4 min | 4.0 mW/cm² | 632.8 | Irradiation 5 days per week until the wounds healed completely |
| Al-Watban et al., 2007 | 57 male Sprague-Dawley rats | *N* = 48 | Diode/DNE | 5, 10, 20 and 30 J/cm²/3.8 to 32.1 min | 20.4 mW/cm² | 532 | Irradiation 3 times per week |
| | | | | | 15.56 mW/cm² | 633 | |
| | | | | | 22.22 mW/cm² | 810 | |
| | | | | | 22.22 mW/cm² | 980 | |
| Carvalho et al., 2010 | 30 male Wistar rats | *N* = 15 | GaAlInP/ Continuous | 4 J/cm²/24 s | DNE | 660 | DNE |
| Rocha et al., 2012 | 30 Non-obese diabetic mice (NOD) | *N* = 7 | GaAs/Pulsed | 3.8 J/cm²/20 s | DNE | 780 | Irradiation immediately after surgery and a second application 48 h after the surgical procedure |
| Noudeh et al., 2010 | 19 male Wistar rats | *N* = 5 | GaAlInP/DNE | 10 J/cm²/48 s | DNE | 670 | Irradiation each 3 days totalizing 7 sessions (first irradiation after 3 days from wound induction) |
| | | | GaAlAs/DNE | 12 J/50 s (1.33 J/cm²) | | 810 | |
| de Loura Santana et al., 2015 | 90 female Wistar rats | *N* = 60 | GaAlAs/DNE | 1 J/cm²/26 s (fractioned-dose group); 4 J/cm²/104 s (single dose group) | DNE | 660 ± 2 | Irradiation in single dose or 4 times (days 1, 3, 8 and 10) |
| Kajagar et al., 2012 | 68 volunteers | *N* = 34 | DNE | 2 to 4 J/cm²/DNE | DNE | DNE | Daily irradiation per 15 days |
| Kaviani et al., 2011 | 23 volunteers | *N* = 13 | DNE | 10 J/cm²/200 s | 50 mW/cm² | 685 | Irradiation 6 times per week during two weeks and then in alternated days until the wounds healed completely |
| Feitosa et al., 2015 | 16 volunteers | *N* = 8 | He-Ne/Pulsed | 4 J/cm²/80 s | DNE | 632.8 | Irradiation 3 times per week in alternated days totalizing 12 sessions |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sandoval Ortíz et al., 2014 | 28 volunteers | *N* = 9 | Diode/Continuous | 2 J/cm$^2$/0,18 s (on wound edges);<br>1.5 J/cm$^2$/0,14 s (on wound bed) | DNE | 685 | Irradiation 3 times per week during 16 week or until the wounds healed completely |
| *Burns* | | | | | | | |
| Fantinati et al., 2016 | 100 female Wistar rats | *N* = 25 | GaAlAs/ Continuous | 3 J/cm$^2$ (until 7th experimental day)<br>6 J/cm$^2$ (from 7th day until euthanasia)/DNE | 12 mW/cm$^2$ | 650 | Irradiation in alternated days |
| Ranjbar and Takhtfooladi, 2016 | 30 male Wistar rats | *N* = 15 | GaAlInP/DNE | 3 J/cm$^2$/75 s | 15 mW/cm$^2$ | 685 | Daily irradiation for 5 days (first irradiation after 3 days from burn induction) |
| Dahmardehei et al., 2016 | 13 volunteers | *N* = 13 | DNE/Continuous | 2 J/cm$^2$ (for bed of the ulcers)/DNE | 0.6 W/cm$^2$ | 650 | Irradiation in alternated days for 7 to 10 sessions before graft surgery and 3 to 5 sessions after graft surgery |
| | | | | 6 J/cm$^2$ (for the surrounding)/DNE | 0.2 W/cm$^2$ | 810 | |
| | | | | 10 J/cm$^2$/16 min (intravenous) | 10 mW | Intravenous laser therapy: 660 nm (median cubital vein) | |
| Al-Watban et al., 2009 | 75 male Sprague-Dawley rats | *N* = 60 | Diode/DNE | 5, 10, 20 and 30 J/cm$^2$/3.6 to 32.1 min | 20.4 mW/cm$^2$ | 532 | Irradiation 3 times per week |
| | | | | | 15.56 mW/cm$^2$ | 633 | |
| | | | | | 22.86 mW/cm$^2$ | 670 | |
| | | | | | 22.22 mW/cm$^2$ | 810 | |
| | | | | | 22.22 mW/cm$^2$ | 980 | |

*DNE* data do not exist or it is not clearly specified

nondiabetic rats would respond to this light stimulus, with regard to growth factors as fibroblast growth factors (FGF), platelet-derived growth factors (PDGF) and vascular endothelial growth factor (VEGF). In its analyses, FGF increased significantly in the fibroblast cultures of diabetic rats with regard to nondiabetic ones. PDGF also was stimulated, however not significant, while VEGF had an inhibiting effect. These scholars suggest that the power may have been elevated, since better results were obtained in other studies with lower irradiation values [30].

In this same year, the publication of Esmaeelinejad et al. (2014) [32] with human fibroblasts in vitro supported not only the laser's stimulating capacity but also the damages caused by hyperglycaemia on these cells. Therefore, three mediums of culture were adopted, including the physiologic medium with 5.5 mM/L glucose plus two mediums of glucose high concentration with 11.1 mM/L and 15 mM/L. In these last concentrations, in order to receive irradiation, cultivated fibroblasts remained in this condition or passed to a physiological medium. The laser induced stimulating effect in the samples contained in normoglycaemic and hyperglycaemic medium alike. In the cells that developed in concentrations of elevated glucose, the tested doses (0.5, 1 and 2 J/cm$^2$) favoured viability, morphological modification and proliferation, separating spindle shape from fibroblasts and making them more elongated. Independently from the therapy, when comparing the group with concentration of 5.5 mM/L to the 11.1 mM/L group, the authors observed that the excess of glucose brings damages for the cells, made evident by the statistical decrease of proliferation and viability added up to the slight prolonged and less dispersed format.

## 6 Surgical Wounds

The realization of a surgical procedure involves risks inherent to the act, in higher or lesser degree depending on the complexity. The cure of the resulting incisions is considered as primary wound healing or of primary intention and occurs more rapidly, because the tissue edges are newly approximated and sutured, needing little tissue synthesis. However, when a significant quantity is lost in excision surgeries, the completion of repair is more prolonged and occurs secondarily or through secondary intention. Other damages caused to the skin and to deeper layers such as burns and ulcers also fit in this last case, although these generally remain open until their repair, due to a lack of suture [33, 34].

When a diabetic undergoes this type of intervention, be it incisive or excisive, the possibility of complications is considerably high and may occur during surgery or in postoperatory. In the acute phase of the recovery, dehiscence and /or infection of the generated wound are among the risks that these patients potentially develop compared to healthy people in the same condition (postoperatory). Abnormal wound healing made more difficult by the occurrence of a dehiscence tends to increase the period of hospitalization as well as the probability of contracting a hospital infection. The risks are still impending after discharge, in domestic context, where the care with the injury must also be constant.

Another important aspect generally associated with postoperative phase is pain, showing discomfort during rest as well as mobilization. Moreover, when left untreated, an infection initially located can evolve through blood stream and spread throughout the entire body. This situation known as sepsis represents a severe and potentially lethal case [33, 35, 36].

The prolonged tissue repair can still contribute for the formation of excessive scars classified as hypertrophic or keloidean and that develop due to a mismatch in ECM in its degradation/deposition relation. They are considered difficult to treat, being therefore the adoption of prophylactic cares during wound management more effective. For many people the satisfaction with their appearance is directly related to their self-esteem, which may be affected due to an alteration in the body image of an unaesthetic scar. Depending on its extension and localization, it is also capable of affecting functionality [37, 38].

Considering all of these elements, using therapeutic cares that aim at accelerating the healing of the surgical site may contribute substantially to reducing complications and consequently morbidity and mortality in diabetics.

In studies that involve hyperglycaemic animal models, laser effects in the repair process are generally studied by means of inducing wounds that appear similar to diabetic ulcers, and a circular excision is therefore performed and remains open to receive therapy or act as control. In other cases, incisions are made and these appear similar to surgical wounds. To verify the action mechanisms of the infrared laser, Dancáková et al. (2014) [9] created these two types of wounds on the back of each rat of their experiment. Traction resistance was higher in the nondiabetic control group. However, among diabetics, it was significantly higher in the group that received laser compared to the placebo. Furthermore, the formation of granulation tissue and collagen was higher in the treated group.

The surgical excision needs higher quantity of granulation tissue formed in the proliferative phase with regard to the incision to complete the wound healing cycle. This is why the authors suggest that the ideal LLLT is the one which better adjusts to the necessities of each injury, and there may be an inverse relationship between wavelength and intensity. In their results, they showed that there were differences between the cicatrization of both wounds. In the open injuries of the irradiated diabetic group, the quality of formed tissue appeared similar to the nondiabetic. In the incision this similarity to the nondiabetic control did not occur, although resistance has significantly increased [9].

After the incision followed by suture in diabetic rats, Güngörmüs et al. (2009) [39] realized a total of five applications with a 808 nm laser and a 10 J/cm$^2$ dose, with one application being immediate after injury and the following with a 2-day interval. The first histological evaluation performed 10 days postoperation demonstrated that reepithelialization was faster in the treated diabetic group, indicating not only the healing potential of this resource but also an effective treatment schedule under these parameters.

Later, Sharifian et al. (2014) [28] utilizing pulsed laser, 890 nm wavelength and 0.2 J/cm$^2$ dose, obtained in their results a significant enhancement of basic fibroblast growth factor expression gene (bFGF) in the diabetic and nondiabetic group when compared to control. At days 4, 7 and 15, progress analyses of the cicatrization process evolution were performed, and a significant increase of macrophages in the first check and of fibroblasts in the following was determined. This is an important finding, because as previously described, fibroblast deficient proliferation in diabetic people plays a decisive role in delaying the cure. In this case, the laser accelerated wound healing also through a stimulating effect on the fibroblasts when producing growth factors.

The study suggests that laser photobiomodulation may be related to growth factor stimulation such as the one studied (bFGF). Although it was not an aim of this research, these authors also observed that in diabetic and nondiabetic animals alike nonirradiated injuries also showed improvement, suggesting a possible systemic effect, once the stimulated growth factors would act in other areas through blood stream [28].

The production of reactive oxygen species (ROS) occurs physiologically in some metabolic activities of the organism, as for neutrophils and macrophages during their defence activity after an injury. However in diabetic patients, they are produced beyond regular need due to the hyperglycaemic chronic medium, and the oxidative stress generated may cause cellular damages compromising the functional activities and, consequently, cicatrization. The same may occur due to an excess of reactive nitrogen species (RNS) such as nitric oxide. Based on that, Tatmatsu-Rocha et al. (2016) [40] making use of a GaAs 904 nm laser reported that collagen showed to be more organized and that oxidative and nitrosative stress decreased, proving antioxidant effects. With daily applications of LLLT, there was an increase of fibroblast quantity and new vessels.

As a result of DM complications, among which vascular types, many patients are elected for myocardial revascularization surgeries. Lima

et al. (2017) with a 6 J/cm$^2$ dose and AlGaInP laser verified following to 8th day of surgery that irradiation favoured wound healing, as well as dehiscence prevention, which occurred in three non-treated individuals [35].

## 7 Diabetic Ulcers

Diabetic ulcers are wounds characterized by difficult healing that develop with higher prevalence in lower limbs and frequently become chronic. The union of local and systemic factors is responsible for initiating the injury as well as delaying the cure. In diabetics the main aetiologies are of ischaemic and neuropathic origin plus mechanical causes [6].

Over the years, the deleterious effects of hyperglycaemia in the endothelial wall favour the onset of the peripheral vascular disease. In this pathology, there is an atheroma plaque formation that reduces the vessel lumen and generates tissue ischemia. Adherence molecules that initiate the formation of this plaque are in bigger concentrations in diabetics.

On the other hand, nerve involvement leads to somatic and/or autonomic disorders. In somatic neuropathy, pain, temperature, pressure and vibration sensibility may result abolished or reduced. Diabetic patients may also show dry skin as a result of autonomic neuropathy, a sympathetic alteration that reduces lower limb sudoresis and predisposes to fissure. It is still possible to develop articulatory abnormalities resulting from the abnormal motor component. This combination of factors contributes to ulcer formation, which tend to arise in prominent bone areas subject to pressure or trauma, be it during walk or due to footwear (Fig. 2) [41].

Diabetic ulcer management is a challenge for patients and health professionals alike because it has shown very resistant to conventional therapy. Multiple causes require intensive care associated with resourced and multidisciplinary assistance. Without adequate treatment, ulcers tend to progress until affecting deeper layers, with significant tissue loss and enhancement of the exposed area. Infected and non-cicatrized ulcers are responsible for serious complications, being lower limb amputation one of the main dangers, whose occurrence is from 10 to 20 times higher in diabetic population, according to the WHO [5].

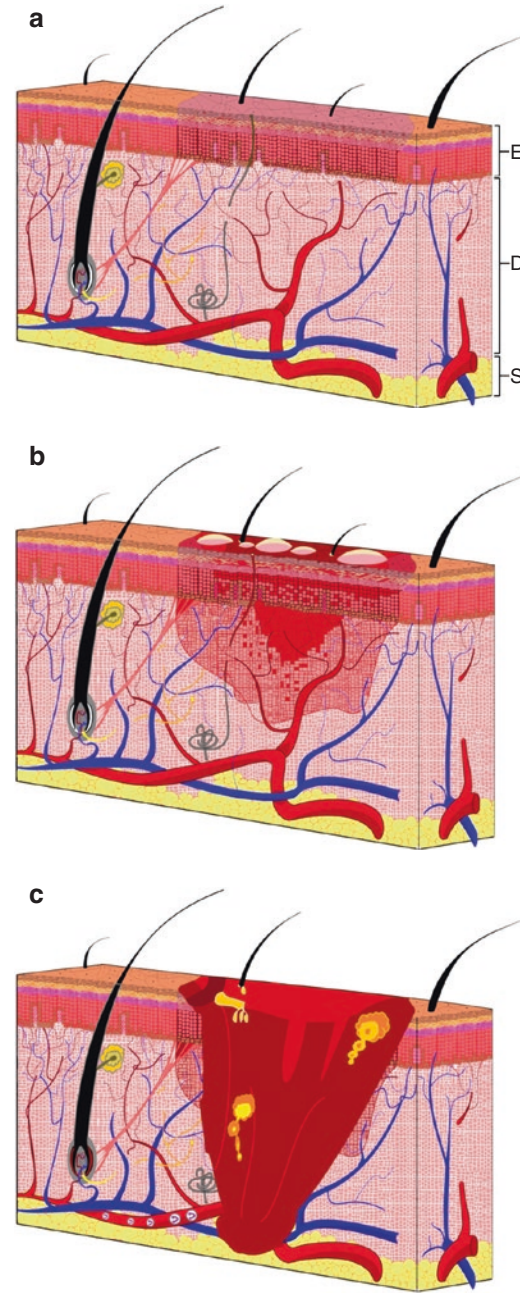


**Fig. 2** Burn degrees. (**a**) First degree. (**b**) Second degree. (**c**) Third degree. *E* epidermis, *D* dermis, *ST* subcutaneous tissue

These elevated indexes and their individual and collective repercussions highlight the necessity of more effective actions. This is related not only to therapeutic modalities, but similarly to another decisive component for curing diabetic ulcers which is the time of intervention. In this context, laser therapy used by an increasing number of researchers has showed significant results in wound healing acceleration and even in infection management.

Using the GaAlAs laser (635 nm), Kilík et al. (2014) [30] tested three power densities and verified that 5 and 15 mW/cm$^2$ were more effective in rat injuries, promoting anti-inflammatory action, favouring collagen and new blood vessel synthesis. However, nondiabetic and diabetic group reacted differently to the irradiation. In the diabetic group, laser therapy was not so expressive in the initial phases, as much as subsequent to stimulating the angiogenesis and to significant collagen deposition, enhancing maturation phase. The nondiabetic group showed inflammatory phase acceleration and, subsequently, of the following phases too. Results indicated that when using 635 nm wavelength, 15 mW/cm$^2$ power density promotes results even more remarkable in diabetic wound healing for secondary intention.

The He-Ne laser also generated positive effects in animal models. Hegde et al. (2011) [42] showed that in epidermis and superior dermis injuries, the stimulation generated by LLLT in the wound healing process is received by cells that may be found in these superficial skin layers, which would explain the efficacy of utilizing low wavelength lasers. Moreover, the authors reported that after testing five doses (1–5 J/cm$^2$) with 632.8 nm, they verified that the 3 J/cm$^2$ fluency realized immediately after the injury caused the acceleration of wound closure in the treated diabetic group, decreasing this period on average 15 days when compared to the non-treated group. They also suggested that the wound contraction observed in the studied tissue may have resulted from fibroblast photostimulation which favoured not only its migration towards the injured area but also its differentiation in myofibroblasts that act in the contraction.

In diabetic patients, hyperglycaemia significantly reduces collagen production. In the study of Maiya et al. (2009) [13], this decrease in animal models occurred by half after 2 weeks following the induction of diabetes. In this experiment comprised of intervention and placebo group, the inquiry was centred around the effects of He-Ne laser in diabetic excisional wounds testing seven different doses (3–9 J/cm$^2$). In addition to other assessed indicators, researchers realized a biochemical analysis of hydroxyproline, an amino acid involved in collagen fibre constitution. The researchers obtained stimulatory results in 3 and 6 J/cm$^2$ doses and, mainly, in 4 and 5 J/cm$^2$ where a higher and more organized collagen deposition occurred with regard to the placebo group. By contrast, energy densities between 7 and 9 J/cm$^2$ made the wound healing process slower.

Eissa and Salih (2017) [26] also conducted a study using the same wavelength, with 4.0 mW/cm$^2$ power density, and demonstrated that a daily irradiation for 5 days during 3 weeks was able to decrease wound healing time by half. While in the group with no treatment, skin repair occurred between 40 and 60 days, in the irradiated group, it was complete after 21 days. The injuries showed infection; however, even so, the laser was effective and already in the second session, a smaller wound diameter could be noticed.

Another inquiry that provided good results concerning wound healing acceleration was led by Al-Watban et al. (2007) [43] in which diode lasers were used in four distinct wavelengths and repeating dosimetry of 5, 10, 20 and 30 J/cm$^2$ for each tested group. From the findings it was observed that the repair process was superior in irradiated wounds, being the best outcomes obtained in the ones using 633 nm and a 10 J/cm$^2$.

In the study of Carvalho et al. (2010) [44] realized with nonirradiated and irradiated diabetic rats with a InGaAlP laser (660 nm) and energy density of 4 J/cm$^2$, three assessment moments were realized (days 3, 7 and 14) after wound induction. Collagen quantity was superior in all the analyses of treatment group, remaining as a significantly more organized matrix towards wound healing. The treated group showed lower inflammatory infiltrate when compared to the control, in addition to the considerable formation of granulation tissue.

Rocha et al. (2012) [45], through two applications of GaAs laser and 3.8 J/cm$^2$ fluency, noticed that after 7 days following wound induction, cicatrization in the treated group showed to be faster, in addition to the reduction of inflammatory infiltrate, higher quantity of blood vessels and hair presence, while in the control the characteristics still highlighted the initial phase of repair with intense inflammation and formation of fibro-necrotic tissue. As a result of these findings, which, even if favourable, were not statistically significant, a cyclooxygenase 2 enzyme (COX2) expression resulted significantly reduced by inflammatory cells and, in the irradiated group, a higher concentration of TGF-β2, an inflammatory cytokine which contributes to synthesis modulation and ECM degradation. By contrast, the research led by Noudeh et al. (2010) utilized a 670 nm wavelength for the wound bed and a 810 nm wavelength for the edges and did not obtain better statistics in ulcer closure [45, 46].

Despite some limitations, laser therapy appears to be remarkably effective in wound treatment. However, in experiments conducted on human beings, the treatment regimen may frequently be unviable or obstruct adhesion to the treatment for countless reasons, mostly due to the number of sessions. Soon, protocols that have a lower number of attendances, but that do not compromise the treatment efficacy, may contribute to reducing outflows and favouring the adhesion of patients that need this intervention.

In this context, de Loura Santana et al. (2015) [47] using GaAlAs laser studied whether the treatment aided by this resource would be more effective with a single application or multiple doses fractioned in smaller amounts. They observed that the effects of both doses were similar and that the irradiated diabetic groups showed higher myofibroblast concentration and collagen fibre organization. The irradiated ulcers did not finish the healing cycle before control group; however the authors demonstrated that wound healing showed 40% faster in the initial phase of repair, and because of that they indicated that its application in the immediate postoperative may contribute to more precocious tissue restoration.

According to Kajagar et al. (2012) [48], ulcers in DM patients are rather worrying, because they generally show great resistance to conventional treatment. These researchers examined the effects of laser therapy combined with conventional treatment in dealing with chronic ulcers. Overall, patients realized 15 daily sessions with energy density varying between 2 and 4 J/cm$^2$ according to the affected area. While in the control group, which received only conventional treatment, the rate of wound contraction was 11.87%, in the irradiated group, it was significantly higher, reaching 40.24%, proving that photostimulation can act together with other therapies (asepsis, debridement, bandage, drug therapy and self-care). The authors also included the patients' education, teaching them not only cares to be adopted with ulcers but also with other DM factors which also interfere in wound healing such as eating habits and lifestyle.

Kaviani et al. (2011) [49] also obtained good results associating LLLT to conventional treatment. They observed that wound irradiation with a 10 J/cm$^2$ energy density and 685 nm reduced the time of total wound healing in approximately 3 weeks compared to placebo group. During this research, it was related the occurrence of volunteers in both groups that did not continue participating in the trial due to complications, including infections and death. Two patients of the placebo group needed hospitalization and amputation due to gangrene. Lower limb amputations are important diabetic ulcer complications. This type of surgery is performed with the primary purpose of preserving the patient's life; however, it is a complex procedure, and many patients die in few years due to the adversities of this intervention, since the surgical procedure.

Although reduction in sensitivity is a common characteristic in DM patients, in other cases it is possible that the ulcer be followed by pain. In that case, Visual Analogical Scale (VAS) applied by Feitosa et al. (2015) [50] contributed to assessing the treatment progression. Under a 3 weekly session regimen, using 4 J/cm$^2$ tissue repair was significantly higher and pain decreased 4 points on average on pre- and post-intervention, while it

remained practically unchanged in the group without laser. Volunteers of the control group received instructions to realize the daily asepsis of the ulcers; however its size progressed expressly, which led to transfemoral amputation of a patient. Unfortunately, this is a case scenario that happens frequently in virtue of the injuries resisting to conventional therapy.

The inconveniences generated by a chronic injury may have repercussions on quality of life and this must be considered. Even if the focus of many studies is centred on wound closure, preventive aspects need to be addressed incisively and include the patient's education, approach adopted in the randomized clinical trial by Sandoval Ortiz et al., 2014. Volunteers had diabetic ulcers degree I or II localized in the lower limbs (legs or feet) and underwent previous assessment of life quality through a questionnaire (EuroQoL-5D). Over the course of 4 months, the ulcer edges were photostimulated with 2 J/cm$^2$ and the centre with 1.5 J/cm$^2$ 3 times per week. Results did not show significant differences, while in the health assessment regarding the evaluated dimensions for life quality, all reported having "some problem" in specific items, even though these had not been severe problems [51].

## 8 Burns

Burns are the third major cause of accidental deaths in the USA [52]. This injury type may be caused by heat, freezing, electricity, chemical products, radiation or abrasion, and, when it occurs in diabetic individuals, it represents an important public health issue, with a significant increase in mortality rate. In individuals with DM, the prevalence of this injury type is higher in lower limbs. In order to determine severity, prognosis and treatment, total body surface area (TBSA) is generally used, in addition to injury degree and depth assessment [53–55].

In the clinical practice, burns are classified according to the severity of injury in the epidermis, subcutaneous tissue and underlying structure. In their study Cancio et al. (2017) [56] cited the following categorization: superficial thickness burns, which correspond to first-degree burns; partial thickness burns, equivalent to second-degree injuries; and total thickness burns or third degree (Fig. 3). Such injuries are considered highly complex and need specialized care, since hyperglycaemic people already have abnormal wound healing characteristic of DM. In addition to the previously described benefits, laser therapy is able to contribute to bone restoration

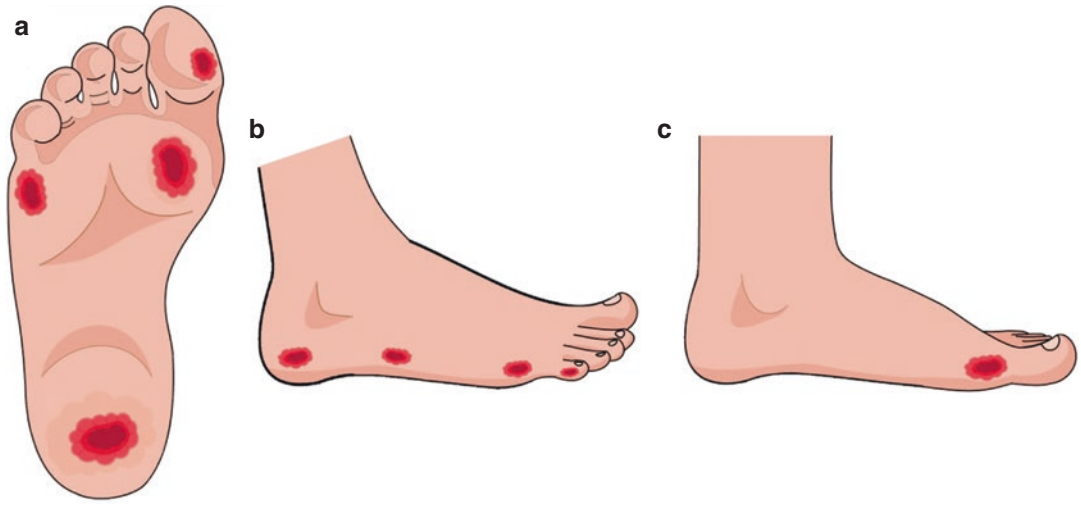


**Fig. 3** (**a**–**c**) Areas of bony prominences with prevalent formation of foot ulcers. Adapted from [6]

through osteoblast stimulation, enhancing its utility as regards burn injuries [56–58].

In order to demonstrate the utility of low-intensity laser therapy in these situations, Fantinati et al. (2016) [58] induced a third-degree burn on the back of the studied animals, but previous to photostimulation they adopted prophylactic measures of infection, realizing immediate cleaning of the damaged area and applying silver sulfadiazine followed by occlusive bandage, daily sterilized until the end of the experiment. A surgical debridement was also realized on the second day. The occlusion aimed as well at avoiding traumas and the formation of crusts for dehydration, while the removal of the devitalized tissue contributed to the incidence effect being more effective. The radiation was emitted with GaAlAs laser in two distinct doses, the lower dose being used in the inflammatory phase of the repair process (3 J/cm$^2$) and the double in the following phases (6 J/cm$^2$).

Over the course of 1 month, four evaluations through photographic and microscopic medium enabled determining a significant increase of wound contraction in the group of diabetic and nondiabetic rats treated with laser compared to the controls, nonirradiated. Furthermore, the angiogenesis was expressive in the first assessment, and the amount of collagen was also higher in the treated group. The authors demonstrated that a significantly higher formation of necrotic tissue occurred in the rats that did not receive the treatment, with more precocious appearance in the diabetic group (on day 14) with regard to nondiabetic group (on day 30) [58].

*Staphylococcus aureus* (*S. aureus*) is a bacteria frequently associated with nosocomial infections. Based on this fact, Ranjbar and Takhtfooladi (2016) [59] searched the effects of laser therapy in third-degree burns infected by this pathogen. Through their experiments, they demonstrated the stimulating and inhibiting effects of the laser for different types of cells using the same parameters and models. Despite of the statistical difference having occurred just at the end of the treatment (after 21 days), the size of the burnt area was significantly reduced in the treated group with regard to the control. Moreover, there was a considerable increase of skin resistance. Another relevant result provided by the irradiation was the bactericidal effect, in which the *S. aureus* decreased significantly. The control of the infection acts as anti-inflammatory, since it operates simultaneously on the reduction of microorganism proliferation and phagocytosis increase for the defense cells.

In very extensive burns, physiologic wound healing cannot replace the lost tissue, and common surgical interventions are not able to reconnect the edges of the caused wounds, thus resulting many times in ulcers. This is why grafts constitute the main instrument used in these situations. In diabetic patients the likelihood of rejection is high, and, among other complications, it may evolve towards amputation of the affected limb.

A promising treatment suggested by Dahmardehei et al. (2016) [8] is the union of laser with grafting surgical procedures. In a hospital specialized in burns, these researchers selected diabetic patients with ulcers caused by third-degree burns that had been elected to amputation in virtue of the complexity of the injury or for the occurrence of dehiscence after *grafting* surgery. Initially these patients received laser therapy as a preparatory therapy for the realization of split thickness skin graft (STSG) surgical procedure. After realizing approximately seven to ten sessions, there was a considerable formation of granulation tissue, which served as support for the skin grafts. Then, aiming to prevent dehiscence, three to five sessions were realized postoperative. Within 2 months, all volunteers showed cure, without manifestation of collateral effects.

However, an important aspect to be highlighted regarding laser therapy concerns the approach to these patients. Divergently with regard to what is generally to be found in literature, each patient received simultaneously three distinctive parameters. On the wound bed a 2 J/cm$^2$ dose and a 650 nm wavelength in the margin of 6 J/cm$^2$ with 660 nm. Additionally, the median cubital vein was punctured, and through a fibre optic inserted within the needle, a 10 J/cm$^2$ with 660 nm intravenous irradiation was applied. This type of intervention is denominated intravascular laser blood irradiation (ILBI), and it is used with the aim of obtaining systemic effects

that favour wound healing and includes, in addition to the benefits of habitual application, support in the control of glucose rate and cholesterol and stimulation of hormone liberation such as insulin and glucagon, among others. Independently from the group to which they belonged, all the volunteers received conventional treatment for ulcers. The patient's education, included in the methods, needs indeed to be part of the treatment routine of all therapeutic modalities, once the treatment's success is intimately related to the patient's collaboration [8].

Considering that the choice of the wavelength is one of the most important parameters, Al-Watban et al. (2009) [60] compared the efficacy of red and infrared lasers in burn cicatrization in diabetic animal models. Regarding the treatment regimen, a 3-day frequency per week was elected after proving its efficacy through previous studies. This choice also took into account the registers in literature that argue in favour of the ideal treatment being the one distributing the doses in different sessions (approximately 3–4 sessions per week) instead of realizing few sessions with high energy densities. Biostimulating effects were obtained in all of the wavelengths, promoting burn repair. However, the percentage of wound healing with invisible lasers was of 50.68%, while the visible reached a 78.37%, showing superiority based on the adopted parameters.

## Conclusions

Based on the described cases, LLLT potential has been highlighted in healing processes that show abnormal wound healing due to DM, including surgical wounds, ulcers and burns. Among the obtained benefits are wound healing acceleration, inflammation reduction, cellular proliferation and migration enhancement, fibroblast and angiogenesis increase, collagen synthesis stimulation and organization, skin resistance improvement, growth factor induction, infection reduction, pain easing and antioxidant effect. 810 nm, 632 nm and 660 nm were the prevailing wavelengths and continuous emission mode outweighed pulsed mode. The majority of studies showed energy densities between 1 and 6 J/cm$^2$. A 5 J/cm$^2$ dose was the more frequent, followed by 4 J/cm$^2$ and 1 J/cm$^2$. Regarding the treatment regimen, daily irradiations or alternated days were highlighted, preferably with immediate irradiation after injury. The authors demonstrated that laser is a promising therapy, whose results may be improved when associated with other conventional resources such as asepsis, debridement, bandages, drug therapy and patient's education.

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# Enzymatic Debridement of Chronic Nonischemic Diabetic Foot Ulcers

Jaime E. Dickerson Jr.

## 1 Introduction

DFU are a frequent and serious complication of diabetes mellitus, with an annual incidence rate of 1–4% and a lifetime risk of 15–25% [1–3]. The often poor prognosis for individuals suffering from diabetic foot ulcers (DFU) has led some to equate these chronic and recurrent wounds with cancer as a major cause of morbidity and mortality [4]. Key causative factors include peripheral neuropathy, large vessel disease, deformity, callus, and trauma [1, 2, 5]. DFU are generally critically colonized with one to many species of bacteria [6, 7] and are at high risk of developing frank infection. When infected they are a major cause of hospital admissions and lower limb amputations [1–3]. It is estimated that from 40 to 70% of all non-traumatic amputations of the lower limbs occur in patients with diabetes and that 85% of lower limb amputations in diabetic patients are preceded by DFU [5, 8, 9]. Aside from the significant morbidity and mortality, DFU-related amputations carry immense social and psychological consequences [10, 11]. The associated economic burden is also great; 20–40% of total healthcare resources spent on diabetes management can be attributed to DFU and sequelae [12]. Although significant progress has been made, the treatment of DFU remains a great challenge.

Generally accepted treatment of DFU includes off-loading, including total contact casts, specialized shoes, or other adaptive equipment (e.g., crutches) to minimize pressure on the wound; antibiotic/antimicrobial treatment (systemic and/or topical) when indicated for infection and to decrease the bacterial burden in the wound; and appropriate dressings to maintain an optimal wound environment for healing [13]. Effective wound management strategy is based on the concept that a clean wound, with minimal exudates, and a completely granulated wound bed is most likely to heal. Wound bed preparation that creates such a state is a critical element of DFU management [14, 15].

An essential part of wound bed preparation is the removal of the nonviable material through debridement [16–18]. Removing devitalized tissue is one aspect of debridement. Equally important is the reduction of bacterial load and biofilm, removal (or reactivation) of senescent cells, and otherwise modulating the wound bed environment. Active methods of debridement include mechanical, surgical, biological, and enzymatic, while the passive maintenance of a moist wound environment is thought by some to promote debridement by endogenous proteases.

Currently only collagenase is available as an approved product for enzymatic debridement. Prior to 2008 there were several marketed

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enzymatic debriders, notably those which contained papain; however, these were taken off the market at the FDA's direction for safety reasons, including anaphylactic reactions, and also because none of these had gone through an FDA approval process. Clostridial collagenase ointment (CCO, SANTYL®, Smith & Nephew, Inc., Fort Worth, TX) was approved by the US Food and Drug Administration in 1965 and is indicated "for debriding chronic dermal ulcers and severely burned areas" [19]. Approval of CCO came close on the heels of the Kefauver-Harris Amendment of 1962 which mandated that new drugs be proven efficacious in "adequate and well-controlled investigations" [20]. Despite approval after the 1962 law, the clinical data supporting CCO's marketing authorization is not comparable to the extensive multiphase testing now standard for a new drug or biologic. An evaluation of the efficacy of CCO must therefore be based on those studies published over the decades since its introduction. In this chapter the published clinical data from studies evaluating CCO in the treatment of DFU will be reviewed, as well as the available evidence shedding light on mechanism of action.

## 2 Clinical Experience

While clostridial collagenases have been used for decades for the debridement of burns [21–23], decubitus ulcers [24–26], and venous leg ulcers [27], the scope of the following discussion will be limited to DFU.

Altman et al. in 1978 [28] reported on outcomes for 30 patients with diabetes mellitus for at least 10 years and with a neuropathic ulcer on the plantar surface treated with CCO. An initial "mechanical" debridement was performed, if needed, at baseline, and CCO was applied in a thin layer, once daily, until "sufficient debridement had taken place." Wound closure was not an endpoint in this study, and the ulcer outcomes beyond "sufficient debridement" were not reported, a design that closely reflects the approved labeling. Twenty of the thirty ulcers (66%) had excellent wound debridement, defined as ulcer sufficiently debrided with new granulation tissue seen in less than 2 weeks. An additional six (20%) were rated as "good," achieving this goal between 2 and 4 weeks and 1 "fair" with debridement requiring greater than 4 weeks. Three patients were lost to follow-up.

Healthpoint Biotherapeutics (later acquired by Smith & Nephew) acquired CCO in 2006 and shortly thereafter embarked on a "hypothesis-generating" series of relatively small clinical studies designed to better understand the efficacy of CCO in the treatment of DFU and to explore this efficacy mechanistically (Table 1). These included comparisons versus various standard care modalities including serial sharp debridement [29], the CCO vehicle [30], standard care as selected by the clinical investigator [31], and hydrogel [7, 32]. An additional study compared CCO with products containing silver [33]. The exploration of mechanism involved analysis of wound bed exudate before and after treatment with CCO and histological study of

**Table 1** Clinical investigations of clostridial collagenase ointment (CCO, Santyl®)

| No. | Study | Clinicaltrials.gov identifier | Main goal | *N* |
|---|---|---|---|---|
| 1 | Versus vehicle [30] | NCT01143714 | Effect on wound area | 54 |
| 2 | Versus hydrogel [32] | NCT01143727 | Effect on inflammation | 17 |
| 3 | Versus saline moistened gauze+serial sharp debridement [29] | NCT01056198 | Effect on wound area | 48 |
| 4 | Versus investigator selected SC [31] | NCT01408277 | Effect on wound area | 55 |
| 5 | Versus vehicle [34] | NCT01197898 | Effect on wound edge | 10 |
| 6 | Versus hydrogel [7] | NCT02111291 | Effect on wound area, granulation, inflammation | 207 |
| 7 | Versus silver containing products [33] | NCT02581488 | Effect on wound area, infection rate | 102 |

the advancing epithelium at the wound edge [32, 34].

With a generally small number of patients, none of these trials were powered to show statistical superiority of CCO over the comparator. What was shown was a percent reduction in ulcer area for CCO that was consistently between 50 and 70% at the end of study, generally 12 weeks (Table 2), and that was at least 15% better than the comparator. Note that the percentage reduction in ulcer area for the CCO-treated groups was statistically significant in each of the studies.

The efficacy of repeated and frequent sharp debridement has been tacitly accepted by most wound care practitioners although conclusive evidence demonstrating a benefit is scant [35]. It is interesting that when CCO was used in conjunction with weekly sharp debridement (study 4), only a relatively modest incremental reduction in ulcer area was observed over use in the absence of weekly sharp debridement (study 3), 61% versus 54%. The additional observed benefit could also be a result of the additional 2 weeks of CCO treatment in study 4 (6 weeks versus 4 weeks).

Wound appearance was also assessed in these studies, primarily with a modification of the Bates-Jensen Wound Assessment tool. In all instances, wound appearance improved significantly for both groups, and between-group differences were not distinguishable statistically although CCO-treated ulcers had generally better scores numerically.

Excessive and prolonged inflammation may result in a non-healing chronic or "stalled" wound [36]. Defining a stalled wound as one failing to achieve a 10% reduction in area relative to baseline, between 4 and 22% of ulcers treated with CCO could be considered "stalled" by the end of study (overall = 15%) compared to between 21 and 25% (overall = 23%) for the standard care control groups [30]. For wounds failing to progress beyond a chronically inflamed condition, a therapy promoting resolution of the inflammation may be able to facilitate the resumption of healing.

In these studies, there was a consistent trend toward more rapid reduction in wound area and fewer "stalled" wounds yet with the finding that the appearance of the CCO-treated wounds could not be distinguished from the controls [7, 29, 31, 32]. The obvious conclusion is that the enzymes present in CCO are likely eliciting cellular and biochemical responses that modify the wound bed in such a way as to promote healing and that these responses go beyond the visible removal of eschar, slough, and other nonviable materials.

In in vitro experiments, Riley and Herman [37] demonstrated that keratinocytes grown on endothelial cell extracellular matrix (ECM) pretreated with purified clostridial collagenase resulted in a doubling in proliferation relative to cells grown on untreated matrix. Including collagenase in the growth media resulted in an additional increase in proliferation. Utilizing a

**Table 2** Percentage reduction in ulcer area for CCO-treated ulcers

| No. | Study | Mean percentage reduction in ulcer area at the end of study[a] (%) | Delta versus comparator (%) |
|---|---|---|---|
| 1 | Versus vehicle [30] | 49 | 15 |
| 2 | Versus hydrogel [32] | 70 | 28 |
| 3 | Versus saline moistened gauze+serial sharp debridement [29] | 54 | 62[b] |
| 4 | Versus investigator selected SC [31] | 61 | 15 |
| 6 | Versus hydrogel [7] | 65 | 14 |
| 7 | Versus silver [33] | 62 | 22 |

[a]Study duration was generally 12 weeks except for study 2 (4 weeks). Treatment duration was either 4 weeks (Studies 1, 2, 3), 6 weeks (Study 4), or 12 weeks (Study 6)
[b]Delta versus comparator exceeds CCO response because control wounds increased in size on average

scratch assay technique to assess keratinocyte migratory activity, the cells grown on collagenase-treated ECM were about eightfold more motile than those grown on untreated ECM. It is noteworthy that substituting another proteolytic enzyme, papain (with urea), in these experiments did not stimulate proliferation or motility. On the contrary, these cellular processes were inhibited, suggesting that some specific peptide fragments resulting from the cleavage of collagen and other matrix proteins [37] by clostridial collagenase may be bioactive. The hypothesis that specific bioactive peptide fragments may be in play is further supported by Galperin et al. [32]. LPS-activated cultured human dermal fibroblasts and endothelial cells both produce lower levels of interleukin-6 (IL-6) when the culture media contains clostridial collagenase proteolytic digests of both collagen III and collagen I. Endothelial cells also produce less tumor necrosis factor-alpha (TNF-α) under these culture conditions. Intact collagen I or collagenase enzyme alone did not affect IL-6 or TNF-α levels [32].

If there are specific clostridial collagenase proteolytic peptides derived from collagen and other ECM component proteins that can promote healing responses in wounds, it should be possible to isolate these and evaluate their efficacy. Sheets et al. [38], using liquid chromatography/tandem mass spectrometry, characterized over 100 peptides from CCO-digested ECM derived from either human dermal microvascular endothelial cells (HMVEC) or human dermal fibroblasts (HDF). Of these 14 (all 12–25 amino acids in length) were selected for synthesis and evaluation in functional assays. Several of these were found to stimulate proliferation of endothelial cells, keratinocytes, or fibroblasts and also tube formation in Matrigel in vivo. Interestingly, some of these synthetic peptides were found to promote wound reepithelialization in a murine model similar to Santyl digests although there was little overlap in the identity of the peptides. This could be due to species differences in the ECM substrates or alternatively to the subsequent action of other enzymes (e.g., clostridial clostrapain) present in Santyl.

## 3 Modulation of Wound Inflammation

Inflammation is a normal and requisite component of successful wound healing [39]. Neutrophil influx is important for control of microbes through the release of reactive oxygen species and in the degradation of matrix and necrotic material with the production of various proteases. Macrophages recognize and phagocytize apoptotic neutrophils and are thought to be key sources of growth factors [40, 41]. However, prolonged and chronic inflammation is a hallmark of the chronic, non-healing wound.

Analysis of wound fluid is an attractive way of assessing the overall wound microenvironment in a noninvasive way and can provide clues as to healing or non-healing status [42]. In a small but provocative clinical study, Galperin et al. [32] explored the effect of CCO on the inflammatory environment of mildly inflamed DFU. Wound exudate was sampled at baseline, prior to treatment with CCO, after 2 weeks of treatment, and after 4 weeks. Exudate was collected using filter paper discs from ulcers that had been gently washed with saline and blotted with gauze. Sharp debridement was not allowed in this study. A multiplex bead-based immunoassay system was used to measure the level of 22 different analytes, 11 of which had been a priori categorized as pro-inflammatory (e.g., IL-1β, TNF-α, MMP-1) and 11 as pro-resolution (e.g., IL-10, TGF-β). Plotting the pretreatment and posttreatment levels for each analyte and each patient with a pre- and posttreatment sample as an ordered pair results in the graphical representations seen in Fig. 1. Points falling above the diagonal correspond to an increase in the level of that marker after treatment; below the line, a decrease. It is visually apparent that analytes associated with inflammation tended to decrease for DFU treated with CCO, while those associated with resolution of inflammation increased. Concentrations of both pro-inflammatory and pro-resolution analytes remained clustered around the line of no change for the control hydrogel-treated DFU. The change in the inflammatory profile of the wound microenvironment may be indicative of

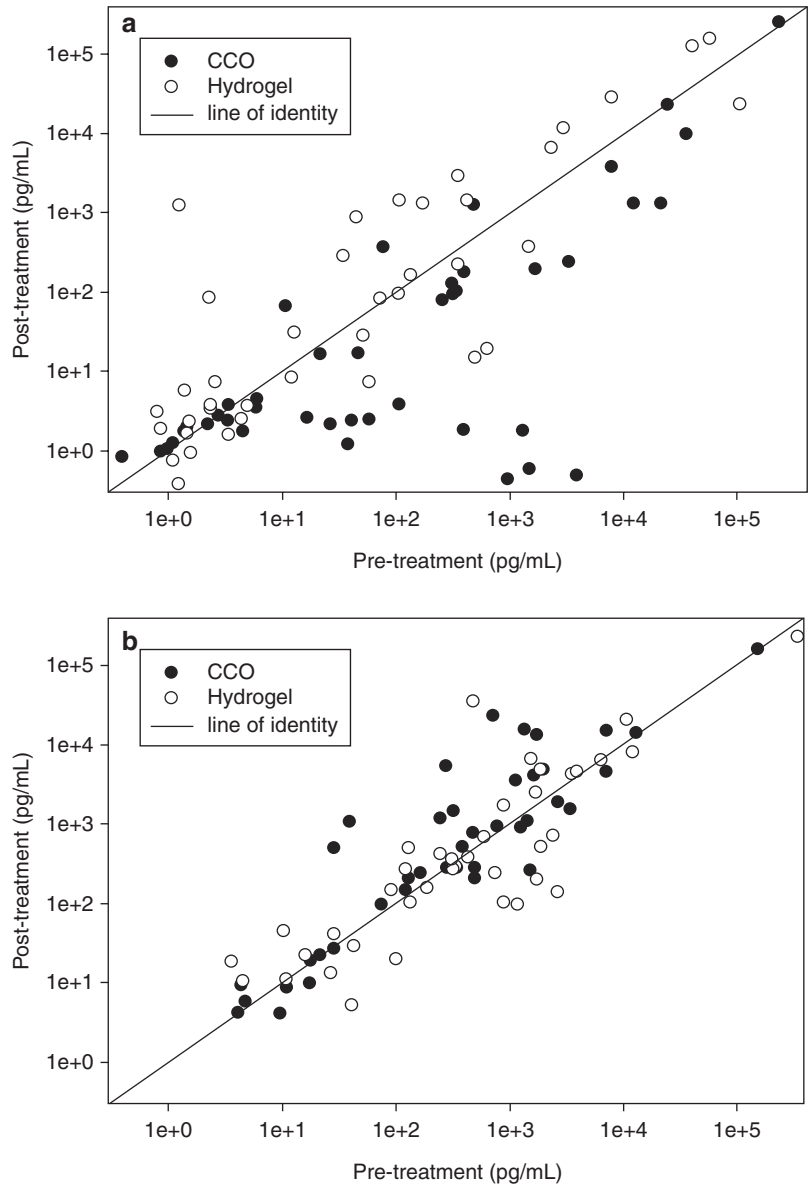


**Fig. 1** Pretreatment and posttreatment concentrations of wound fluid analytes for each patient plotted as ordered pairs. (**a**) Analytes associated with inflammation. (**b**) Analytes associated with resolution of inflammation. *CCO* clostridial collagenase ointment [32]

alterations in the phenotypic character of resident macrophages. In normal wound healing, macrophages are predominantly of the M1 pro-inflammatory phenotype for wounds in the transient inflammatory phase, switching to the pro-resolution (M2) phenotype in response to various signals or through efferocytotic activity as healing progresses [43, 44]. It is not currently known if CCO treatment potentiates a shift in the macrophage population.

## 4 Infection Control

DFU are at high risk of infection with the consequent risk of serious sequelae including amputation, sepsis, and death. Frank infection necessitates aggressive treatment with systemic and local antibiotics. DFU that do not exhibit the clinical signs of infection are, however, generally contaminated with multiple organisms often at fairly high levels [6, 7]. Payne et al. [45] showed

that in a chronically infected granulating rat wound model with bacterial levels >$10^8$ bacteria/g of tissue, treatment with CCO or a papain-urea ointment resulted in 2–3 log unit reductions by day 7 of treatment (papain-urea > CCO). Moreover, wound closure rates were significantly accelerated compared to saline-treated controls and were similar for the two enzymes suggesting that the enhanced closure was a result of the decreased bioburden [45]. The authors concluded that these enzymatic debriders were safe to use without concomitant topical antimicrobial therapy.

In an effort to mitigate the risk of infection in DFU, many practitioners choose dressings containing silver because of its known antimicrobial properties. In a recent study comparing CCO to investigator-selected standard care, 17 of the 27 patients in the standard care arm were treated with some type of silver dressing [31]. The incidence of adverse events for ulcer infection was essentially the same for the CCO-treated (10.7%) and the standard care-treated DFU (11.8%) despite the preponderance of silver dressings in the standard care arm [46]. A direct comparison between CCO and silver dressings was carried out by Motley et al. [33] finding that adverse events for ulcer infection occurred twice as frequently in the silver group (21.6% versus 9.8% for CCO). In a study with a similar patient population, Jimenez et al. [7] compared CCO to a hydrogel, a comparator that has no active pharmaceutical ingredient and no known antimicrobial activity. In this trial the incidence of study ulcer infections was 8.5% for CCO and 14% for the hydrogel. It is interesting to speculate that while silver may provide some protection from infection in DFU (albeit not more so than CCO), the presence of silver ions in the wound bed may be detrimental to healing as evidenced by the lower overall reduction in wound area when compared to CCO (Table 2). Conversely, an agent such as a hydrogel, while not inhibiting reepithelialization, does not provide protection against infection. This may explain, in part, the better outcome for CCO (Table 2). In support of this hypothesis, and of the notion that clostridial collagenase may have inherent properties that promote wound healing, burn wounds treated with CCO (+ polymyxin/bacitracin) achieved debridement faster and healed faster than burns treated with silver sulfadiazine [23]. Jimenez et al. [7] also assessed bacterial load through punch biopsy at baseline. While there was a trend (not significant) toward a negative association between bacterial load and ultimate closure, it was noted that essentially all of the DFU were critically colonized at baseline preventing definitive conclusions.

## Conclusions

The only current option for enzymatic debridement in the USA is CCO. Other less specific proteases, once available, have been removed from the market due to their unapproved status and because of safety reasons. There has been an effort to gain approval for a bromelain-based debrider (EscharEx™, MediWound Ltd.) for use in chronic, hard-to-heal wounds including DFU; however it is not clear from publically available information when this could be expected [47]. It is also not clear that a different enzyme with different specificities, kinetics, and pH and temperature optima would provide similar (or better) benefits as CCO. It is likely that a less specific protease would more aggressively and rapidly debride the wound, but as we have seen, removal of nonviable tissue is only one part of the story. CCO, in addition to debridement activity, appears to potentiate cellular responses. The release of stimulatory peptide fragments that are specific products of ECM and collagen cleavage by CCO have been shown not only to effect proliferation and migration, which are key to wound healing, but also may modulate the inflammatory status of the wound. Healing is a cellular process dependent not only on the signals provided by the environment in the wound bed but, equally important, on the ability of the resident cells to respond appropriately to those signals. A clean wound bed is obviously important for healing; however how a clean wound bed is achieved may be of greater importance.

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# Part III

# Negative Pressure Wound Therapy

# History of Negative-Pressure Wound Therapy (NPWT)

Melvin A. Shiffman

## 1 History

There is a long history to the development of measures we now use as negative-pressure wound therapy (NPWT). Cupping was described in Ebers Papyrus about 1500 BC and was used in 1000 BC in China. Archaeologists have found jars that might have been used for fire cupping about 1000 BC that is the time of the end of the Shang Dynasty era (1600?–1046? BC) and the beginning of the Zhou Dynasty (1046–256 BC) era. One can see that negative pressure was already determined to be beneficial in treating open wounds and other sorts of disorders [1]. Cupping was used around 600 BC in Babylon and Assyria and 400 BC Greek using vacuum with heated copper bowls over wounds to remove blood and fluids. Hippocrates (460–370 BC) and his followers used collection vessels whose openings were heated and applied over wounds.

Chinese cupping dating from 281 AD was an ancient Taoist medical practice and was widely used in the courts of Imperial China at the time. Its administration was first recorded by Ge Hong in an ancient treatise called Zhouhou Jiuzufang or Handbook of Prescriptions for Emergencies that dates to about 300 AD. Ge Hong and other medicine men used animal horns for cupping. That is why in some medical tracts of the empire, cupping was referred to as the horn technique of healing used for draining pustules [1].

Cupping is the term applied to a technique that uses small glass cups or bamboo jars as suction devices that are placed on the skin. There are several ways that can create the suction in the cups. One method involves swabbing rubbing alcohol onto the bottom of the cup, then lighting it, and putting the cup immediately against the skin. Suction can also be created by placing an inverted cup over a small flame, or by using an alcohol-soaked cotton pad over an insulating material (like leather) to protect the skin, then lighting the pad, and placing an empty cup over the flame to extinguish it. Flames create the heat that causes the suction within the cup and are never used near the skin and are not lit throughout the process of cupping, but rather are a means to create the heat that causes the suction within the small cups [2].

The negative-pressure dressing has been used since the nineteenth century for wound care purposes [3]. In 1907 Dr. E. Klapp used a suction pump for removal of infectious materials in tuberculosis lesions in a patient with advanced tuberculosis [4]. Bier ignited alcohol in a glass and placed a rubber tube on the skin prior to applying the heated cupping glass. This technique was reported in 1908 by Meyer and Schmieden [5]. In 1947 Russia used suction for the postoperative exudates by using gauze and wall suction. In 1952 the use of NPWT with natural sponge, rubber sponge, foam rubber, cellulose

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sponge, gauze, cotton, and other filler materials was patented in Germany.

In 1904 Sauerbruch [6] started to work on his most important surgical invention: the negative-pressure chamber. This chamber for the first time enabled operations on the open chest. The patient's head protruded outside a negative-pressure chamber while the patient's body, together with the surgeon, was inside the chamber. However, positive-pressure ventilation was developed by Brauer (1865–1951) [7] at the same time. This became an established procedure in clinical practice [8]. The machine was then miniaturized by the surgeons during the World War I. Sauerbruch [9] invented a portable bell which, put over the chest, isolated the thorax and the surgeons' hands only. Several clinical notes from the same author in his autobiography describe further refinements to the bells allowing the treatment of infected wounds, especially on legs [10].

In the 1970s in Russia the principle was to apply a transparent flexible top under which a vacuum was created mostly by wall suction [11, 12]. Jeter used suction to treat wounds utilizing a gauze dressing and wall suction in 1985 and this was reported in 1989 by Chariker et al. [13] to assist wound healing and exudate management.

From 1986 to 1991 the Kremlin Papers were published that identified unique properties of negative pressure [14–17]. Davydov et al. [14] demonstrated the use of the negative-pressure dressing for purulent lactation mastitis on a series of 97 patients while Kostiuchenok et al. [15] demonstrated in a control study on 90 persons the superiority of surgical debridement of infected wounds after negative-pressure dressing compared with surgical debridement alone.

Fleischman et al. [18] described vacuum sealing for the treatment of soft-tissue injury and in 1995 discussed the indications, technique, and results of vacuum sealing [19]. In 1997 Argenta and Morykwas [20] published their technique using a foam wound filler and pump, called vacuum-assisted closure (VAC) therapy. VAC® was marketed and distributed by KCI (Kinetic Concepts Inc.) and Medical (San Antonio, TX). Stawicki et al. [21] reported on four refractory cases of hepatic cirrhosis using VAC therapy. They concluded that postoperative use of VAC in conjunction with optimization of medical therapy and judicious tapping of ascites provides a safe and effective method to control ascitic fluid leaks and promote definitive tissue sealing in patients with hepatic cirrhosis.

The general technique for use of NPWT includes wound coverage with a non-adherent dressing film, and then a dressing or filler material to fill the contours of the wound that is sealed with a transparent film [22]. A drainage tube through the transparent film is connected to the dressing and connected to a canister on the side of the vacuum pump or vacuum source.

Medicare began covering pumps in 2001 that only included KCI's pump [23]. Beginning in 2005 Medicare expanded its coverage to include several new pump models that are manufactured. In 2007 the models of pumps selected were Medela Vario, Bluesky Versatile 1, Bluesky VISTA Vaersatile 1, and Boehringer Engenex. There were 13% malfunctions of 215 of the beneficiaries and these were repaired or replaced.

## 2 Contraindications and Cautions

Sandoz [24] found that there were specific disorders that were a contraindication for negative-pressure wound therapy. These included:

1. Wounds involving untreated osteomyelitis.
2. Wounds exposing blood vessels, nerves, anastomotic sites, or organs, or with an unexplored fistula.
3. Wounds including open joint capsules.
4. Skin malignancy and excised skin malignancy except for palliation.
5. Wounds with necrotic tissue: Excise first.

He also stated to use with caution in the following circumstances:

1. Wounds with visible fistula: Isolate fistula to prevent deterioration.
2. Wounds with exposed bone or tendon: Isolate bone or tendon from direct pressure by

protecting with a liner dressing to prevent drying out.
3. Clotting disorder or anticoagulant use because of risk of bleeding.
4. Compromised microvascular blood flow to the wound bed because of risk of further compromise of vascular supply.

## 3 Discussion

There is some literature on NPWT that is on aspects of the procedure [25–31]. Some of the disorders in the literature treated by NPWT are on arthroplasty [32], burns [33, 34], cerebrospinal fluid leakage [35], critical wounds [36], diabetic wound [37], dog bites [38], flaps with congestion [39], high-pressure injection injuries [40], infection [41–45], loop ileostomy reversal [46], mediastinitis [47], necrotizing fasciitis [48, 49], nonhealing wounds [50], open abdomen [51–66], open-fracture wounds [67–69], pilonidal sinus [70], pyoderma gangrenosum [71], scalp reconstruction [72], skin graft engraftment [73], soft-tissue defects [74], spine surgery [75], vascular surgery [76], and ventral hernia repair [77, 78].

**Conclusions**

There are many different disorders in the literature which have NPWT for part of the treatment. It has been successful in most of them.

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# The Use of NPWT in Treating Electrical Burn Wounds

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## 1 Introduction

### 1.1 Electricity and Electrical Burn

Electrical burns are relatively uncommon; in adults they usually occur in occupational settings, whereas in children they occur accidentally [1]. In the United States approximately 1000 deaths per year are caused by electrical injuries, the mortality rate being around 3–5% [2].

Electricity is the movement of electrons, which comprise the current, from atom to atom, across a potential gradient from high to low concentration through a conductive material. The voltage represents the magnitude of this potential difference. Amperage measures the volume of electrons flowing across the potential gradient.

Resistance is a measure of how difficult it is for the electrons to pass through a material [3]. The resistance of the human body to electricity is relatively high on the outside and low on the inside; it varies depending on the electrolyte and water content of the tissue through which the electrical current is being conducted. Skin resistance varies on the moisture content, thickness, and cleanliness. All internal tissues offer low resistance, excluding the bone which is a poor conductor of energy. Muscles, nerves, and blood vessels have low resistance due to their high electrolyte and water content and are good electricity conductors. Bones, tendons, and fat have higher resistance. Electricity creates heat, following the path of least resistance through the body (Table 1) [4, 5].

The severity of an electrical burn depends on many factors and can be classified depending on the type of the circuit (electrical current can flow in direct (DC) or alternating current (AC)), duration, resistance of tissues, voltage (low or high), amperage, and pathway of the current [3, 4].

High-voltage direct current (DC) often causes a single-muscle contraction, throwing the victim

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**Table 1** Body tissues resistance

| | |
|---|---|
| Least resistant | Nerves |
| | Blood vessels |
| | Mucous membranes |
| | Muscle |
| | Dry skin |
| | Tendon |
| | Fat |
| Most resistant | Bone |

away from the source, while the same voltage of alternating current is considered to be more dangerous because the cyclic flow of electrons causes muscle tetany and prolongs the exposure to the electrical source [4, 6]. The degree of tissue destruction is directly proportional with the duration of contact with high-voltage current [4].

Contact with high-voltage current can be associated with an electric arc, which is formed between two bodies of sufficiently different potential that are not in direct contact (e.g., a highly charged source and the ground). The arc consists of ionized particles. The temperature of these particles and their surroundings can be as high as 4000 °C [7]. When portions of the arc touch the patient, deep thermal burns occur, the electric arc remaining the cause of most high-voltage injuries.

"Entry" and "exit" are commonly used terms to describe electrical injuries and the pathway that the current takes can determine the severity of the injury and the tissues at risk: disruption of cardiac rhythm, direct myocardial injury, respiratory arrest, paralysis, sensory and motor deficits, seizures, memory loss, cataract, strong muscle contractions resulting in scapular fractures or shoulder dislocations, flash burns, and blood vessel, nerve, and muscle destruction [4, 5, 7].

The incidence of low-voltage burns is currently declining but high-voltage injuries, particularly in adolescent males, remain an unsolved problem [8].

## 2 Sequelae After Electrical Burn

"There are 2 possible consequences of electrical injury: the person either survives or dies" [5]. Efforts are directed towards preventing additional tissue loss, managing a potential compartment syndrome, or handling the necrotic tissue. Electrical injury often results in high rates of morbidity [4, 5]. The long-term sequelae after electrical burn can be neurologic injuries, psychological trauma, ocular deficiencies, pain, etc.

Viable surgical reconstructive techniques for soft-tissue defect covering, such as muscle flaps, free flap transfers, and cross-leg techniques, are frequently used in treating sequelae after electrical burns. Free flaps are considered to be the gold standard when the treatment of leg wounds is needed, because of their ability to cover large defects. Reverse-flow flaps are useful to cover defects of the lower leg and the ankle. When this type of flaps are not available to use, cross-leg flap can be a useful technique (Fig. 1) [9].

In some cases, traditional reconstructive surgical techniques can be insufficient when covering a complex soft-tissue defect. The muscle flap can prove unsuccessful in managing a large defect due to nonhealing of the flap, flap necrosis, infection, hematoma, inadequate debridement of the necrotic tissue, use of a traumatized muscle graft, or unrealistic objectives for the muscle flap coverage (Fig. 2) [10].

Soft-tissue defects that are difficult to cover by muscle flaps and free tissue transfer are often a challenge for the practitioner. In some cases, when the patient cannot be a candidate for free flap surgery the use of negative-pressure wound therapy (NPWT) is an effective alternative that can minimize the traditional reconstructive surgery meth-

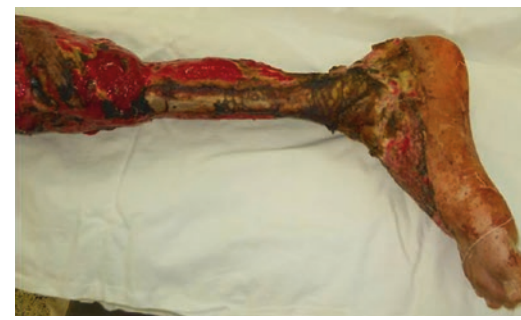

**Fig. 1** A 15-year-old male, victim of a high-voltage electrical injury, suffered major third- and fourth-degree burns on 60% of the body surface. After repetitive excision of the necrotic tissue, the distal extremity of the right leg—lower half of the tibial bone, lower half of the fibula, and internal and external malleoli—became exposed circularly due to the massive, circumferential soft-tissue defect [10]

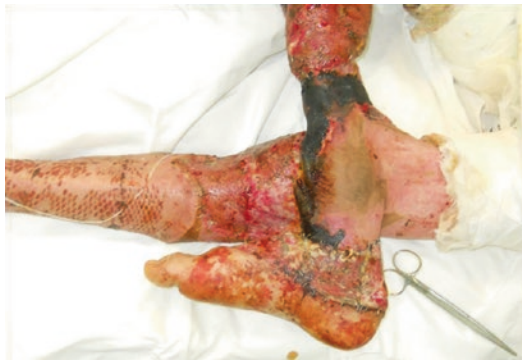

**Fig. 2** For reconstruction several surgical techniques had been used. The soft-tissue defect was partially covered using an internal twin muscle flap and a cross-leg technique covering the posterior defect using a contralateral thigh muscle flap, which division was performed after 21 days

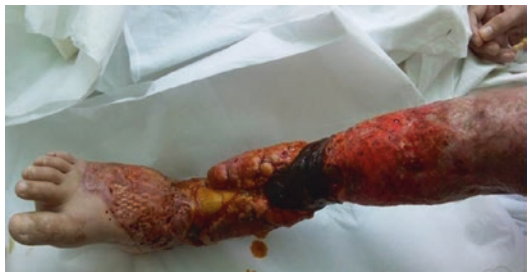

**Fig. 3** Muscle flaps could not cover the entire surface of the defect, due to flap necrosis and unrealistic expectations for the muscle flap coverage

ods and can reduce the surface of the soft-tissue defect by filling it with new formed granulation tissue, creating a skin graft receptor bed [11, 12].

In their retrospective study over a period of 12 years, published in 2006, Parret et al. [13], found out that the free flap use decreased from 42% during the first period to 11% in the last 4 years of the study, when NPWT started to be extensively used. NPWT can reduce both the need for flap transfer and the size of the flap (Fig. 3).

## 3 Negative-Pressure Wound Therapy

NPWT, also referred to as VAC therapy (vacuum-assisted closure) or micro deformational wound therapy (MDWT), has begun to play an increasingly important role in the global landscape of wound treatment. For the last 15 years, this type of therapy intends to augment and improve the traditional methods of approaching these pathologies bringing numerous benefits on morbidity, mortality, as well as aesthetic benefits [12, 14].

The VAC therapy applies subatmospheric pressure to the wound bed, using a computerized device that produces controlled suction, via a connective port. The subatmospheric pressure helps the wound healing through mechanisms that ultimately result in wound contraction, fluid drainage, prevention of bacterial growth, and granulation tissue formation [15–17].

The negative-pressure wound therapy aims to create a perfect environment for wound healing. The mechanisms of action include macro deformation of the wound, micro deformation at the wound-wound filler surface, fluid drainage—thus reducing edema, improvement in local blood flow, creating a moist environment that facilitates wound healing [18], reduced inflammation, improvement in cell proliferation [19], influence in hemostasis, stimulation of angiogenesis, granulation tissue formation, alteration in bacterial burden, and affecting of cellular responses in division, migration, and differentiation (Table 2) [14, 20, 21].

The macro deformation of the wound refers to the contraction and size reduction of the wound due to the centripetal forces that NPWT induce, shrinkage that is also caused by the collapse of the foam pores. Micro deformation refers to the interaction between wound bed and NPWT contact layer, the undulated wound surface induced by the porous material of the foam [11].

The mechanical deformation starts a signaling cascade, leading to wound healing [22]. Fluid

**Table 2** NPWT mechanisms

| |
|---|
| Creates a sterile, moist environment that facilitates wound healing |
| Facilitates fluid removal/exudate draining |
| Improves local blood flow |
| Stimulates angiogenesis |
| Stimulates granulation tissue formation |
| Reduces inflammation |
| Decreases bacterial load |
| Stimulates wound contraction |
| Affects cellular responses |

removal optimizes tissue perfusion by reducing the compression on the capillaries, thus allowing increased blood flow to the wound area [23, 24].

## 4 Technique

### 4.1 NPWT System

The negative-pressure wound therapy system has four major components: a wound filler material, an airtight vacuum seal, a connecting tube, and a vacuum pump [14]. The vacuum device has an incorporated canister, where the fluid is collected and which is equipped with an alarm system that notifies the practitioner when the canister is full.

Contraindications for the use of NPWT include eschar with the presence of necrotic tissue, untreated osteomyelitis, malignant cells in the wound, direct use on exposed blood vessels and nerves, nonenteric and unexplored fistulas, exposed anastomoses, and exposed organs. Some characteristics to consider before using this treatment include high risk of hemorrhage (including patients on anticoagulants or platelet aggregation inhibitors), infected wounds, friable vessels and infected blood vessels, sharp edges in the wound, spinal cord injuries, circumferential dressing application, proximity of the foam to vagus nerve, and patient weight and size [14, 25].

#### 4.1.1 Sequence of Procedure (Table 3)

1. Wound Bed Preparation (WBP)

For wound bed preparation, the necessary supplies can be organized into five categories: anesthetic, sterile field, irrigation, debridement, and dressing (Table 4).

The management of any traumatic wound starts with thorough irrigation using sterile saline, in order to clean the wound and facilitate inspection. Lavage intends to clear the debris from the wound and lower the bacterial burden. Bacterial clearance can be improved by early irrigation. On his study on an animal model, Owens concluded that earlier irrigation in a contaminated wound resulted in a superior bacterial removal: irrigation within 3 h lowered the bacterial load by 70%, within 6 h 52%, and 37% at 12 h (Fig. 4) [10, 26].

**Table 3** Sequence of NPWT procedure

| |
|---|
| 1. Wound bed preparation (irrigation, debridement of necrotic tissue) |
| 2. Placement of contact layer and foam |
| 3. Creating an airtight seal |
| 4. Application of NPWT |

**Table 4** WBP necessary supplies

| |
|---|
| 1. *Anesthetic* (local anesthetic, distraction techniques, anxiolytics, and/or sedation) |
| 2. *Sterile fields* (sterilizing solutions, sterile drapes) |
| 3. *Irrigation* (sterile saline) |
| 4. *Debridement* (gauze for mechanical scrubbing, forceps for tissue handling, scalpel) |
| 5. *Dressing supplies* (saline-moistened gauze, antimicrobial impregnated dressings, NPWT, biological dressings) |

A proper debridement of the devitalized, necrotic tissue must be obtained, to facilitate healing and decrease the risk of infection at the wound site, with due regard to vital anatomic structures, hemostasis, and wound hygiene. NPWT cannot be applied over necrotic, devitalized, or infected tissue. A devitalized tissue, by absent or tenuous blood supply, is poorly penetrated by systemic antibiotics and provides a good environment for bacterial proliferation [10, 27].

Profuse lavage of the wound is recommended each time the change of the dressing is performed. If required, a swab culture for microbiology should be taken before saline lavage (Fig. 5) [28].

2. Placement of Contact Layer and Foam

The VAC therapy requires a contact material that enables the negative pressure to reach the wound bed. The wound filler material, as part of the commercial NPWT systems, is available as foam wound filler and gauze wound filler [29].

The foam wound filler is custom cut by the practitioner to fit the wound. Several types of foam are available:

(a) Polyurethane (PU) black foam, hydrophobic and reticulated, made of highly interconnected cells [14], allows even

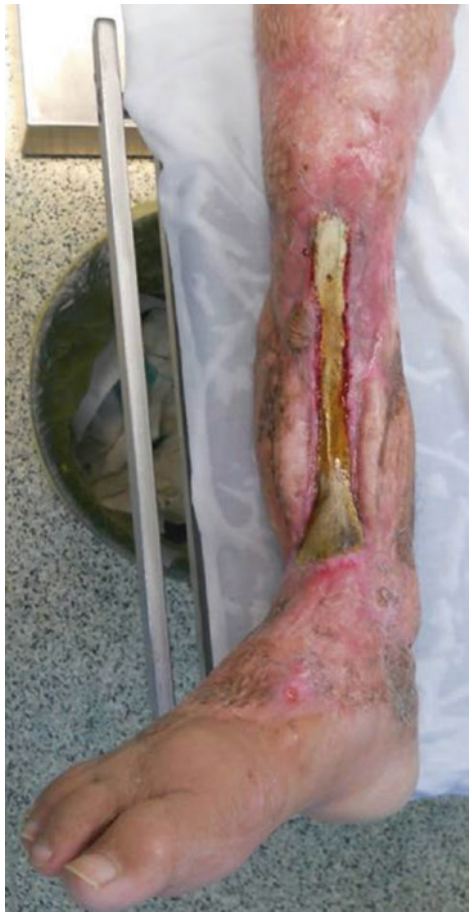

**Fig. 4** A couple of months later, the patient was transferred to our department presenting a chronic wound. Clinical examination of the right leg revealed two soft-tissue defects, tibial bone exposed on an area of 15/3 cm in the lower half, the peroneal malleolus exposed and had a surface of 7/2.5 cm [10]

distribution of the negative pressure across the wound bed [30] and improves fluid drainage [14] and wound contraction [31]. This type of foam is often used in wounds with large fluid drainage and when stimulation of the granulation tissue formation is wanted [14].

(b) Silver-coated foam that can be used in the surgical therapy of infected wounds because of the antimicrobial effects of silver nanoparticles that can destroy bacterial cell walls and inhibit enzymes for bacterial cell replication [32].

(c) Polyvinyl alcohol white foam (PVA), hydrophilic, with higher tensile strength than the PU foam [30]: This type of foam can be used when growth of granulation tissue is less needed [31], because of the increased density and the smaller pores. It is recommended in wound with delicate underlying structures (e.g., tendons, blood vessels) that need to be protected [14, 33].

The foam can be used in association with a silver nanoparticle contact layer that has the ability to reduce wound infection rates, decrease the frequency of dressing changes, diminish pain levels, and promote wound healing. Also, a silicone wound layer may be used to reduce trauma and pain at dressing changes, prevent the ingrowth of the new formed tissue in the foam reticules, protect the delicate wound structures, and facilitate the formation of granulation tissue (Figs. 6 and 7) [12].

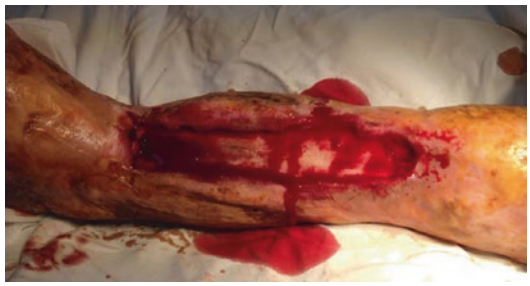

**Fig. 5** Wound bed preparation: The aspect of the leg after wound-edge excision and debridement of devitalized bone tissue: removal of the outer layer of the anterior cortex and the whole anterior cortex in some regions, causing the bone to bleed [10]

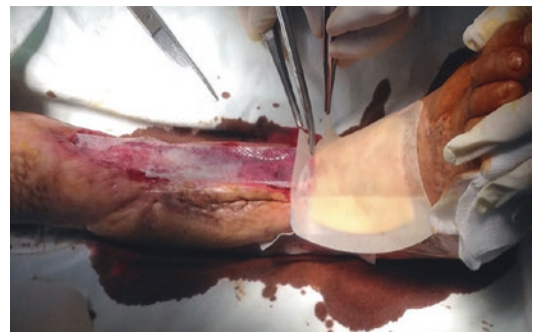

**Fig. 6** The use of silicon contact layer to facilitate granulation tissue formation and hydrocolloid dressing to protect the intact skin

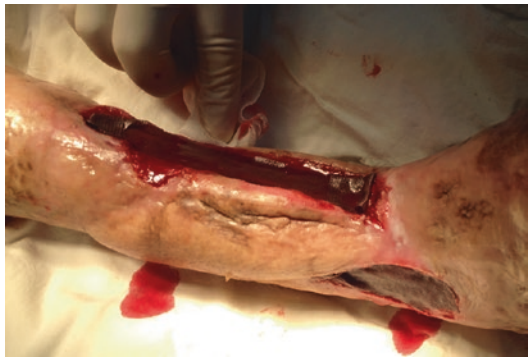

**Fig. 7** The use of nanocrystalline silver dressing to reduce the bacterial burden of the wound

3. Creating an Airtight Seal

The second component of the negative-pressure wound therapy is creating an airtight seal over the wound and the wound filler, thus facilitating the suction to the wound bed. This can be done with an adhesive occlusive dressing. Depending on the anatomical location of the wound, this process can be sometimes difficult and application of skin adhesive to maintain the seal is needed [34]. The sealing dressing must entirely cover the wound filler and the wound. Special care must be taken for the wound edges, ensuring that these are clean and dry (Fig. 8).

4. Application of NPWT

Third component, the non-collapsible tube is embedded in the foam through an incision made in the sealing dressing, geometrically fitted for the connecting device.

The vacuum pump is a computerized device that creates negative pressure at the wound site via the canister and the tube. There are several types of vacuum pumps. The traditional pump is usually portable and the canister in use with this pump can hold from 300 to 1000 mL wound exudate. It usually incorporates a

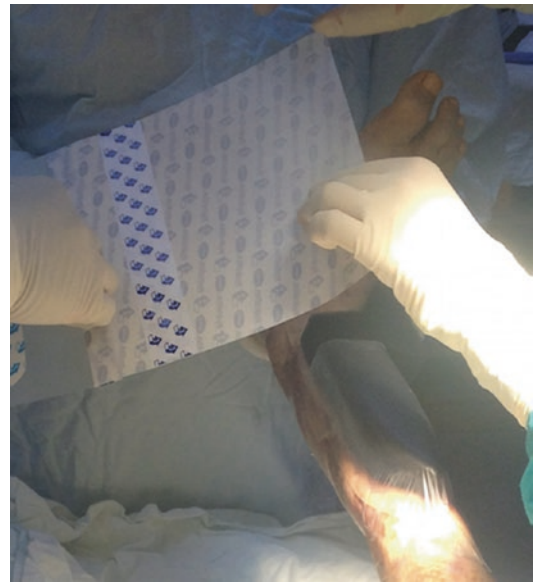

**Fig. 8** Airtight sealing of a chronic wound of the leg using plastic drapes

computerized alarm system that detects inadequate seal, excessive fluid drainage, blockages of the tube, etc. [15]. It is electrically powered and has a rechargeable battery. From this device the practitioner can choose what type of pressure to use, continuous, intermittent, and variable, and the amount of pressure applied to the wound.

The NPWT pump delivers negative pressure to the entire wound bed. The amount of pressure applied can vary between 25 and 200 mmHg depending on the wound and the wound filler type. In clinical practice the amount of pressure that is usually used is 125 mmHg.

After the application of NPWT, the dressing must be changed in an interval of 2–4 days. Dressing changing can be a painful maneuver; local or general anesthesia is recommended. Avivement (surgical trimming of wound edges before suturing them) of the wound and lavage must be performed each time (Fig. 9).

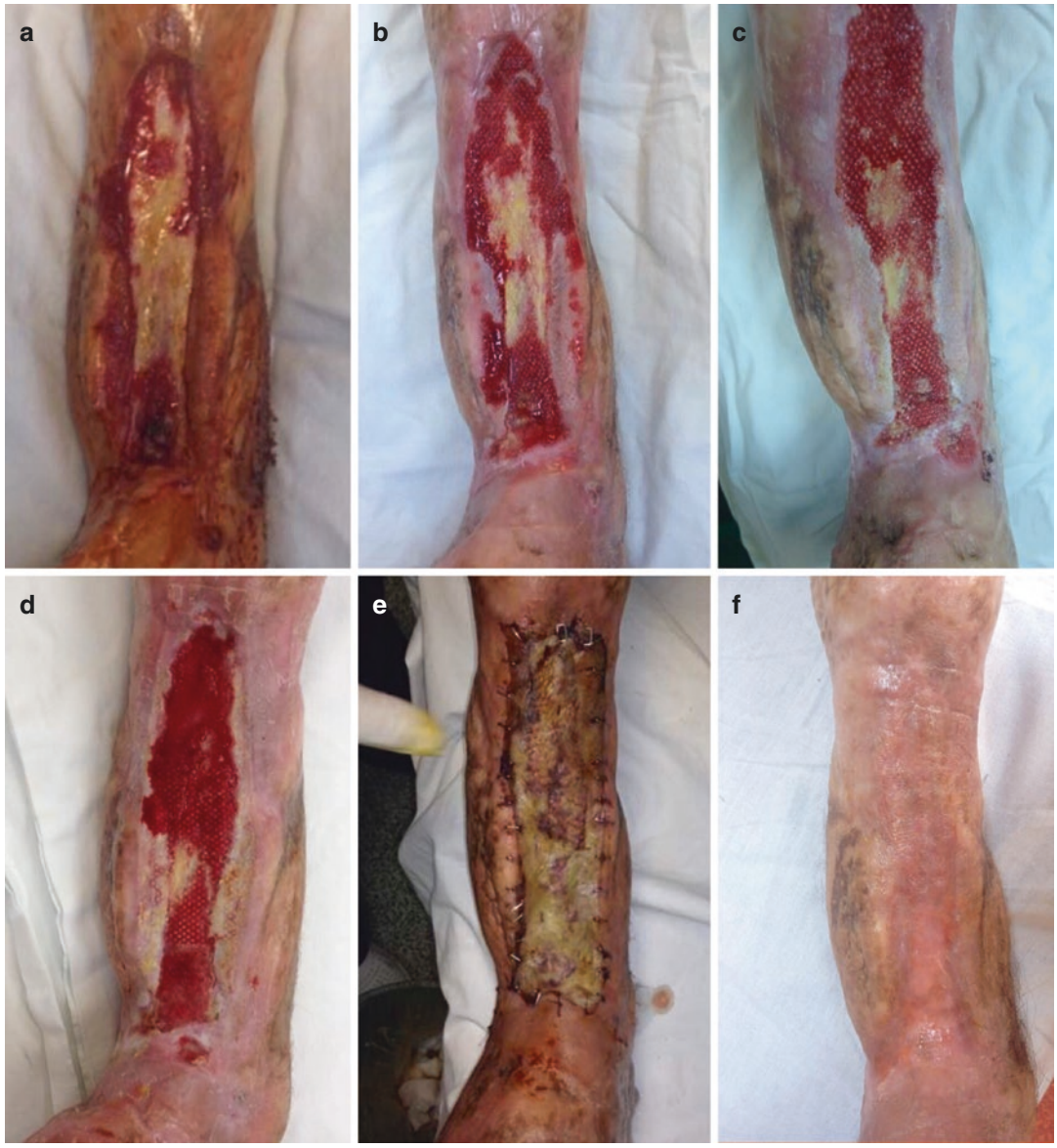


**Fig. 9** Stages in granulation tissue formation during NPWT, final wound coverage, and wound healing. (**a**) On the sixth day of using the NPWT the contraction of the wound edges could be observed. (**b**) After 15 days of using the NPWT granulation tissue covered the proximal and the distal ends of the wound. (**c**) Day 25 of NPWT. (**d**) Day 33 of NPWT. (**e**) Split skin grafting. (**f**) Healed wound

## 5 Discussion

Compared to conventional burns, high-voltage burns are characterized by an increased morbidity and worse potential for rehabilitation. During the early posttraumatic period, the surgical management of these particular burns is represented by repetitive debridements and necrectomies. Mortality in this type of injury is remarkably high, even with the aggressive approach to remove necrotic tissue.

In burns, conventional surgical debridement is the gold standard. Although full excision was historically standard practice in excision of burns, nowadays tangential excision, removal of necrotic tissue by sequential layered excision of devitalized tissue until the level of healthy, bleeding, vitalized tissue, has replaced full excision. Timing of debridement is an important aspect as well; tangential excision facilitates early debridement by intraoperatively determining the depth of the burn. In electrical burn wounds the intraoperative determination of the burn depth is hard to obtain [10].

Muscle flaps (reverse flow flaps, cross-leg, free microvascular flaps, etc.) can be used in an attempt for limp salvage [35].

The normal healing process includes hemostasis, inflammation, cell proliferation, and cell maturation. These phenomenons that appear in the healing process of an acute wound do not apply entirely in the processes involved in the healing of chronic wounds. The delayed healing of chronic wounds is due to a failure to progress through these phases, the sequence of events becoming disrupted at one or more of the steps of the healing process. Usually chronic wounds are "stuck" in the inflammation phase of healing as a barrier defect that has not healed in 3 months [36, 37].

Usually chronic wounds include, but are not limited to, diabetic, venous, pressure foot and leg ulcers. These type of chronic wounds have a different pathophysiology than an acute wound or a traumatic chronic wound and the modalities of treatment and means of healing differ. A good understanding of the differences between different types of chronic wounds should lead to better healing rates and improve treatment management. The primary challenge in treatment of a chronic wound is to overcome the factors that sustain a delayed healing and to have a comprehensive approach to wound care. Chronic lower extremity wounds include leg and foot ulcers due to a vascular disease, diabetes, neurological foot, chronic venous insufficiency, arterial disease, neuropathy, and prolonged pressure [13, 38].

Some of the factors that contribute to the delayed healing include prolonged and massive inflammation, unremitting infection with drug-resistant microbes, and lack of epithelialization. A major step forward in managing the problems of wound healing is concerned by the wound bed preparation, allowing the practitioner to identify hypoxia, increased bacterial load, presence of necrotic tissue, and alteration of the matrix. Wound bed preparation can accelerate the endogenous healing of the wound or facilitate the effectiveness of other therapeutical strategies [36, 37].

NPWT protocol when treating electrical burn sequelae wounds is determined by the goal of treatment (granulation tissue growth to cover a soft-tissue defect, fluid drainage and edema removal, flap or graft immobilization), pressure values, pressure modes, and type of dressing.

The duration of the negative-pressure wound therapy depends on the goals of treatment. When the device is used to stimulate the formation of the granulation tissue, for preparing a skin graft receptor bed, the therapy can continue until the soft-tissue defect has been covered and the granulation tissue has reached the skin level so the skin graft can be safely placed.

The amount of pressure applied to the wound can vary, as well as the modes of pressure available. A series of basic animal studies demonstrated that the blood flow levels increased when 125 mmHg negative pressure was applied [39]. Other animal studies concluded that wound

contraction and fluid removal are directly proportional with the level of negative pressure until reaching a steady state. Maximum wound contraction was observed at a pressure of −75 mmHg and the maximum fluid drainage from the wound was at −125 mmHg [40].

NPWT can be delivered in continuous, intermittent, or variable modes. The continuous pressure mode is the most commonly used, during which the pressure level is constant. In the intermittent mode the negative pressure is switched on and off repeatedly. Studies suggest that the intermittent pressure therapy results in faster healing by stimulating the formation of granulation tissue (by mechanically stimulating the wound bed and increasing blood flow to the wound edges), but is painful. Variable pressure was introduced to decrease the amount of pain by creating a smooth transition between the two modes of negative pressure [41]. Variable pressure is the most indicated pressure mode when formation of granulation tissue is needed as well as the management of pain. Special attention concerning pain management during treatment and dressing changes must be given.

The placement of the foam and contact layer is an important aspect for successful NPWT. The foam must be cut to size to fit the wound and the contact layer, if used, must be inserted into all undermined areas and must fill all irregularities of the wound. Placing the foam directly on top of intact skin should be avoided.

In some cases there are more than one soft-tissue defects in the same anatomic region. In order to use a single-vacuum port a foam bridge that connects both areas can be created. The healthy skin between them must be protected using a seal drape or a hydrocolloid dressing (Fig. 10).

The adverse effects when using this type of treatment are represented by pain and discomfort, skin irritations caused by allergies from the adhesive sealing drapes, excoriation of the skin if the foam is not correctly cut to size, and sometimes odor from the dressings or the canister. Common complications of NPWT include bleeding, infection, foam-tissue adherence, and foam retention in the wound [28].

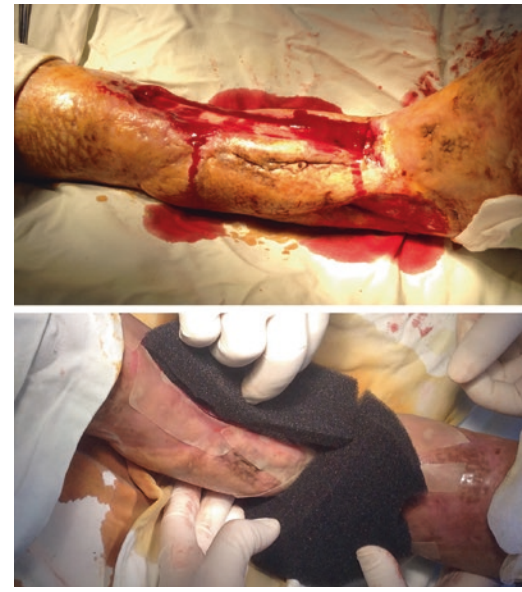

**Fig. 10** Foam bridge that connects the two soft-tissue defects in order to use a single-vacuum port

A potential and serious complication of NPWT is bleeding from the wound site. When preparing the wound for applying the negative-pressure wound therapy, removal of the devitalized tissue must be done, until healthy, bleeding tissue is revealed. When removing devitalized bone, coagulation in the remaining bone during suction can be sometimes difficult and severe hemorrhage can occur. Special care should be taken concerning this aspect. Also, removal of devitalized bone tissue can affect the mechanical resistance of the remaining bone. Considering this aspect, one of the complications that can occur after debridement of the necrotic bone tissue can be the fracture of the bone at the wound site (Fig. 11). This situation needs special care, immobilization of the leg, and therefore a more difficult access to the wound that can endanger wound's healing.

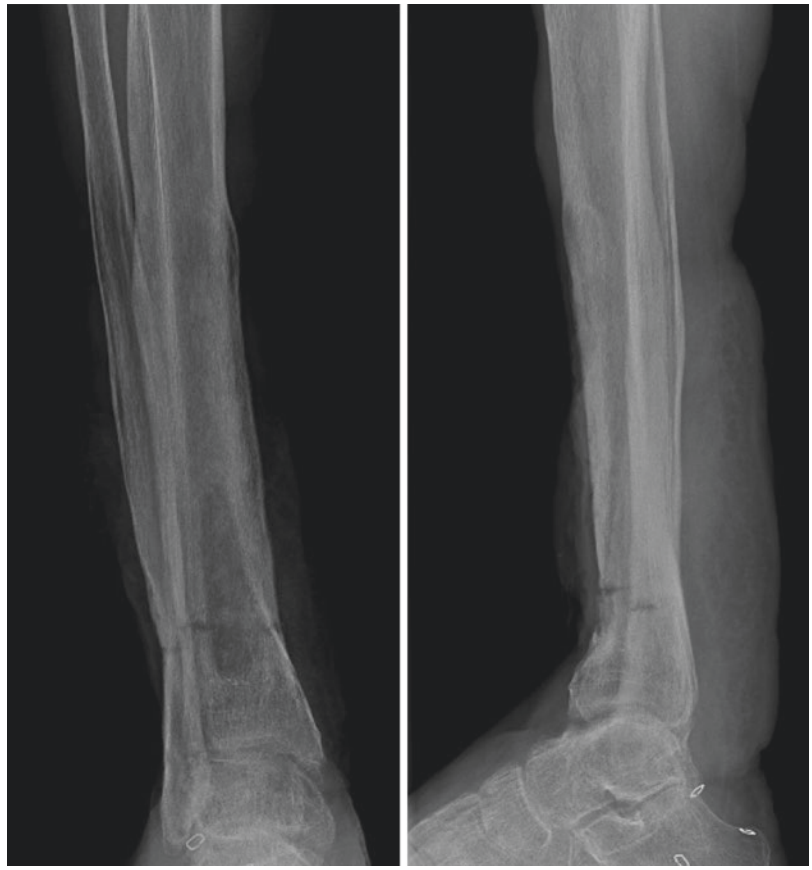

**Fig. 11** Undisplaced fracture of lower tibia and fibula after the resection of the devitalized bone tissue

## Conclusions

The care of traumatic electrical burn wounds requires prompt evaluation, pain management, irrigation, debridement, and application of appropriate dressings. Initial management of electrical burn wounds should intend to optimize function and minimize long-term scarring.

The treatment of delayed deep and extensive soft-tissue defects is a challenge for the practitioners, especially if the patient's overall condition is poor. In some cases, by using the NPWT it is possible to cover major soft-tissue defects, thus avoiding the amputation of the limp. Considering these results, there is a trend in using fewer muscle flaps and more delayed closures, using skin grafts on a receptor bed created by VAC therapy.

This newer and simpler technique used for covering of exposed bone tissue can question the gold standard of plastic reconstructive surgery that utilizes muscle flaps as the only way to cover these defects.

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# Negative-Pressure Wound Therapy as Prophylaxis for Surgical Site Infection in Perineal Wounds

Patrick B. Murphy and Michael Ott

## 1 Introduction

Abdominal perineal resection (APR) is most commonly performed in the setting of locally advanced low rectal cancers. Several other indications also exist such as severe inflammatory bowel disease and treatment failure or recurrence of anal cancer [1]. Despite increased emphasis on intestinal continuity the APR still remains a frequently performed procedure. Following APR one of the most devastating complications patients can experience is infection or dehiscence of the perineal wound [2]. Significant efforts and innovation have been applied to reduce perineal wound complications but it remains common and a challenge to avoid and manage [3]. The significant patient morbidity is mirrored by the magnitude of healthcare costs to treat perineal complications. Such complications include abscess, dehiscence, persistent sinus tracts, and hernia [4]. While the morbidity to patient and cost to the healthcare system are great, perhaps most important related outcome is the demonstrated increased local recurrence of cancer in patients with significant perineal complications. This increase in local recurrence attributed to perineal wound complications is likely secondary to significant delays in the initiation of required adjuvant therapy [4].

The large cavity created following removal of the rectum and anus is at high risk due to a number of factors. The dead space created in the pelvis easily accumulates fluid and blood postoperatively by gravity as the most dependent area of the abdominal cavity. The dead space is created by the rigidity of the pelvis making it impossible to remove all of the dead space. The effects are compounded on the skin incision as often there is significant tension on the pelvis floor muscles, adipose, and skin in the perineal wound. Several other unavoidable factors influence wound healing in a negative way including patient factors, disease-related factors, and treatment- or surgical related factors (Table 1). Despite increased vigilance against surgical site infections (SSI) the incidence of perineal SSI following APR ranges from 16 to 60% depending on the report and patient population considered [5, 6].

Several strategies to decrease SSI in the perineal wound have been implemented. Historically, Miles in 1908 [7] when first proposing APR recommended leaving the wound open with packing to allow the dead space to close by secondary intension because of such a high risk of infection. Since then many others have advocated numerous strategies to limit infection including primary closure, partial closure, drains, local antibiotic infusion,

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**Table 1** Risk factors for perineal complications

| |
|---|
| **Patient** |
| • Diabetes mellitus |
| • Smoking |
| • Obesity |
| • Malnutrition |
| **Disease** |
| • Inflammatory bowel disease |
| • Immunosuppressive therapy |
| • Tumor size |
| **Treatment** |
| • Neoadjuvant radiation therapy |
| **Surgical** |
| • Fecal contamination |
| • Flap closure |

omental plugging, and muscular flaps to decrease perineal SSI [3]. Negative-pressure wound therapy (NPWT) has gained attention in the last decade in wound management revolutionizing complex wound care and management of the open abdomen. As a direct extension of the benefits of NPWT surgeons have experimented with NPWT on a closed incision as a postoperative dressing attempting to decrease wound infections in at-risk wounds. The use of NPWT as a prophylactic treatment for SSI through application in sterile conditions, in the operative theater, after primary closure is an extrapolation based on the success seen in complex wound. These closed incision systems were derived from standard NPWT using surgeons' own innovation. In response to this innovation several incisional NPWT kits are now commercially available from numerous manufacturers and are typically single use only. Criticized for the cost of such disposable devices, "homemade" NPWT devices have been described effective at significantly less cost [8, 9]. The cost of prophylaxis NPWT in reducing SSIs is offset by the high costs of SSI, mostly realized in the potential reduction in length of stay, further operative procedures, and home care for open wounds.

The biomechanical profile of NPWT has been elucidated through both in vitro and in vivo models. NPWT promotes angiogenesis and modulates the local inflammatory environment around wound healing [10]. The application of negative pressure reduces lateral force and helps [10] maintain the integrity of the wound and improves approximation of the tissue faces. Negative pressure improves perfusion to the skin edges and on a microscopic level promotes cell proliferation and angiogenesis. The connected drainage system allows the dressing to remain on the patient for longer compared to nonnegative pressure dressings which can become soaked through. This has the potential to limit bacterial contamination. In clinical studies incisional NPWT has demonstrated a reduction in seroma/hematomas which may help explain the effect in prevention of SSI [10].

## 2 Technique

All-in-one incisional NPWT devices are straightforward to apply under sterile conditions in the operative theater and add <10 min to operative time. The authors' experience is with continuous NPWT commercial devices but descriptions of "homemade" setups are available. One concern with simplified incisional NPWT for this location is the placement of the pressure-sensing pad and suction tubing. The pressure pad and tubing must be offset to ensure that the patient is not required to lie on the tubing which may create pressure sores. For the perineal wound we recommend the setup in Fig. 1.

A single layer of non-adhesive gauze is placed of the entire length of incision to protect the incision directly from the NPWT foam. This gauze may be impregnated with silver. Various sizes of foam are available or customizable devices can be used which are cut to the length of incision. Ostomy paste is then used to fill any gaps or crevices around the wound to maintain a closed airtight seal. A second piece of foam is overlapped and runs perpendicular to the incision in order to move the suction tubing away from a pressure-dependent portion of the body (Fig. 1). An occlusive, transparent, adhesive dressing is then placed over the foam. It is important to ensure that hair removal is wide around the incision to prevent seal leaks which may reduce the effectiveness of the negative pressure. This is usually done preoperatively and a 5 cm margin around the planned incision is typically effective. An incision is made over the foam gauze and a pressure-sensing pad is applied with tubing connected to a vacuum unit. Negative 125 mm suction

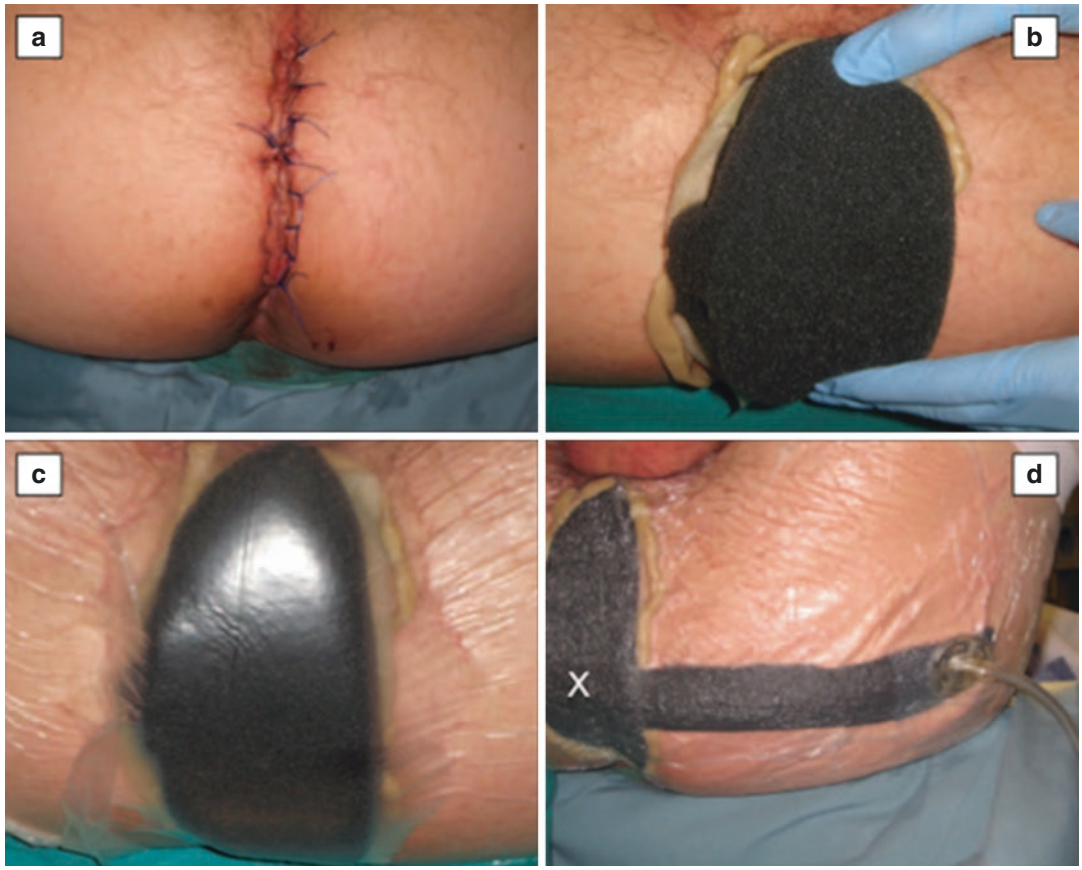


**Fig. 1** Postoperative placement of incisional negative-pressure wound therapy device. (**a**) The incision is closed with interrupted 2–0 polypropylene suture in an interrupted vertical mattress fashion. (**b**) The incision is surrounded with ostomy paste and covered with a piece of petrolatum emulsion-infused gauze (adaptic). An oval piece of suction polyurethane foam placed on top of the gauze. (**c**) The suction foam is covered with a layer of adhesive tape to create an airtight seal. (**d**) An extension piece of polyurethane suction foam is placed across the gluteal region to the lateral thigh. A window in the adhesive overlying the incision is created below the extension piece of suction foam (point X). A second piece of adhesive tape is placed over the top of the extension foam, and a window is placed in its most lateral aspect where the suction through the traction piece is applied

is then applied continuously. Newer devices have a stronger vacuum than earlier models and in general poor hair removal is responsible for leaks followed by body contours around the incision. If air leaks are identified the dressing can be reinforced with more occlusive adhesive dressing or a stronger vacuum unit can be used in order to maintain negative pressure despite the leak.

Generally the NPWT dressing is removed on POD #5 or at the time of discharge if earlier. Our institution does not send patients home with incisional NPWT. Other reports have used both longer and shorter durations of NPWT and the best length of application is not known.

## 3 Discussion

The evidence for incisional NPWT for primarily closed wounds in prevention of SSI developed from the orthopedic and cardiac surgery literature [11]. Significant decreases in SSI were seen in hip fractures, other high-risk orthopedic fracture repairs, and high-risk median sternotomies. A recent meta-analysis on the use of NPWT for closed incisions demonstrated a reduction in SSI. Authors also commented on a lack of well-designed and appropriately powered randomized control trials using closed-incision NPWT [12].

Our institution compared 27 consecutive patients undergoing APR and placement of incisional NPWT with 32 matched historical controls. We were able to demonstrate a statistically significant reduction of perineal SSI from 41 to 15% in the NPWT group [13]. This reduction in SSI also leads to a decrease in healing time, decrease in further surgical procedures, and decrease in overall cost. More recently, Sumrien et al. [14] also reported similar results. Comparing standard APR to extralevator APR (results in a larger defect) the reported reduction in SSI was 40–9% with NPWT compared to a standard dressing.

Both trials are limited by their small and retrospective designs. Recently published randomized controlled trials in general surgery patients undergoing laparotomy have failed to demonstrate the benefit of NPWT on primarily closed abdominal incisions for SSI prevention [15]. However these studies also suffer from a great degree of heterogeneity. While the evidence supporting the use of NPWT on perineal wounds is of a lower quality than randomized controlled trials, all the evidence that is published to date is supportive.

### Conclusions

Negative-pressure wound therapy on primarily closed incisions has a growing body of literature in many surgical specialties supporting its use to prevent SSI. The perineal wound following APR continues to present challenges to surgeons and wound care specialists due to the high rate of SSI and the morbidity associated. While small, retrospective studies have demonstrated the potential benefit of NPWT on perineal wounds there remains a gap in the literature for prospective and randomized trials.

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# Negative-Pressure Wound Therapy: Principles and Usage in Orthopedic Surgery

Jaiben George, Mhamad Faour, Jared M. Newman, Gannon L. Curtis, Alison K. Klika, Nathan W. Mesko, and Carlos A. Higuera

## 1 Introduction

A wound is defined as a disruption of the anatomical structure and function of an organ, such as the skin, resulting from a pathologic process beginning internal or external to the organ [1]. Acute wounds are those that repair themselves or can be repaired in an orderly and timely process, while chronic wounds heal in a delayed fashion (often >1 month) [1]. Skin acts as a protective barrier, and irrespective of the type and etiology of the wound, restoration of this normal barrier is important to prevent loss of body fluids, infection, and injuries to underlying tissues and organs. Dressings have been traditionally used to cover and prevent contamination of wounds [2]. However, with the increasing nature of wound complexities and the various local and systemic factors that affect wound healing, advancements in the types of wound dressings have been made, which can promote wound healing in addition to preventing contamination.

Negative-pressure wound therapy (NPWT) has become an integral part in the management of different types of wounds over the last few decades. It relies on creating a subatmospheric pressure on the surface of wound which is believed to promote wound healing, especially when there are various factors which can affect wound healing [3]. The negative pressure is typically applied until granulation tissue develops or until the local conditions are favorable for an additional surgical procedure, such as skin grafting. Negative-pressure wound therapy can be used for chronic wounds, acute wounds, and even surgical wounds (incisional NPWT) [4, 5]. However, not all types of wounds may benefit from NPWT, and studies have shown mixed results regarding the added clinical benefits of NPWT [6]. A thorough understanding of the mechanisms, indications, and applications of NPWT is crucial to promote the judicious use of NPWT. In this chapter, we focus on the principles of NPWT, and discuss the current evidence in support of its use in various surgical fields, especially orthopedic surgery.

## 2 History

Approximation of the skin edges and obliteration of dead space have long been recognized as crucial components of wound healing. Use of negative pressure was initially implemented

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in the 1950s to drain the collection of fluid under the skin associated with certain types of surgeries [7, 8]. These devices were composed of subcutaneously placed drains connected to a vacuum device to drain the excess fluid collection, and were reported to prevent fluid collection formation and promote granulation tissue growth [9]. By the late 1980s, scientists in Europe started to apply negative pressure over the surface of wounds with the use of foam and suction tubing [2, 10]. In the 1990s, a series of basic science and clinical studies performed by Argenta [11] and Morykwas [3], highlighting the positive effects of wound deformation, tissue pressure changes, and cytokine stimulation, led to the widespread implementation of NPWT in the present form in the United States. The first commercially available device that provided NWPT was the vacuum-assisted wound closure device and technology (V.A.C.®) (Kinetic Concepts Inc. (KCI), San Antonio, Texas). While the initial application of NPWT was restricted to large open wounds in debilitated patients, the use of NPWT has expanded to include wounds of varying severities and even as a prophylactic measure over surgical incisions. Although a number of negative-pressure device systems have been described, the most popular and widespread clinically used systems consist of delivery of an open-pore foam dressing, which results in the formation of small, domelike structures at the wound surface called microdeformation [12]. Therefore, some authors have suggested the term microdeformation wound therapy (MDWT) to distinguish the commonly used NPWT system from other systems delivering negative pressure [12]. However, in this chapter we use the term NPWT to refer to the commonly used systems that use foam.

## 3 Mechanism of Action

Although a number of theories have been described, the effects of NPWT can be broadly explained by two basic theories [13, 14]. The first one is based on the mechanical stain imposed on the tissues at the macroscopic and microscopic level, which leads to approximation of the skin edges and stimulation of growth of granulation tissue. The second is based on the removal of excess fluid, inflammatory markers, and potentially bacteria from the wound and the surrounding tissues. However, this last one is controversial and is discussed further in this chapter. Apart from these two mechanisms, the application of NPWT on wound beds has many indirect effects on wound healing, like modulation of inflammation, angiogenesis, peripheral nerve response, hemostasis, improved lymphatic clearance, and alteration in bioburden [12, 15–17]. However, the clinical relevance of some of these observed effects is unclear [18, 19].

With the application of the negative pressure, the porous foam shrinks in size and exerts strain on the wound bed, which leads to macro- and microdeformation of the wound (Fig. 1) [12]. Macrodeformation refers to the shrinkage of the size of the wound with the application of the NPWT. The foam used in NPWT systems can reduce in size by approximately 80%, and has been shown to result in a substantial decrease in wound sizes [13]. The extent of the contraction depends on the deformability of the tissue being used with larger shrinkage seen with abdominal wounds, compared to less deformable tissues located in the extremities or in a previously irradiated tissue bed [20]. Additionally, the wound contraction is associated with a paradoxical rise in the pressure of the surrounding tissues presumably due to the tension applied on the tissues by the contracting wound [12]. This can decrease the blood supply and can be detrimental in certain types of wounds, especially in ischemic limbs if circumferential NPWT is administered. In addition to the changes at the macroscopic level, the porous surface of the foam results in an undulated wound surface at a microscopic level [21]. This microdeformation results in strain of the tissue's cytoskeleton, which in turn stimulates cell proliferation, migration, and differentiation [22]. These microscopic changes in the surface of the wound

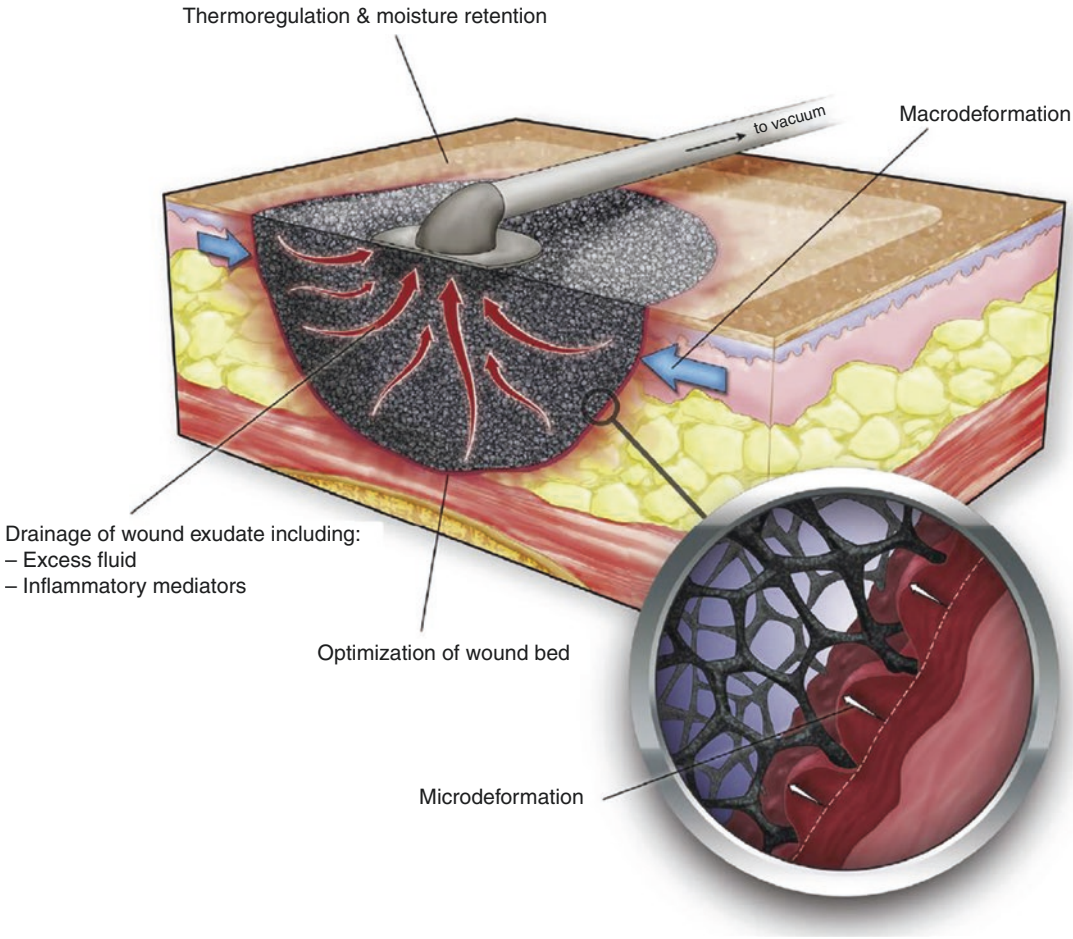


**Fig. 1** The proposed mechanisms of action of negative-pressure therapy. Used with permission [12]

result in faster granulation tissue formation and quicker wound healing [13].

The negative pressure applied over the wounds results in the removal of fluids and clears the wound of toxins and exudates. Removal of fluid relieves the compressive effect of extracellular fluid on surrounding tissues and has been shown to improve circulation in the wound bed [23]. Removal of fluid also reduces the amount of fluid that must be cleared by the lymphatics and induces a local increase in lymphatic density [24]. It is also important to understand that the basic science evidence behind incisional NPWT (application over a primarily closed wound) has also been shown to afford similar benefits as application over open wounds, such as decreased tension on the skin, improved blood flow in the dermal location, and decreased seroma/lymphedema formation [17]. The use of NPWT does not appear to reduce the bacterial burden in the wounds. Some studies have even reported that the use of NPWT can increase the bacterial burden although there was enhanced wound healing with NPWT [18, 19].

## 4 Application of NPWT

NPWT does not replace the basic principles of wound management. Wounds should be thoroughly debrided, and necrotic or infected tissue should be removed prior to the application of NPWT. There are five basic components to the modern-day NPWT system, including wound

filler, tubing, drapes, a pump, and a canister. The most commonly used wound filler is open-cell polyurethane foam and is composed of interconnected cells of size ranging between 400 and 600 μm in diameter [15]. The porous nature of the foam allows the pressure to be evenly distributed throughout its entire surface. Once the wound bed is ready, the foam piece is cut into an appropriate size so that the foam stays within the wound edges. After the application of the foam, a semiocclusive adhesive drape is placed over the wound covering the entire foam to ensure an airtight seal. The drape should have at least 3–5 cm of border to ensure maintenance of a tight seal. A small hole is made in the drape and a non-collapsible tube is placed over the hole and connected to a vacuum pump. The fluid drained from the wound is collected in the canister attached to the pump. The pressure applied by the pump can vary depending on the local wound conditions, and the device can be programmed to provide both continuous and intermittent negative pressure. The standard suction pressure is 125 mmHg, as optimal granulation tissue formation has been reported with this pressure [25]. However, other pressures have been reported depending on the size of the wound, location, and predisposition to bleeding. The most common mode of negative-pressure application is the continuous mode, but intermittent suction (for periods of 5 min separated by 2-min intervals) may be associated with greater stimulation of granulation tissue formation [3, 26]. However, intermittent therapy is not routinely used, as sudden and frequent changes in pressure can create varying discomfort for patients. Despite this, it is recommended to advance from continuous suction to intermittent suction in acute wounds, after the initial 48 h, unless there is uncontrolled pain, suction leaks, or an uneven wound surface. The duration of use of NPWT depends on the type of wound and the treatment goals. Chronic wounds often require prolonged treatment with NPWT, sometimes over a period of months, and NPWT might be continued until satisfactory outcomes are obtained. The negative-pressure dressing should be changed once every 48–72 h to prevent fluid saturation of the foam, which can decrease the effectiveness of the treatment. Newer dressings, however, such as the incisional NPWT dressing, can be placed over closed wounds for up to 7 days without changing. For infected wounds, dressings may need to be changed more frequently, though the clinician should be cautious about the use of these dressings over grossly infected wounds.

## 5 Advancements in NPWT

Since the initial introduction of V.A.C.® in the 1990s, significant advances have been made in the field of NPWT to cope with the expanding indications. One major challenge of the NPWT therapy is the maintenance of a tight seal so the negative pressure can be delivered. Automated alarm systems are currently available which can detect inadequate seal. Additionally these electronic systems can detect excessive fluid output and can be programmed to deliver negative pressure at various intervals. Two major advancements in the field of NPWT have been the availability of incisional NPWT and negative pressure with instillation.

Surgical wounds are closed with either sutures or staples and heal by primary intention. Surgical incisions from trauma-related surgery, total joint arthroplasty, cardiothoracic surgery, vascular surgery repair in the setting of known ischemia, major soft-tissue rearrangement plastic surgery interventions, and neurosurgical procedures are at high risk of wound dehiscence and increased risk of surgical site infections, all being studied in the setting of these recent advancements of NPWT. Traditionally, negative pressure has been used to treat complex open wounds, which usually heal by secondary intention. However, with the increasing popularity of NPWT, the indications for NPWT have extended as a prophylactic measure in the management of closed surgical incisions (incisional NPWT). Currently, there are

commercially available NPWT dressings that can be applied over surgical wounds, such as Prevena™ (KCI, San Antonio, Texas) and PICO (Smith and Nephew, St. Petersburg, Florida) [27]. Compared to the traditional NPWT devices, Prevena and PICO are composed of lightweight portable suction devices that allow patients to remain ambulatory with the dressing. The PICO system is different in that it does not have a canister and the fluid is lost by evaporation [28]. In a meta-analysis by Hyldig et al. [29], NPWT significantly reduced the rate of wound infection and seroma when applied to closed surgical wounds compared with the standard postoperative dressings. However, there was heterogeneity between the included studies, meaning that no general recommendations could be made. Also, they reported that a relatively large number of patients were lost to follow-up in the control groups and length of follow-up might have been inadequate to detect surgical site infections [29]. Although, conclusive evidence regarding the benefit of incisional NPWT is lacking, it is believed that they may be beneficial for surgeries in high-risk patients such as those with medical histories characterized by diabetes, obesity, active smoking status, an immunocompromised state, active dialysis, or previously irradiated wounds.

Maintaining a moist wound environment facilitates the wound healing process by prevention of tissue dehydration and cell death, accelerated angiogenesis, and increased breakdown of dead tissue and fibrin. Negative-pressure wound therapy with instillation has recently been introduced in various settings. This technology combines the traditional negative-pressure system with a method to intermittently instill a solution into the wound [30]. In addition to keeping the wound bed moist, it also enables the controlled delivery of topical anesthetic and antiseptic solutions over the wound bed. First, the instillation fluid drips by gravity through a tube to saturate the foam and then the fluid is allowed to bathe the wound for a predetermined period of time (from 1 s to 1 h). Then, the vacuum is applied through a separate (suction) tubing (5 min to 12 h), thereby removing the irrigation fluid and wound exudate and collapsing the sponge. Suction is continuously maintained until the entire cycle is repeated according to the amount of time programmed into the unit. The instillation solutions include normal saline, bacitracin, povidone-iodine, polyhexanide, acetic acid, antifungals, antiseptics, silver nitrate, local anesthetics, and insulin, depending on the type of wound and desired effects [30, 31]. Alcohol-based solutions and solutions that contain alcohol are contraindicated for use with NPWT with instillation as alcohol is not compatible with wound tissue [32, 33]. Hydrogen peroxide solutions are also contraindicated with this system due to the effervescent nature of this solution [30, 32]. The NPWT dressing is a closed system and any effervescence produced by the hydrogen peroxide may lead to air emboli. In addition, hydrogen peroxide is considered highly cytotoxic and deleterious to wound healing [34]. In a study by Gabriel et al. [35], patients with complex infected wounds treated with instillation of silver nitrate and negative pressure had significantly fewer days of treatment and experienced earlier wound healing compared with the control group. In a retrospective study by Timmers et al. [36], patients with osteomyelitis of the pelvis or lower extremities who received instillation NPWT using polyhexanide had a significantly lower rate of infections compared to patients who were treated with gentamicin-impregnated beads only. As contaminated traumatic wounds are at a high risk for infection, NPWT with antimicrobial instillation may potentially be useful in those cases. Strong evidence supporting the prophylactic use of antimicrobial solutions in contaminated wounds, however, is lacking. In a large multicenter randomized clinical trial (RCT) comparing irrigation protocols of open fractures, irrigation with normal saline resulted in lower rates of infection than castile soap solution [37]. In another RCT by Anglen et al. [38], bacitracin solutions did not decrease wound infection rates compared with normal saline irrigation in decreasing wound infection after open fractures, though wound healing problems were higher in bacitracin-treated

patients. Most of the scientific evidences supporting antimicrobial use with or without NPWT have been based on observational cohorts without a control group or based on poorly designed trials. However, in view of the >40% infection rate of contaminated traumatic wounds, NPWT with instillation is expected to be beneficial without any clinically relevant adverse effects [31]. Further prospective randomized studies are needed to clarify this issue.

## 6 Current Evidence

Although the indications for NPWT have rapidly expanded, there is a paucity of high-level evidence supporting the use of NPWT [39]. While NPWT has proven to be beneficial for certain types of wounds like diabetic wounds, sternal, and abdominal wounds, the benefits are unclear for vascular wounds and surgical wounds [4]. A large number of studies including RCTs and meta-analyses of RCTs have been published in this field and have provided mixed results partly owing to the heterogeneity in terms of wound types, outcome variables, and outcome assessments [40]. Conflict of interest in NPWT-related research is also a matter of concern as most studies were sponsored by the two main device manufacturers [15, 29]. Additionally, a number of RCTs studying the effects of NPWT were not published and the lack of access to unpublished study result data raises doubts about the accuracy of the available evidence [41]. Further, we focus on the current evidence in support of the use of NPWT in orthopedic trauma, total joint arthroplasty (TJA), and orthopedic oncology (Table 1). Additionally, the use of NPWT in other fields is also briefly reviewed.

**Table 1** Major uses of NPWT in orthopedic surgery

| Field | Usage |
|---|---|
| Trauma | • To assist wound closure when there is soft-tissue loss<br>• To assist wound closure in open fracture<br>• Closure of fasciotomy wounds<br>• As incisional dressing over contaminated surgical wounds |
| Total joint arthroplasty | • To treat dehisced wounds<br>• To treat ongoing drainage<br>• As temporary coverage, till definitive closure can be performed<br>• As prophylactic dressing over high-risk surgical wounds |
| Orthopedic oncology | • To treat large soft-tissue defects after tumor resection<br>• Contraindicated if wound has known unresected neoplasm<br>• As prophylactic dressing over high-risk surgical wounds (i.e., preoperative radiation) |

### 6.1 Orthopedic Trauma

Since its introduction more than two decades ago, NPWT has had an important impact in orthopedic trauma. The use of NPWT has been adopted in a variety of clinical scenarios in orthopedic trauma, which includes extensive soft-tissue injuries, penetrating trauma, open fractures resulting from high-energy trauma, and fasciotomy incisions. Treatment of traumatic wounds is challenging due to significant wound contamination, need for subsequent debridement, significant edema, or systemic compromising factors from multiple injuries. Negative-pressure wound therapy can be quickly applied and may potentially prevent wound desiccation, minimize microbial contamination, reduce edema, and facilitate wound drainage.

#### 6.1.1 Soft-Tissue Trauma

War wounds pose a challenge to trauma surgeons. These wounds are usually sustained due to energy transfer (gunshots, blasts, and explosives) across multiple tissue planes. These high-energy wounds are heavily contaminated and characterized by extensive loss of soft and/or osseous tissues. Traditionally, these wounds are managed in field hospitals with adequate irrigation and debridement, application of wet-to-dry dressings, and bedside dressing changes. Despite repeated irrigation and debridement of war wounds, wound healing is particularly challenging due to extensive tissue loss, breakdown of traumatized soft tissue, wound necrosis, and infection that requires additional

surgical interventions [42]. A systematic approach to war wounds was thus implemented to include eliminating bedside dressing changes and instituting mandatory interval wound examination, re-debridement, and dressing changes in the cleaner environment of an operating room [42]. Negative-pressure wound therapy is advantageous in such settings by keeping the wound covered while simultaneously promoting wound contraction, controlling wound drainage, decreasing wound edema, and augmenting wound granulation and healing [43, 44]. The ease of the application of NPWT is helpful in war injuries as it allows for the temporary coverage of large soft-tissue defects in hospitals located in or near areas of conflict before the patient can be transported to better facilities.

DeFranzo et al. [45] evaluated 75 patients who had open wounds and extensive soft-tissue damage or breakdown, concluding that NPWT decreased tissue edema by diminishing the circumference of the extremity and, thus, decreased the wound surface area allowing for successful wound closure in 71 out of 75 patients. Leininger et al. [46], based in a field hospital, treated 77 patients who sustained a total of 88 high-energy wounds. All wounds were operated on within 24 h of injury, and were covered with NPWT dressings and set to −125 mm Hg continuous pressure for 2–4 days. They reported no acute wound complications, and no reoperations on those who required skin grafts, and all of the patients had clean and closed wounds. In another study by Helgeson et al. [47], 16 patients who had high-energy complex soft tissue with exposed tendon and/or bone that were not amenable to skin graft were initially treated with a bioartificial dermal substitute regeneration template and NPWT. The authors concluded that NPWT had a beneficial effect on the formation of granulation tissue and as a barrier to reduce potential infection.

Stannard et al. [48] randomized 44 patients who suffered injuries from high-energy trauma and developed wound hematomas into two management groups, pressure dressing or NPWT. Dressings were changed daily in the pressure dressing group and every other day in the NPWT group. They found that NPWT was associated with a shorter duration of wound drainage (1.6 vs. 3.1 days, $p$=0.03) and lower, but not statistically significant, infection rate (8% vs. 16%, $p$ >0.05). Therefore, application of NPWT may offer some advantage in the management of highly complex soft-tissue injuries by promoting wound healing and potentially decreasing incidence of infection.

### 6.1.2 Open Fracture-Related Wounds

Open fractures are challenging for orthopedic surgeons. High-energy trauma results in not only bone fractures, but also large soft-tissue loss or breakdown. These injuries are at a high risk for infection and osteomyelitis. Open fracture infection rates are reported to range from 16 to 66% depending on the type of fracture, severity of the soft-tissue injury, and patient-related comorbidities [49, 50]. The primary goal of surgical treatment for open fractures is stabilization of the fracture, followed by soft-tissue repair. Careful homeostasis and wound coverage are important for reducing the risk of infection. Traditionally, these wounds undergo a series of irrigations and debridement to ensure that all nonviable tissues are removed to allow for subsequent healing by secondary intention with granulation tissue. Theoretically, NPWT may play an important role in the periods between surgical interventions, where it may be more advantageous than the standard wet-to-dry dressings [51].

In an RCT by Stannard et al. [52], 59 patients who had 63 severe high-energy open fractures were randomized to receive either a standard fine-mesh gauze dressing or a NPWT between irrigation and debridement procedures until definite closure was performed. They found that patients treated with NPWT were less likely to develop an infection compared to the control group (relative risk for infection [RR] = 0.199, 95% confidence interval [CI] 0.05–0.87). Blum et al. [53] retrospectively reviewed 229 open tibia fractures where 72% of patients received NPWT and 28% received a conventional dressing, and found a significantly lower deep infection rate in the NPWT

group (8.4% vs. 20.6%, $p = 0.01$). After adjusting for injury severity, NPWT was found to reduce the risk of deep infection by almost 80% (odds ratio [OR] = 0.22; 95% CI, 0.09–0.55; $p = 0.001$).

Virani et al. [54]conducted a RCT to study the effect of NPWT on deep infection and osteomyelitis after open tibia fractures, and they reported a significant reduction in the incidence of infection with use of NPWT compared to controls (4.6% vs. 22%; $p < 0.05$). Wound cultures showed positive growth in 3 patients who received NPWT and 17 in the control group (6.9% vs. 34%; $p < 0.05$), and the probability for infection in the NPWT group for a wound with an open fracture was 5.5 times less compared to controls. However, there was no significant difference in the time required for the wound to be ready for delayed primary closure or coverage. In another RCT by Arti et al. [55], treatment of open fractures with NPWT resulted in a reduction of wound surface volume and lower hospital length of stay. However, the authors did not find a difference in the infection rates. While there are discrepancies in the results of various RCTs evaluating the efficacy of NPWT, overall NPWT appears to have several benefits in the management of open fractures including lowering infection rate, accelerating closure of open wounds, and shortening the hospital length of stay.

### 6.1.3 Fasciotomy Wounds

Compartment syndrome is considered a surgical emergency, with the treatment goal being to decrease the muscle compartments pressure while maintaining tissue perfusion, which is achieved by open fasciotomy. Primary closure of these wounds would theoretically result in more functional and aesthetic outcomes with decreased morbidity. However, due to muscular edema, protrusion of muscles through the fascia, and significant skin retraction, premature primary closure may increase the compartmental pressure and the forced re-approximation under tension may cause necrosis at the wound edges. Healing by secondary intention had been a commonly used technique, but due to the increased risk of infection, longer hospitalization, increased requirements of frequent dressing changes, delay in rehabilitation, significant scarring, and poor aesthetic outcome, it is no longer considered an appropriate intervention. Serial dressing changes are often needed until definitive primary closure is possible. Primary coverage with NPWT creates a closed environment, which in theory protects the wound from outside infection, reduces local edema, and reduces the need for frequent dressing changes until final closure is achieved.

A large retrospective study by Zannis et al. [56] evaluated 458 patients who had 804 wounds, and demonstrated a significantly earlier time to primary closure (NPWT vs. standard = 5.2 vs. 6.5 days, $p < 0.01$) as well as higher rate of primary closure in fasciotomy wounds treated with NPWT compared to standard wet-to-dry dressings. On the other hand, Kakagia et al. [57] in an RCT comparing NPWT with the shoelace technique (gradual suture approximation technique to facilitate wound closure) found no difference in wound infection rates between the groups. They found that the wound closure time was significantly prolonged in the NPWT group compared to the shoelace method group, and the cost of treatment was also increased in the NPWT group. Although NPWT has become increasingly popular for the closure of fasciotomy wounds, the efficacy of these dressings to decrease infection and shorten time to closure remains uncertain.

### 6.1.4 Incisional Wounds

The outcomes of NPWT are promising in the management of surgical incisions and prevention of the development of hematomas in closed wounds.

Stannard et al. [48] evaluated NPWT as an adjunct to healing of surgical incisions after fractures that were at high risk for wound complications in terms of wound drainage. They showed that NPWT was associated with a significant reduction in the duration of wound drainage (1.8 vs. 4.8 days; $p = 0.02$). They also showed similar results in a larger randomized controlled trial where they prospectively evaluated the role of NPWT for the prevention of wound dehiscence and infection after high-risk lower extremity trauma in 249 patients who had 263 fractures [58]. In this study, incisional NPWT was applied to the closed surgical incisions in 141 patients, whereas standard postoperative dressings were applied to 122 control patients. The infection rate was significantly lower in the NPWT group

compared to the control group (9.7% vs. 18.9%; $p = 0.049$). Similar results were also reported in an RCT by Nordmeyer et al. [59] who compared NPWT to standard dressing after dorsal stabilization of spinal fractures in 20 patients (10 in each group). The NPWT reduced the development of postoperative seromas, nursing time, and material required for wound care. Overall, the use of NPWT appears to be beneficial in the management of surgical incisions in the trauma setting following fixation of high-risk fractures. Negative-pressure wound therapy has been reported to reduce wound drainage, postoperative infection, development of seromas/hematomas, and time and costs related to wound care [60].

## 6.2 Total Joint Arthroplasty

Total joint arthroplasty (TJA) is a common procedure with approximately one million total knee arthroplasties (TKA) or total hip arthroplasties (THA) being performed annually in the United States [61]. Periprosthetic joint infection (PJI) is a serious complication of TJA with the incidence reported to be from 1 to 2% [62]. The incidence of PJI is even higher after revision surgeries, and can be up to 20% [63]. Approximately 25% of PJIs occur within the first month following the surgery and these early infections are usually associated with wound complications like drainage and wound dehiscence [64]. It has been reported that each day of prolonged wound drainage can increase the risk of wound infection by 42% following THA and by 29% following TKA [65]. Therefore, over the past decade, there has been increased attention placed on NPWT as an effective technique to help prevent wound complications following TJA.

The predominant use of NPWT in arthroplasty is in the form of incisional NPWT dressings. Although a number of observational studies have described the utility of negative-pressure dressings on surgical incisions following TJA, the results of different studies on this topic are inconclusive. In an RCT by Howell et al. [66], no benefits were observed with the use of incisional NPWT in TKA patients at high risk for prolonged wound drainage. However, a higher incidence of blister formation was observed in the NPWT group leading to premature cessation of the trial. But, later RCTs have shown some beneficial effects with the use of incisional NPWTs. In a study by Pachowsy et al. [67], the authors randomized 19 patients undergoing primary THA for osteoarthritis into either a group receiving standard wound dressing or a group receiving NPWT, and showed decreased volume of postoperative seromas on day 10 in the NPWT group (NPWT vs. standard: 1.97 mL vs. 5.08 mL, $p = 0.021$). Although reduction of postoperative seromas can theoretically lead to increased blood flow, better apposition of the wound edges, and decreased risk of drainage, there is currently no evidence to suggest that reduced seroma can decrease rates of clinically relevant complications such as PJI [60, 67]. The use of incisional NPWT has also been reported to decrease wound dressing changes and to eliminate excessive hospital stay following primary TJA [28, 60]. In an RCT of 220 patients undergoing primary TKA/THA, Karlakki et al. [28] found that the use of incisional NPWT decreased the amount of wound drainage and eliminated prolonged length of stay. In another RCT by Manoharan et al. [68] the use of incisional NPWT following primary TKA was associated with improvement in wound leakage and better wound protection, although no benefit was found with respect to hospital cost and wound healing.

Although studies have shown that the use of incisional NPWT can decrease wound exudates, decrease in wound infection after primary TJA has not been reported with the use of NPWT. This might be due to the fact that the incidence of PJI is very low compared to the incidence of other wound complications like wound drainage. In an RCT by Gillespie et al. [69], the authors did not find a decrease in surgical site infections with the use of NPWT in patients undergoing primary THA. Furthermore, they suggested that a definitive trial would require approximately 900 patients per group to demonstrate a decrease in SSI after primary arthroplasty. Even though current evidence suggests that wound complications place patients at a higher risk for the development of PJI, there is uncertainty around the benefits of NPWT following elective arthroplasty for decreasing the infection rate [69]. The reasons for the differences in the results of various RCTs are probably related

to the heterogeneity of the patient population in terms of the type of arthroplasty (primary or revision) and the indication for arthroplasty (fracture or osteoarthritis) [60, 70]. Although NPWT may not have an added clinical advantage over the standard occlusive dressing in primary elective arthroplasty, it might be helpful in certain high-risk populations like patients who undergo revision arthroplasty. For example, the findings of a comparative study by Cooper et al. [70] suggest that incisional NPWT may decrease wound complications and SSIs in patients who undergo revision hip and knee surgery. The benefits of NPWT may be even more apparent after revision surgery for PJI or in patients with preexisting wound issues. While strong evidence to support the prophylactic use of NPWT in primary or revision arthroplasty is lacking, there are a number of ongoing clinical trials, which might help to better understand the indications for incisional NPWT in TJA.

In addition to the use of incisional NPWT as a prophylactic measure, NPWT can also be used to treat chronically infected, dehisced, or draining wounds in the setting of knee or hip arthroplasty (Fig. 2). In a retrospective study of 109 patients who had persistent drainage after primary THA, Hansen et al. [71] showed that majority of the patients (76%) had cessation of the drainage after being treated with NPWT. Therefore, NPWT can potentially avert morbid surgical procedures which are traditionally performed for persistent drainage. Hansen et al. [71] also demonstrated that patients who failed NPWT therapy and required a subsequent surgical procedure had success rates similar to the published literature, indicating that NPWT might be safely considered as a first-line treatment modality for persistent drainage [72]. Treatment of PJI involves extensive debridement of soft tissues, which can often compromise the soft-tissue coverage required for primary closure, especially for the knee. Therefore, NPWT can be used in such instances to promote granulation tissue formation and to act as a bridge until definite closure can be performed. The benefits and mechanism of action of NPWT dressing in such settings are similar to other open wounds. The availability of instillation therapy offers the additional advantage of providing topical antimicrobial solutions, which may help in the clearance of infections, although the benefits of this remain unclear [73]. Even though NPWT dressing is widely used to treat wound drainage and other wound-related complications after arthroplasty, the majority of studies describing the use of NPWT to treat wound-related complications were performed without a control group. Therefore, the clinical superiority of NPWT over the traditional dressings in terms of faster wound healing and improved infection clearance has not been established. It is reasonable, however, to assume that NPWT can at least decrease the number of wound dressing changes in actively draining wounds, and can remove some tension on the wound edges, and keep them better approximated under lower stress.

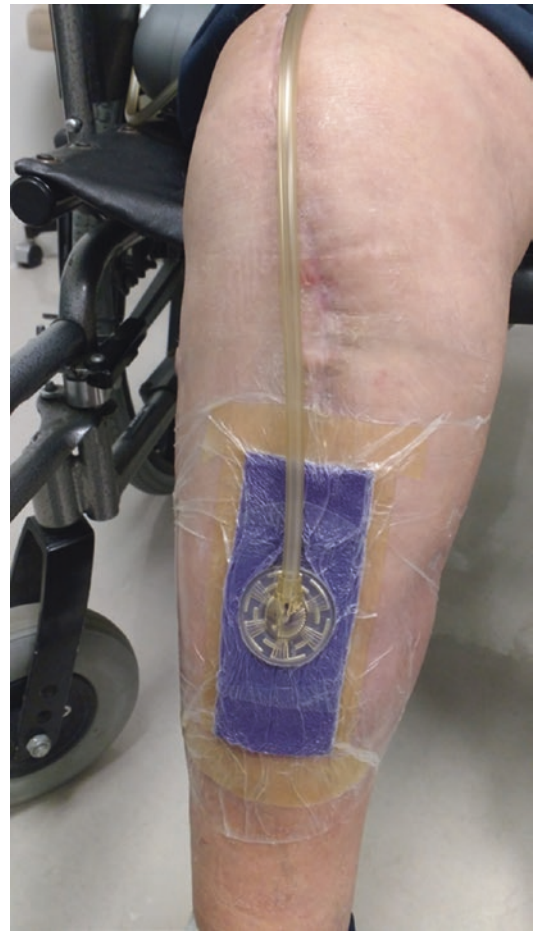

**Fig. 2** Negative-pressure dressing applied over a patient who developed postoperative drainage from the distal portion of wound after a complex revision knee arthroplasty. The tubing is connected to a portable suction device allowing the patient to be ambulatory with the dressing

## 6.3 Orthopedic Oncology

Bone and soft-tissue sarcomas are relatively uncommon cancers, but over the past decade, the estimated incidence increased from 12,000 to 15,000 new cases per year [74, 75], with the most common soft-tissue sarcomas occurring on the extremities [76]. Historically, the treatment for sarcomas of the extremities was limb amputation; however, there was a shift towards limb salvage procedures with adjuvant chemotherapy and/or radiotherapy [77–79], which has been associated with more patient satisfaction [80], improved physical function [81], and less disability [82]. Limb salvage procedures involve wide surgical margin resection, sometimes necessitating soft-tissue defect, bone defect, or vascular reconstruction in order to minimize recurrence risk and maximize long-term limb function [83–86]. Particularly with soft-tissue sarcoma, wide excision, in combination with neoadjuvant or adjuvant radiotherapy, has been shown to have positive effect in time to local recurrence and overall survival [87]. Despite the benefits of limb salvage procedures, tumor resection and radiotherapy can lead to significant wound complications, which can be a cause of significant morbidity [88]. Surgical resection of bone and soft-tissue sarcomas is often difficult due to involvement of the adjacent fascia and neurovascular structures [77], and depending on the location of the tumor and the surrounding tissues involved, patients may have large open wounds with soft-tissue defects [89]. Despite the benefits conveyed regarding local recurrence, radiotherapy also is strongly associated with various wound-related complications, with a higher rate of wound complications (~30–40%) with neoadjuvant radiation as compared to adjuvant therapy (~20–25%). One study reported on 202 patients who had preoperative radiotherapy and then had surgery for soft-tissue sarcoma of the lower extremity ($n$ = 119), upper extremity ($n$ = 32), trunk ($n$ = 36), and head and neck ($n$ = 15) [90]. The overall wound complication rate was 37%, and a second surgery for the wound complications was required in 16.5%. Similarly, Kunisada et al. [91] evaluated 43 patients who underwent preoperative radiotherapy followed by surgery for soft-tissue sarcomas of the lower leg ($n$ = 28), upper arm ($n$ = 8), and trunk ($n$ = 7). They reported a high complication rate, with preoperative radiotherapy-associated acute skin toxicity that occurred in 84% of cases, and a postoperative wound complication rate of 44%, of which 23% required an additional surgery.

Resection of large bone or soft-tissue tumors can lead to massive soft-tissue defects that cannot be closed at the time of surgery. Bickels et al. [92] reported on 62 patients who underwent resections of either bone or soft-tissue tumors and were left with a large soft-tissue wound defect after surgery, debridement from wound complications, or radiation-associated skin necrosis. Twenty-three of these patients had a NPWT device placed for a mean of 14 days (range 7–19 days), and were followed for a median of 19 months (range 12–27 months). Their outcomes were compared to a similar cohort of 39 patients who were treated prior to the surgeon's use of NPWT. Compared to historical controls, the patients who were treated with the NPWT had a decreased rate of additional surgical wound procedures and a higher rate of primary wound closure, and had shorter hospital length of stay. The soft-tissue defect area decreased by a mean of 25% in those who received NPWT.

In those patients with large soft-tissue defects from resection of bone and soft-tissue tumors, incisional NPWT allows for improved healing and primary wound closure [89]. In addition to the use of negative-pressure dressings, silver has been added to the dressings in order to prevent surgical site infections [93]. Siegel et al. [93] reported on 42 patients who suffered from massive soft-tissue loss resulting in large extremity and/or pelvic wounds and compared a plain NPWT dressing to a NPWT with silver dressing. Tumors were the etiology in 14 of the patients; 11 patients underwent local radiation and 12 patients had immunosuppression either from chemotherapy or from a transplant. The etiology in the remaining patients was infections in 22 and trauma in 6 patients. The patients who had the NPWT with silver dressing had a decreased length of stay compared to the patients with the NPWT alone (7 vs. 19 days, $p < 0.033$). Compared to the patients who only had the NPWT, the NPWT plus silver dressing patients

had to undergo fewer surgeries prior to flap coverage (62% vs. 19%, $p = 0.024$) and had required fewer surgical debridements (7.9 vs. 4.1, $p < 0.001$). It seems that the addition of silver to NPWT dressings may have a positive effect for wound healing in such patients. Additional studies are needed to have definitive conclusions.

### 6.4 Other Major Indications

Perhaps, one of the first indications of NPWT was the treatment of chronic wounds. Chronic wounds pose a great challenge to the medical community, and with the increasing prevalence of bed-ridden patients and those with chronic conditions such as diabetes mellitus and peripheral vascular disease, more patients are being diagnosed with chronic wounds. These wounds are difficult to heal, and may be due to the continuous exposure to the external environment, which can result in colonization with bacteria and fungus. Negative-pressure wound therapy, though, has revolutionized the management of chronic wounds. The primary goals of NPWT in chronic wounds are to achieve wound closure (by surgical or secondary intention), reduce the wound size, improve patient quality of life, manage wound fluid and edema, and prevent wound deterioration. However, the effectiveness of NPWT in achieving these goals depends on the type of wound. Currently there is strong evidence to support the use of NPWT in diabetic foot ulcers. In a multicenter RCT, Armstrong et al. [94] reported that treatment of diabetic foot wounds with NPWT led to a higher proportion of healed wounds, faster healing rates, and potentially fewer re-amputations than standard care. In another multicenter RCT, a greater proportion of foot ulcers achieved complete ulcer closure with NPWT, suggesting that NPWT is more effective than the standard dressings [95]. There is a moderate amount of evidence supporting the use of NPWT in pressure sores and venous stasis ulcers. In an RCT by Vuerstaek et al. [96], the use of NPWT was associated with faster wound healing of venous ulcers and resulted in lower costs. Although a few RCTs have suggested some benefits with the use of NPWT in pressure ulcers, the overall quality of evidence is low and the clinical effectiveness of NPWT is inconclusive [97]. There appears to be no benefit with NPWT in the setting of chronic ischemia ulcers [4]. The benefits of NPWT are usually seen in large edematous wounds, while the wounds arising in the setting of arterial insufficiency are usually in the toes, without much swelling unless there is an associated infection [98]. Additionally, as most of the wounds related to arterial insufficiency are small and surrounded by nonviable tissue, surgical debridement might be preferred over NPWT, which may explain why the literature on the treatment of vascular ulcers is limited [99]. The use of NPWT in an acutely ischemic leg may even have detrimental effects as excessive negative pressure may further compromise blood flow [4].

In addition to major orthopedic indications for NPWT, other areas of application that have been studied include open abdominal wounds, sternal wounds, and skin graft host environments [4, 100–102]. While not the scope of this book chapter, these large defects and scenarios can mimic many of the situations in orthopedic surgery and add important insight into the applications for NPWT in the treatment of major appendicular and axial wound concerns.

## 7 Adverse Events

There have been few complications associated with NPWT, and they can often be avoided or minimized with proper application. The most common complications of NPWT are skin related, which can range from a simple rash to a large blister. Blister formation is an important adverse effect with the use of incisional NPWT due to the direct application of negative pressure over the normal skin. In an RCT by Howell et al. [66] the study was prematurely interrupted when a total of 60 patients were enrolled and a significant difference in blister formation about the knee was detected between the NPWT group and the control group. In order to address the issue of blistering, a non-adherent dressing has been recommended for use over unprotected skin to avoid direct contact with the foam [15, 66]. The study by Howell et al. [66] was one of the initial studies

that used an incisional NPWT dressing and blistering was not found to be an issue in the subsequent studies, where the normal skin was protected [103]. Allergy to the components of the NPWT dressing (e.g., adhesive or silver) can also cause skin rashes. The skin of patients who have been treated with immunosuppressive drugs may be fragile and more prone to desiccation from the use of negative pressure [104, 105].

If the sponge is left deep in a wound for prolonged periods (more than 48 h), it can be difficult to extract because of the overgrowth of exuberant granulations. Extraction of the sponge may be associated with minor bleeding due to the highly vascular granulation tissue. To prevent the ingrowth of granulation tissue, dressings are recommended to be changed every 48–72 h. Since this is not an issue with incisional dressings, NPWT can be kept over wounds for longer periods (7 days or longer). Although NPWT is used in tumor surgeries to help with wound closure and prevent wound complications, the effects of negative pressure on neoplasms are unknown. As NPWT is known to stimulate the cytoskeleton and promote granulation tissue, it is thought to maybe have stimulatory effects on the neoplasm as well. Therefore, NPWT is contraindicated for use over neoplastic wounds. However, NPWT may be used for wound closure after resection of deep or superficial tumors. Patients on anticoagulants and those with a history of a bleeding disorder may develop hematomas from the application of negative pressure, especially when wounds are large, and these patients need to be monitored. Lower levels of negative pressure can be used in such cases. When NPWT is used in deep and tunneling wounds, care should be taken to remove the entire piece of foam from the wounds when dressing changes are performed.

## 8 Cost-Effectiveness

Although the vast majority of the literature supports the efficacy and safety of NPWT, it is important to know whether NPWT is cost effective compared to conventional dressings. A number of studies have suggested NPWT to be a cost-effective method and most insurance companies cover the commercially available NPWT devices. In a study of more than 1000 patients with advanced-stage pressure ulcers, Philbeck et al. [106] demonstrated that wounds treated with NPWT healed faster (97 vs. 247 days) and at a lower cost ($14,546 vs. $23,465) compared to the traditional dressings, suggesting that NPWT is cost effective. However, the cost-effectiveness of NPWT is not fully established for all of the current uses of NPWT. When NPWT is used as a prophylactic agent on surgical incisions, the cost of NPWT ranges from $15/day to $495/week depending on whether the device is a self-made or a commercially tailored for incisions [107]. Since one of the major reasons for the use of incisional NPWT is to prevent surgical site infections, use of prophylactic NPWT might be cost effective due to high costs associated with infections such as PJI [108]. Since NPWT is changed less frequently than wet-to-dry dressings, NPWT can be less labor intensive for hospital staff and may result in overall reduction of cost [109]. The quality of the current evidence supporting the use of NPWT to prevent infection is low and cost-effective analyses are limited [107]. Nevertheless, NPWT is expected to be cost effective at least in patients with well-established risk factors for infections.

The majority of the negative-pressure dressings applied in North America are commercially available preparations [110]. However, these devices can be expensive and may not be readily available throughout the world. Nguyen et al. [110] demonstrated that standard gauze sealed with an occlusive dressing and connected to wall suction was able to achieve similar outcomes to the commercially available devices, but at a lower cost. Further studies are needed to establish such cost-effectiveness.

### Conclusions

Negative-pressure wound therapy continues to gain popularity in various specialties including orthopedic surgery, since the indications for its use have grown dramatically since it was first introduced. While efforts have been made to provide an evidence-based guide for its use, this has been limited by a lack of good-quality evidence. The majority of support for the use of NPWT comes from retrospective studies

that either fail to compare it to other wound management techniques or are underpowered with both heterogeneous and small patient populations. The majority of the published literature concludes that NPWT is an effective technique but requires more prospective research to support its use. Currently, NWPT is considered superior to traditional dressings for the management of chronic wounds and pressure ulcers. Additionally, in orthopedic surgery, trauma patients experience the most benefit with the use of NPWT especially when there are large soft-tissue defects precluding primary closure. The NPWT is also used as prophylactic dressing after hip and knee arthroplasty in high-risk patients although this is based on observational data. One of the key problems with research in the field of wound healing is founded in the fact that wounds are very difficult to standardize—varying in size, shape, position, and chronicity. Objective assessments of wound healing are not easy to define and labeling wounds based on arbitrary scales is not evidence based. Furthermore, adequate wound healing relies on multiple local and systemic factors and consequently wounds vary from one another. Although the efficacy of NPWT in wound healing is well established, well-designed randomized controlled trials tailored to a specific patient population characterized by a specific wound environment dilemma are needed to give definitive answers regarding the clinical superiority of NPWT over the conventional less expensive dressings.

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# Negative-Pressure Wound Therapy for High-Risk Wounds in Lower Extremity Revascularization

Patrick B. Murphy and Adam Power

## 1 Introduction

Lower limb revascularization is indicated for acute limb ischemia and critical limb ischemia. The latter manifests as rest pain and nonhealing ulcers. The three general approaches for limb revascularization are open surgical with bypass, endovascular with angioplasty and stenting, or a hybrid approach of open and endovascular. The incidence of peripheral vascular disease (PVD) is approximately 12%, largely related to atherosclerosis [1]. One-third of these patients will not respond to and progress with conservative therapy and will require an intervention. Lower limb revascularization represents a substantial cost to the healthcare system and related complications lead to prolonged hospital stays, readmission, and potential need for reoperation [2].

Surgical site infections (SSI) represent a challenging and common complication after lower limb revascularization. Despite a "clean" wound classification, SSI after lower limb revascularization has a reported incidence of 20–25%, significantly higher than the expected rate of 1–4% for "clean" wounds [3–5]. The development of a SSI can significantly impact graft failure, need for amputation, and mortality [2, 6]. The pathophysiology of peripheral vascular disease and related comorbidities put patients at higher risk of SSI. Obesity, diabetes mellitus, ischemic tissue loss, and prior surgery have been identified as significant risk factors for postoperative development of SSI [7]. While many surgical techniques and prosthesis are available, Table 1, lower limb revascularization techniques which require an infra-inguinal incision are especially at risk of SSI. A number of different techniques have been attempted to reduce SSI including topical antibiotics, antibiotic-impregnated grafts, supplemental oxygen, and platelet-rich plasma [7–10].

**Table 1** Lower limb revascularization techniques requiring groin incisions and graft choices

| |
|---|
| Procedures requiring groin incisions |
| Femoral endarterectomy |
| Bypass |
| Distal limb (tibial/popliteal) |
| Femoral-femoral |
| Aortobifemoral |
| Axillary femoral |
| Graft types |
| Greater saphenous vein |
| Polytetrafluoroethylene (PTFE) |
| Dacron |
| Bovine pericardium |

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Negative-pressure wound therapy (NPWT) has gained attention in the last decade as a prophylactic measure for SSI in orthopedic and trauma surgery [11–13]. Originally indicated for postoperative care of wound infection, NPWT can potentially reduce the incidence of SSI by being applied to primarily closed incisions. Incisional NPWT devices are available from a number of manufacturers and are often criticized for the associated costs. However, the recent development of simplified "all-in one" devices and the rising costs of SSIs have raised the question of the cost-effectiveness of NPWT dressings. "Homemade" devices have been described to have significant cost savings but can limit portability.

The biomechanical profile of NPWT has been elucidated through in vitro and in vivo models. The application of negative pressure reduces lateral force, helps maintain the integrity of the wound, and improves approximation of the tissue faces [14]. Negative pressure improves perfusion to the skin edges and on a microscopic level promotes cell proliferation and angiogenesis. The connected drainage system allows the dressing to remain on the patient for longer compared to nonnegative pressure dressings which can become soaked through. This has the potential to limit bacterial contamination. In clinical studies, incisional NPWT has demonstrated a reduction in seroma/hematomas which may help explain the effect in prevention of SSI. Improvements in patient comfort have been demonstrated.

## 2 Technique

The infra-inguinal incision is best suited for all-in-one incisional NPWT which requires no additional assembly other than to remove the adhesive cover and place the bandage over the wound (Fig. 1). Prior to sterilization and incising the skin, it is important to ensure that all hair is removed from the area as non-removed hair can interfere with the vacuum seal. We recommend at least 5 cm around the planned incision. After the incision is closed with either staples or sutures the NPWT dressing can be placed under sterile conditions. A number of manufacturers market all-in-one devices and the ease of application adds <5 min to the operative time. The authors' experience is with a continuous suction device which provides 125 mm Hg of suction. "Homemade" devices or those which require more assembly have been described and the basic principles are the same [15, 16]. A single layer of nonadhesive gauze is placed of the entire length of incision. This gauze may be impregnated with

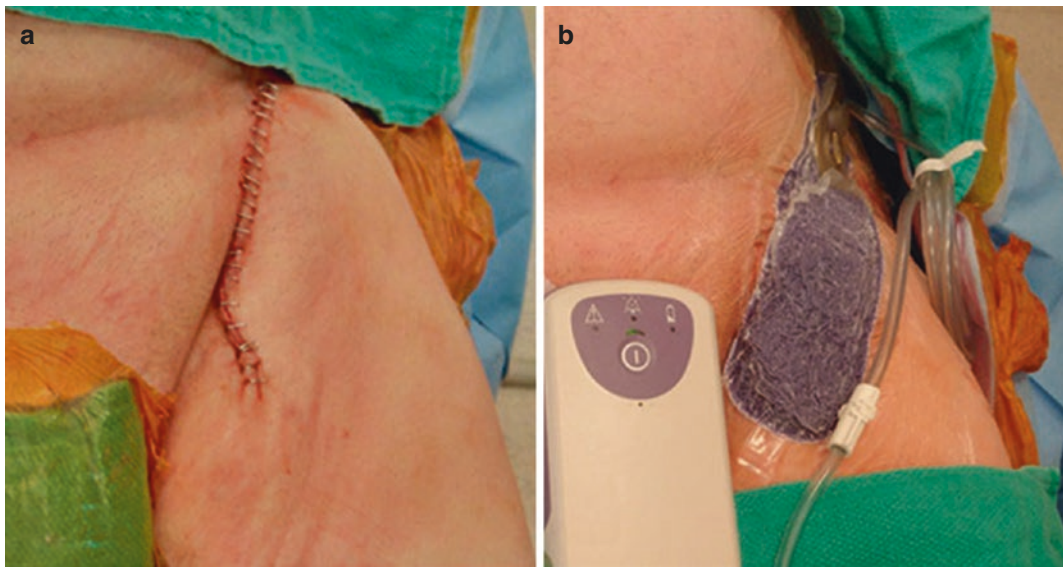


**Fig. 1** (**a**) Infra-inguinal incision closed with staples prior to placement of NPWT dressing. (**b**) NPWT dressing in place with attached vacuum

silver. Various sizes of gauze are available or customizable devices can be used which are cut to the length of incision. An occlusive, transparent, adhesive dressing is then placed over the gauze. An incision is made over the foam gauze and a pressure-sensing pad is applied with tubing connected to a vacuum unit and 125 mmHg suction is then applied continuously. Newer devices have a stronger vacuum than earlier models and in general poor hair removal is responsible for leaks followed by body contours around the incision. If air leaks are identified the dressing can be reinforced with more occlusive adhesive dressings or a stronger vacuum unit can be used in order to maintain negative pressure despite the leak.

Generally the NPWT dressing is removed on POD #5 or at the time of discharge if earlier in keeping with the manufacturer's directions. Our institution does not send patients home with incisional NPWT. Other reports have used both longer and shorter durations of NPWT and the best length of application is not known.

## 3 Discussion

The first evidence for NPWT in lower limb revascularization was reported in 2013 by Matatov et al. [11]. This retrospective study in 115 groins demonstrated a SSI reduction from 30 to 6% with NPWT applied to primarily closed infra-inguinal incisions in the operating room. The NPWT had no deep infections or infected grafts compared to 14% of patients in the standard dressing group. While the results of the study were promising, our own institution was unable to demonstrate a similar effect. We randomized 102 patients and demonstrated a non-statistically significant 90-day SSI rate reduction from 22 to 13% in the NPWT group. This study was underpowered secondary to a lower than expected baseline SSI incidence and a lower than expected difference between groups. A nearly 10% reduction in SSI would likely offset the preoperative costs associated with the prophylaxis application of an all-in-one device given the significant costs associated with SSI in this group of patients including readmission to hospital, reoperation, graft failure, and home wound care.

**Conclusions**

Preliminary results of studies within the vascular literature suggest that NPWT may play a role in reducing wound infection following lower limb revascularization. Commercial devices are available to aid in the ease of application, but more inexpensive, "homemade" options are described. The costs associated with such devices may be justified through the reduction in SSI and the associated in- and out-of-hospital costs burdened by wound infections. Larger, prospective trials are required to fully justify these prophylactic interventions but early evidence suggests efficacy in reducing infra-inguinal SSI following lower limb revascularization.

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# Clinical Experience with Negative-Pressure Wound Therapy Combined with Silver-Impregnated Dressing in Mixed Wounds

Peter Bukovčan and Ján Koller

## 1 Introduction

The management of wounds with problematic healing, for example, with massive infection, and extensive tissue loss with/without exposure of deep structures (muscles, tendons, bones), where conservative and conventional surgical treatment methods are insufficiently effective, attracts considerable attention of many clinicians. In order to achieve healing of these problematic wounds, advanced wound care treatment methods are necessary. Negative-pressure wound therapy (NPWT), introduced by Morykwas [1] offers treatment options for the management of problem wounds. This non-pharmacological treatment method utilizes physical principles (subatmospheric pressure) for wound environment manipulation to enhance natural wound healing processes, leading to accelerated wound healing. To date, NPWT has proven its versatility in the management of both acute and chronic wounds in a wide range of indications: open fractures [2], extensive tissue loss, severe wound infections, destructive burn injuries [3] and frostbite, enhancement of skin graft and skin substitute take [4, 5], salvage of compromised flaps [6], wounds with massive edema and/or venostasis, trophic ulcers (venostatic, arterial, neuropathic, diabetic [7], postirradiation), pressure sores [8], and sternal or abdominal dehiscences [9, 10]. Nevertheless, it must be emphasized that NPWT cannot replace surgical debridement, which should always be performed before applying topical negative pressure. However, the indication and contraindication criteria for NPWT usage should be borne in mind and strictly followed as with any other treatment method. In NPWT, several mechanisms act in concert and exert beneficial effects: stimulation of blood flow, angiogenesis and granulation formation, derivation of soluble wound healing inhibitor substances from the wound area, mechanical forces pulling the wound edges together, reduction of tissue edema, and reduction of bacterial contamination. The latter is considered to be one of the key factors in wound healing. To stimulate wound healing, according to the literature [11, 12], the authors attempted to enhance this antibacterial effect by using silver-impregnated dressing in conjunction with NPWT. Silver products have two key advantages: they are broad-spectrum antibiotics and are not yet associated with drug resistance [13]. Silver has both bactericidal effects via oxidation of the cell membrane and bacteriostatic effects by inhibiting bacterial replication through damage to DNA [14]. To prove that the antimicrobial

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activity and clinical effectiveness of this combination are not the same, different types of wounds were included in the retrospective study, which also gives more insight into problem wound management. This chapter is about the author's approach, treatment algorithm, interesting clinical findings, and results of the retrospective study, discussion, and conclusions, with aim to share the knowledge and clinical experience with faithful readers.

## 2 Technique

### 2.1 NPWT Treatment System

The whole NPWT treatment system is a closed, airtight system consisting of a sterile hypoallergic polyurethane foam (sponge) connected to the source of subatmospheric pressure by the tube which in addition to negative-pressure distribution also serves for derivation of wound exudates to a container attached to the device. Patients included in this study were treated using the Vivano negative-pressure therapy system (Paul Hartmann AG, Heidenheim, Germany), which consists of the VivanoTec® negative-pressure unit and VivanoMed® wound dressing kit. The fine-pored flexible polyurethane sterile foam dressing can be trimmed and shaped to ensure contact with all wound surfaces for equal distribution of subatmospheric pressure. Sponge placed on the wound surface is sealed using the adhesive drape Hydrofilm®. After the creation of a small hole through the drape in the center of the sponge surface, the silicone adhesive port is airtightly attached. The opposite end of the tube is connected to the negative-pressure unit with a detachable collection container (300 or 800 mL). The pressure unit is a subatmospheric pressure device equipped with touch screen to adjust the intensity, duration, and frequency (continuous or intermittent) of the subatmospheric pressure to the wound surface. In authors' department, particular preference has been given to intermittent negative pressure provision; during treatment a continual change occurs between two negative pressure values at specified intervals: negative pressure of 125 mmHg for 5 min followed by a negative pressure of 20 mmHg for 2 min. Intermittent negative pressure increases blood flow to the wound tissue more effectively and the proliferation of granulation tissue is higher over continuous negative pressure [1, 15].

Polyurethane foam as a wound contact layer can lead to granulation tissue ingrowth, with possible damage to the underlying tissues, and increased bleeding and pain [16] caused by necessary sponge change/removal. Additionally, the application of the foam directly on exposed deep structures (bones, tendons, nerves) is not recommended. Because of these reasons, and with the intention to enhance bacterial contamination reduction, we decided to use a nonadherent wound contact layer with antimicrobial properties. In this study, NPWT treatment was combined with a polyamide tulle dressing chemically coated with metallic silver and impregnated with nonpetroleum triglyceride-based ointment (Atrauman AG, PAUL HARTMANN Ltd., Heywood/Lancashire, UK). On contact with wound exudate, the silver ions responsible for antimicrobial properties are activated.

#### 2.1.1 Inclusion Criteria

All patients with nonhealing or any type of problem wounds hospitalized at the Department of Burns and Reconstructive Surgery of the Comenius University and University Hospital Bratislava Ružinov, who have been treated using NPWT from September 2011 to December 2013, were eligible for inclusion in this retrospective study. Performance of this study was approved by the Ethics Committee of Ružinov Hospital Bratislava.

#### 2.1.2 Treatment Details

Each patient was examined thoroughly regarding patient's history, associated illnesses, medications use, nutritional status, vascular system with regard to wound-area perfusion, and compensation of diabetes, if present. Wound swabs were taken for bacterial wound culture. Prior to the admission of each patient to the hospital, wound examination was always performed together with wound assessment, including a search for wound-edge undermining or wound fistulas. When indicated, ultrasound examination, fistulography,

and/or bone X-ray examinations were also performed. The treatment plan usually consisted of three phases:

First, preparatory phase included the above-stated examinations as well as laboratory findings and auxiliary examinations. Following a detailed wound assessment, a decision on whether to use NPWT treatment was made. Regular dressing changes with topical antimicrobial treatment of heavily contaminated, mainly chronic wounds were performed to reduce the bacterial load.

Second phase was represented by surgical debridement of the wound with subsequent application of the NPWT system. The surgical intervention started with wound exploration followed by thorough debridement with the removal of all devitalized and necrotic tissues (necrectomy). Meticulous hemostasis of bleeding points was of utmost importance. The silver-impregnated non-adherent mesh was applied to the wound followed by foam application, sealed with adhesive drapes and connected with the negative-pressure unit. When treating two independent wounds both suitable for NPWT treatment, for example on the same leg laterally and medially, the foams covering the wounds could be connected using an "overbridging" foam and sealed with adhesive drapes together to create a single closed system, which was thereafter connected to a single negative-pressure unit (Figs. 1 and 2). Immediately after NPWT system application, the treatment was initiated by the application of a continuous negative pressure of 125 mmHg. After 2–4 h it was changed to an intermittent negative pressure of 125 mmHg for 5 min alternating with a negative pressure of 20 mmHg for 2 min. Depending on the wound characteristics and amount of exudate, the first sponge change and wound exploration were done after 3–5 days. The wound was debrided repeatedly, where relevant, before every NPWT system reapplication. The main goals of NPWT treatment should include the achievement of a clean wound surface with a sufficient amount and quality of granulation tissue, decreased bacterial contamination, reduced edema, and feasibility of conservative or surgical wound closure.

In third phase, once an acceptable wound condition was achieved either surgical wound closure was performed (foam removal was performed in the operating room) or the wound was considered to have sufficient capacity to heal using conservative treatment methods.

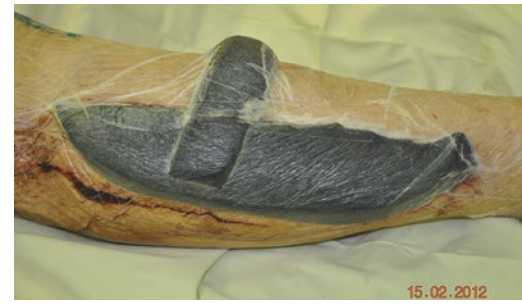



**Fig. 1** "Overbridging" foam connecting two independent wounds

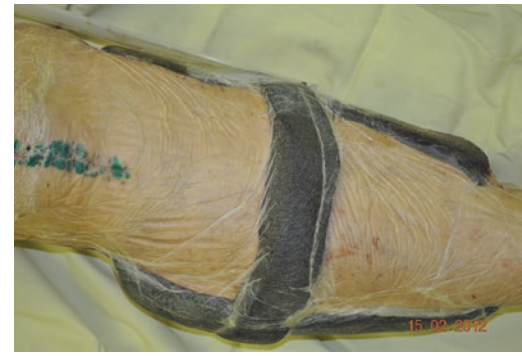



**Fig. 2** "Overbridging" foam connecting two independent wounds

### 2.1.3 Evaluation Criteria

Information obtained for the evaluation criteria included the following: demographic data, time interval between wound development and NPWT initiation, wound characterization (i.e., wound etiology, localization, diameter, wound bed, wound margins, and periwound area characterizations), wound cultures before and after NPWT, duration of NPWT and the number of sponge changes for each patient, wound appearance after NPWT, types of surgical wound closures following NPWT, healing time calculated from the end date of NPWT to the date of achieving a healed wound after the wound closure, and length of hospital stay. Data are presented as the means ± standard deviation (SD). A value of $p < 0.05$ was considered statistically significant.

# 3 Results

## 3.1 Demographic Data

Between September 2011 and December 2013, at the Department of Burns and Reconstructive Surgery, Comenius University and University Hospital Ružinov, Bratislava, Slovak Republic, 50 patients with 54 wounds were treated and included in a retrospective study. Of the 50 patients, 35 (70%) were males and 15 (30%) were females (Table 1). As in our study the patients with both acute and chronic wounds were included; the time interval between wound development and NPWT varied from 14 to 600 days with a meantime interval of 71.6 ± 97.3 days (95% confidence interval of the SD: 81.27–121.24).

**Table 1** Patient demographics

| Sex | *N* | Mean age ± SD (years) | Age range (years) |
|---|---|---|---|
| Male | 35 | 49.9 ± 18.6 | 19–77 |
| Female | 15 | 63.3 ± 17.8 | 24–81 |
| Total | 50 | 55.4 ± 18.7 | 19–81 |

*SD* standard deviation

## 3.2 Wound Characterization

The wound etiology was considerably heterogeneous, mostly represented by posttraumatic and diabetic defects (Table 2). The majority of wounds were lower extremity wounds with nearly equal distribution on the leg and foot,

**Table 2** Wound etiology

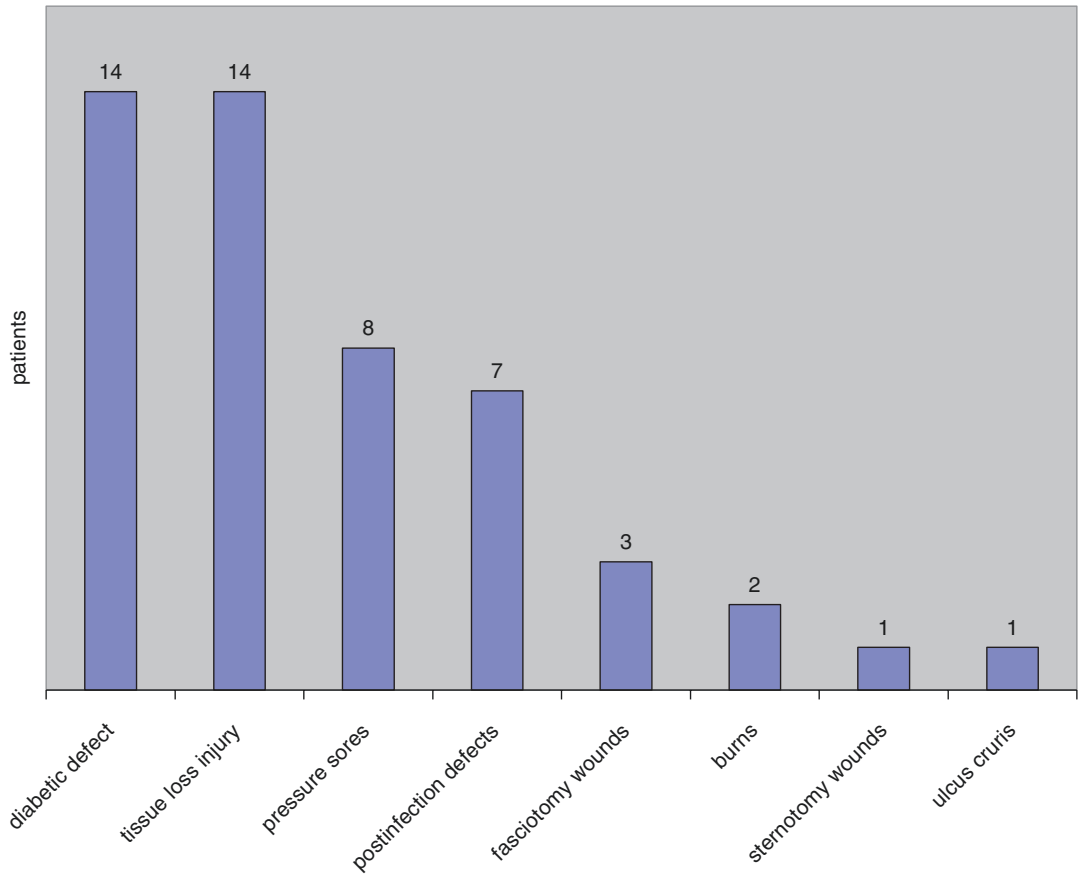
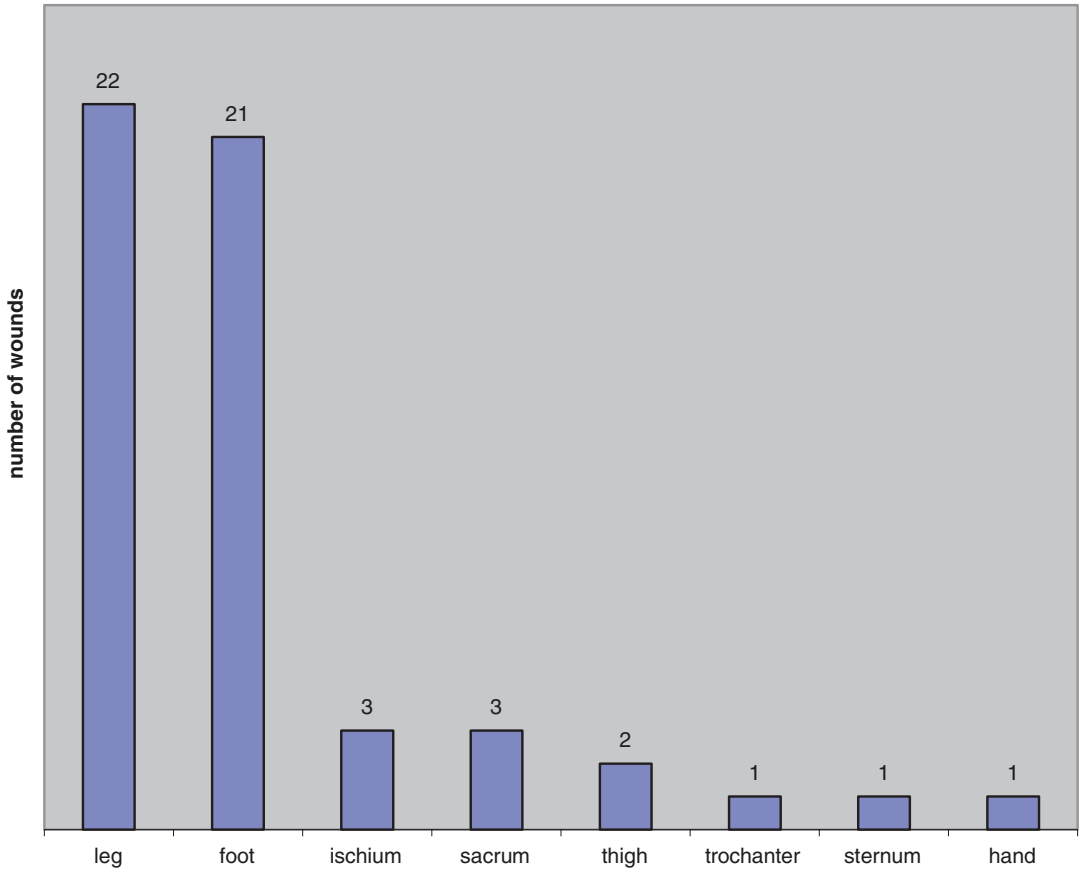


**Table 3** Localization of 54 wounds in 50 patients




followed by other less frequent wound locations (Table 3). Among the 50 patients in the present study, three with wounds after fasciotomies had two wounds located on the lateral and medial sides of their lower legs and one patient with inveterate burns had two independent wounds located on each leg, so there were in total 54 wounds in 50 patients. The average wound size was 97.2 $cm^2$ (range 1–375 $cm^2$). Despite some wounds having a small surface area, they were usually complicated with extensive wound-edge undermining and affections of deep structures which were exposed following necrectomy. In addition to wound size, the wound bed, edges, and periwound areas were included in the wound assessment (Table 4).

**Table 4** Wound characterization

| Wound characterization | №. of wounds |
|---|---|
| *Wound bed* | |
| Necrotic tissue | 21 |
| Fibrinous slough | 29 |
| Small amount of granulation tissue | 4 |
| **Total** | **54** |
| Deep structures exposed | 26 |
| *Wound edges* | |
| Edematous | 19 |
| Erythematous | 17 |
| Irregular | 17 |
| Undermined | 14 |
| *Periwound area* | |
| Edematous | 17 |
| Erythematous | 19 |
| Indurated | 4 |

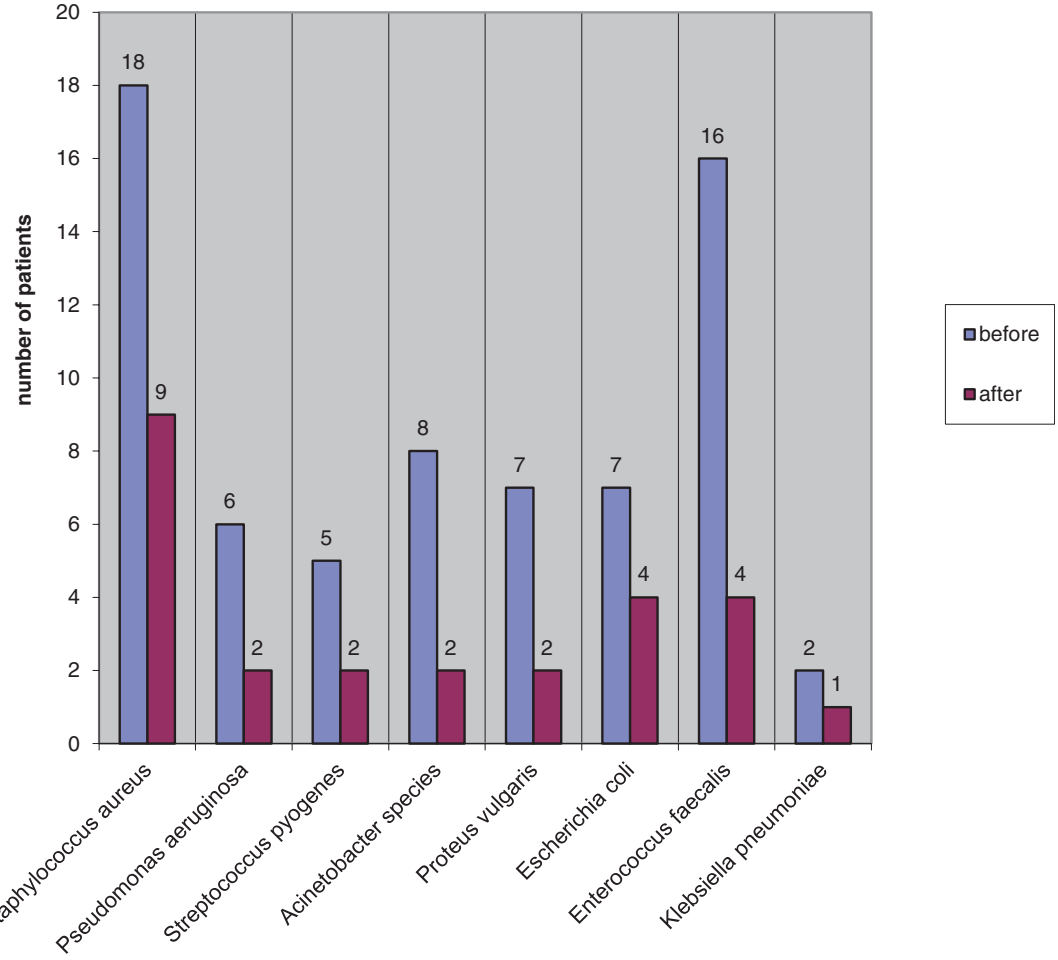
### 3.3 Wound Cultures

Wound swabs were taken before and at the end of NPWT. To assess NPWT efficacy, the wound culture results obtained from swab cultivations before and after NPWT were compared. Table 5 shows the comparison of main pathogenic microbial strains from wound cultures before and after NPWT, where reduction of pathogenic microbial strains after NPWT was observed. To compare the values obtained that were related to each other (before and after NPWT) a paired t-test was performed. A statistically significant reduction of pathogenic microbial strains was observed after NPWT ($p = 0.0038$).

### 3.4 NPWT Treatment

In 50 patients with 54 wounds the mean NPWT duration was 9.2±7.2 days (range 5–36 days). For all 54 wounds in this study, the mean number of foam changes was one per wound. In 16 patients with 17 wounds, where NPWT was repeated, the mean number of foam changes was 2.4 ± 1.94 (range 1–7). A low average number of foam changes and a relatively short NPWT duration in

**Table 5** Bacteriology before and after NPWT treatment. Statistically significantly reduced pathogenic microbial strains in wound cultures after NPWT confirmed by paired *t*-test ($p = 0.0038 < 0.05$)



all patients can be explained by meticulous initial wound decontamination followed by thorough wound debridement performed before NPWT application, and by the performance of the surgical wound closure as soon as the wound surface and conditions allowed for this procedure.

### 3.5 Wound Closure

An appropriate type of surgical wound closure was chosen with regard to clinical conditions, wound appearance after NPWT, and wound localization. Table 6 provides an overview of the treatment methods chosen for wound closure. In 34 wounds the preferred method was a split-thickness skin graft (STSG), selected in cases where the wound bed was well prepared for graft take. Split-thickness skin grafts were meshed to achieve greater wound-fluid drainage and to obtain better graft adherence to the wound surface, thus enhancing skin graft take. This method could be used in many of the cases where deep structures were exposed, for example bones or tendons that were overgrown by healthy granulation tissue that was stimulated by the beneficial effect of NPWT. Closure of the wounds with remaining deep structures exposed even after NPWT required the use of healthy tissue from the wound-adjacent areas. Surgical methods, including direct suture after wound edge mobilization and adjacent fasciocutaneous, muscle, or musculocutaneous flaps or their combinations, were used. These methods were performed much more easily and with considerably greater safety because of decreased tissue edema and increased tissue perfusion after NPWT. Three muscle flaps in combination with fasciocutaneous flaps and one musculocutaneous flap were used in the wound closure of two ischial and two sacral pressure sores, respectively. Conservative treatment was chosen three times in patients with chronic feet defects with a small remaining wound surface (maximum 2 cm × 2 cm).

**Table 6** Wound closure methods

| Method | N |
|---|---|
| STSG | 34 |
| Fasciocutaneous flap | 7 |
| Direct suture | 6 |
| Muscle flap | 3 |
| Musculocutaneous flap | 1 |
| Conservative treatment | 3 |
| **Total** | **54** |

*STSG* split-thickness skin graft

## 4 Wound-Healing Time and Mean Hospital Stay

The wound-healing time was defined as the time interval between foam removal (end of NPWT) and complete wound closure. The mean wound-healing time for 53 of 54 healed wounds was 16 ± 7.53 days (range 7–50). One patient with diabetes acutely admitted with phlegmon in the heel region displayed no progress following initial debridement and NPWT. A second surgical intervention due to progression of infection to the plantar space showed plantar aponeurosis involvement after evacuation of an extensive plantar abscess. After repeated necrectomy and NPWT application, again no healing progress was observed, which led to the suspicion of a compromised blood supply to the affected area. Lower leg arterial sonography showed multiple segmental obstructions of the main supplying arteries, which required correction by percutaneous transluminal angioplasty and stenting of the affected vessels, performed at the National Institute of Cardiovascular Diseases. Following intravascular intervention, the patient was transferred back to the authors' department, where repeated debridements and NPWT, including seven foam changes, were performed until complete wound closure was finally achieved. The mean hospital stay for the 50 study patients was 28 ± 20.8 days (range 10–138). The hospitalization time of patients with more than one wound (particularly those with multiple pressure sores) was related not only to the wounds with NPWT treatment but also to the treatment of all other wounds, for which NPWT was not used. Therefore, the mean length of hospital stay did not fully reflect NPWT efficacy. For this reason, the wound-healing time was considered to be more suitable.

## 5 Discussion

As the results have shown, majority of the patients included in this study had wounds that were contaminated, undermined, had exposed deep structures, and had irregular surfaces and wound edges (Fig. 3). The majority of the patients with this type of wound had been transferred to the authors' facility following the failure of conventional wound treatment methods, both conservative and surgical. Although the wound etiology and duration (acute and chronic wounds) were variable, the beneficial effects of NPWT observed were similar to those described in the literature [1]. After thorough surgical debridement and NPWT application, wound fluids were derived, blood flow and angiogenesis were supported, tissue edema was reduced, and granulation tissue growth was induced.

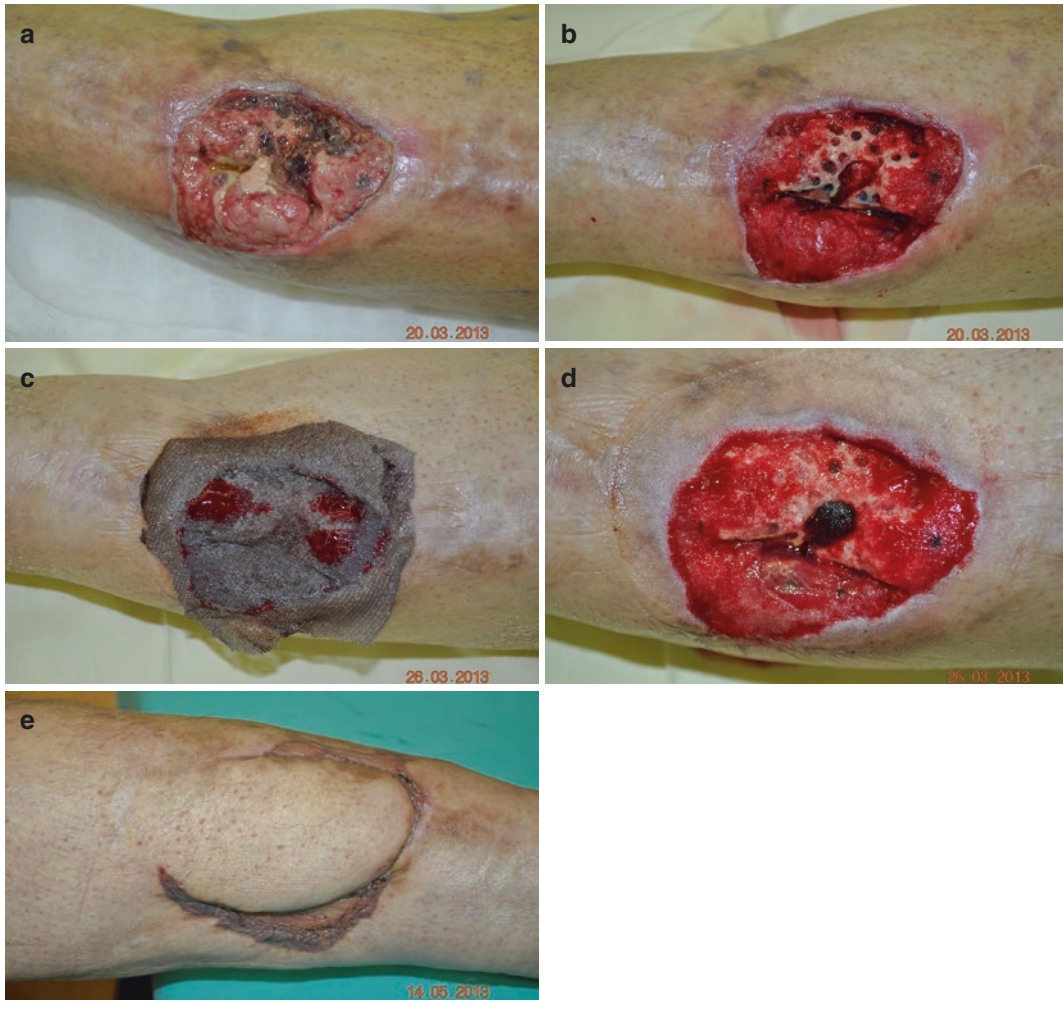



**Fig. 3** (**a**) 67-year-old patient with insulin-dependent diabetes mellitus, hypertension, and ischemic heart disease who sustained tibial fracture 30 years before. After hitting his leg a bone fistula developed and open wound with tibial exposure arose. Patient was transferred from another hospital 17 days post-injury after fistula extraction, debridements, and bone forage. (**b**) Debrided wound prior to NPWT. (**c**) Foam removal after 6 days of NPWT, note silver-impregnated dressing as wound contact layer. (**d**) Wound appearance after silver-impregnated dressing removal, bone almost overgrown by granulation tissue, drill holes after forage filled with granulation tissue. Wound ready for the wound closure procedure. (**e**) Seven weeks after coverage of the bone by fasciocutaneous flap, the rest parts of the wound and the donor site of the flap were covered by STSG

In addition to the clinical assessment of the wounds, wound culture results are equally important regarding the treatment options and outcomes of patients, although it should not be the sole criterion in the decision process. Reduction of bacterial contamination is frequently attributed to the effects of NPWT. Although some studies have shown a significant decrease in the bacterial load of *Staphylococcus aureus* and epidermidis [1], in others [17, 18] NPWT was more effective in the clearance of *Pseudomonas aeruginosa* and nonfermentative gram-negative rods, with no significant difference in *Staphylococcus aureus* clearance. The wound culture results of this study showed a statistically significant decrease in all pathogenic microbial strains after NPWT treatment. Because a significant reduction in bacterial rebound when using NPWT in combination with topical silver dressing was reported previously [11, 19], the results obtained in this study can be attributed not only to meticulous debridement and the effect of NPWT, but also to the antimicrobial activity of the silver-impregnated dressing used as the wound contact layer in all patients. In the authors' experience, the use of a nonadherent dressing as a wound contact layer in combination with NPWT appeared to have further advantages. First, foam removal was easier, with less bleeding, less or minimal pain, and less damage to the underlying granulation tissue. Second, in wounds with irregular edges, the foam could be placed on the wound without any concern that it would exceed the edges of the wound already covered by the dressing, thus avoiding maceration of "healthy" skin. For all the above-mentioned reasons, the authors consider the use of nonadherent silver-impregnated dressing in conjunction with NPWT to be an advancement in wound treatment. No adverse events or reactions related to the silver-impregnated contact layer used during NPWT were observed in our patients, which confirmed the safety of this method.

On completion of NPWT treatment, after foam and nonadherent dressing removal, the dead spaces and skin underminings were reduced or even obliterated, and former wound-surface irregularities were filled with healthy granulation tissue which in many cases overgrew the exposed deep structures (bones, tendons), which facilitated the use of simpler surgical techniques of wound closure, as can be seen in Table 6. This shift from complicated to easier surgical wound closure procedures was observed in accordance with findings of other studies in the literature [20]. Some patients with chronic wounds with multiple comorbidities could benefit from a less invasive operative procedure (e.g., the use of split-thickness skin graft instead of a flap) and a shorter operation time.

In six cases, direct suturing of the wound could be performed after NPWT, resulting from a contraction effect of NPWT on the wound, which pulls the edges together [21]. In two of these patients (wounds on the heel and the knee, respectively, sutured under slight tension), NPWT was placed directly on the sutured wound to enhance the beneficial effect of increased capillary perfusion around wounds induced by negative pressure.

Six patients in the current study were referred to the authors' facility with wounds that had resulted from open fractures of the lower extremity stabilized with external fixation. Following debridement and foam application, it was technically demanding to achieve an airtight seal of the spaces around some of the Steinmann pins using adhesive drapes in the treated area. The authors suggest using of silicone adhesive strips approximately 5–7 cm long, 3–4 mm wide, and 2–3 mm thick, which could be wrapped around the Steinmann pins wherever there is contact between the pins and adhesive drapes to seal any air leaks after connection to the negative-pressure source. Further investigations need to be performed to solve this problem.

To obtain the best possible results, in accordance with our experience, NPWT treatment method should be:

1. Individual—Each patient must be treated as a unique individual. All comorbidities and medications must also be taken into consideration. The whole patient and not the wound alone should be treated (holistic approach [22]).

**Table 7** Contraindications for NPWT

| Contraindications for NPWT |
|---|
| Necrotic tissue with eschar present |
| Untreated osteomyelitis |
| Nonenteric unexplored fistulas |
| Malignancy in the wound |
| Exposed vasculature |
| Exposed nerves |
| Exposed anastomotic sites |
| Exposed organs |

2. Selective—NPWT represents a method of choice for the treatment of only indicated wounds. The contraindications are listed in Table 7 [23]. Additionally, patient risk factors/characteristics must be considered before NPWT use, for example, patients at high risk for bleeding or hemorrhage, on anticoagulants or platelet aggregation inhibitors, with friable or infected vessels, or with other contraindications [23].
3. Complementary—NPWT as a complementary method is an integral part of wound treatment in indicated cases. The position of NPWT in the wound-healing process should be somewhere in the middle, between the initial surgical debridement and the surgical or conservative wound closure at the end.

## Conclusions

NPWT treatment represents a valuable adjunct in accelerating the healing of deep defects or problem wounds in indicated cases and in preparing wounds for successful wound closure, preferably (but not exclusively) using split skin grafting and/or other surgical or nonsurgical methods. The combination of NPWT with silver-impregnated dressing has been shown to be beneficial. Results of this study showed a reduced mean wound-healing time and reduced length of hospital stay.

In NPWT treatment, several mechanisms of actions and factors act in concert and exert several beneficial effects. NPWT offers new options in treating problem wounds in indicated cases. It can provide a good alternative in cases where classical wound treatment methods have been ineffective as a preparation for final surgical wound closure. Nevertheless, initial decontamination and meticulous surgical debridement of the wound prior to the use of NPWT treatment are of paramount importance.

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# Negative-Pressure Wound Therapy in Abdominal Surgery

José Pintor Tortolero and Ramón Cantero Cid

## 1 Introduction

Surgical site infection (SSI) is a common wound complication. It is defined as infection related to a surgical procedure that occurs at or near the incision site within 30 days after the procedure or in the subsequent 90 days in the case of material implantation during the surgery [1].

Different factors such as an increase in the mean age of the patients undergoing surgery, high rates of obesity, and immunosuppression secondary to oncological treatments have led to an increase in its incidence. SSI may lead to increased healthcare costs due to delayed recovery and prolonged hospital stay, repeat surgery, and need for increased wound follow-up.

Negative-pressure wound therapy (NPWT), also known as vacuum-assisted wound closure therapy, refers to wound dressing devices that apply continuous or intermittent subatmospheric pressure to the surface of the wound. The positive clinical effects of negative-pressure wound therapy (NPWT) on open and complicated wounds are well known. Recently, the application of this technique has been extended to the treatment of closed, clean wounds. A growing body of evidence has reported the positive effects of NPWT over closed wounds, particularly in patients with comorbidities which make them prone to develop surgical site infections.

## 2 Technique

### 2.1 Mechanism of Action

There are systemic and local factors that can contribute to a delay in the normal process of wound healing. Systemic factors (malnutrition, wound ischemia) should be identified and corrected as early as possible. Local factors include desiccation, tissue edema, excessive exudate, SSI, and poor tissue apposition (for example, in flap situations). NPWT will act on all these local factors, thus accelerating healing and reducing wound closure time.

### 2.2 Reduction of Tissue Edema

Interstitial fluid accumulation generates an extrinsic compression of the microvascular network decreasing the oxygen supply to the tissue and alters the venous and lymphatic drainage perpetuating the edema. Moreover, wound exudate is rich in matrix-degrading proteases and poor in epithelial growth factors.

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Thus NPWT contributes to wound healing by removing fluid and reducing the formation of hematomas and seromas and by improving the wound microenvironment by removing excess of proteases [2, 3].

### 2.3 Increase of Granulation Tissue

NPWT has been shown to increase the organization of collagen and the expression of vascular endothelial growth factor (VEGF) and fibroblast growth factor 8, thus promoting faster and more effective wound healing [4–6].

### 2.4 Holding Incision Edges Together

In deep open wounds, the sponge's open porous structure imparts the negative pressure homogeneously to the wound surface, reaching all its edges. The wound will deform to join its edges and to firmly adhere any skin flaps present. In closed wounds, NPWT maintains the cohesion of the incision edges, facilitates contraction of the epithelial edges, and helps reduce tensile forces [2, 3, 7–12].

### 2.5 Physical Barrier to Microorganisms

With NPWT, there is less need for dressing changes with respect to conventional techniques, with less possibility of colonization of the wound.

## 3 Devices Used in Negative-Pressure Therapy

### 3.1 Assisted Vacuum Locking System (Renasys®) and V.A.C. Unit®

These devices create a subatmospheric pressure at the wound site through the placement of a polyurethane sponge inside the edges of the wound which is covered tightly with a self-adhering plastic. A small incision is made in the plastic over the sponge and a suction tube is attached which is connected to an empty container (canister), which in turn is connected to an automatically controlled mechanical pump, which generates continuous or intermittent negative pressures up to −125 mmHg. Applying the suction creates an airtight seal that protects the wound, drains fluids through the pores of the sponge, and approximates the edges of the wound, accelerating the healing process. The size of the sponge is reduced slightly in each of the dressing changes, which must be performed every 3 days, such that the edges of the wound gradually approach one another. Transparent self-adhesive film allows monitoring of the status of the wound edges without removing the dressing.

### 3.2 PICO®/Prevena® System

These single-use pocket devices stand out for being disposable, portable, and of immediate application. The device consists of a sponge with a microperforated dressing covered with an adhesive sheet. A suction drain is connected to the sponge and a vacuum applied, with a small tank for collecting fluids. When the system is activated, a negative pressure of 80 mmHg (PICO® device) or 125 mmHg (Prevena® device) is applied to the wound, excessive fluid is extracted if present, and the incision is completely isolated from external contamination. The battery has a duration of 7 days. Light signals inform of its correct operation, or situations of leakage or low battery.

The microperforated dressing is composed of different layers: a silicon adhesive layer which is in direct contact with the wound, an air layer to homogenize the negative pressure, an absorption layer, and a surface layer of water-resistant polyurethane, which allows evaporation but avoids the entry of air with the consequent loss of vacuum. The exudate enters the air layer and is quickly transferred to the absorption layer, where it is stored forming a gel, which will progressively evaporate through the polyurethane layer to prevent the dressing from becoming heavy with the storage of liquid.

# 4 Discussion

## 4.1 NPWT Versus Conventional Systems

Conventional systems used in wound care consist of dressings that have to be changed up to three times daily, which is usually associated with pain with each dressing replacement. NPWT uses dressings that can be changed every 2–3 days and up to weekly. This, in addition to reducing episodes of pain, is associated with fewer manipulations and therefore less risk of SSI. NPWT significantly reduces wound-healing time improving patient quality life and decreasing inpatient stay.

On the other hand, the main complaint of NPWT patients is the discomfort of transporting the pressure pump. Moreover, NPWT devices are more expensive than traditional dressings. However, in recent years, several studies have reported NPWT to be cost effective when compared with traditional dressings due to the lower frequency of dressing changes, shorter duration of treatment, and no need for skilled wound care. Nevertheless, these studies should be interpreted with caution since they have small sample size or are based on the experience in a single center and lack of randomization.

## 4.2 NPWT for Management of Closed Incisions

NPWT has become an important tool in wound management. Since the first studies in pigs by Morykwas and Argenta in 1997, its widespread use has been implemented for the management of the open abdomen and for wounds associated with trauma or major complexity. In recent years, it has been proposed for the treatment of closed surgical wounds despite the fact that its effects in patients undergoing surgery with primary wound closure have been poorly investigated [13–15].

SSI is one of the main postoperative complications in abdominal surgery. It decreases the quality of life of the patient and implies a longer hospital stay and greater economic costs for the healthcare system. In developed countries the incidence is 5%, reaching 50% in high-risk patients. The prevention of SSI has been a focus of surgeons' efforts in recent years. Surgeons of different disciplines have incorporated the use of NPWT into the current standards of prevention of SSI (preoperative systemic antibiotic protocols, preoperative shower, surgical surface washing with antiseptic, and sterile surgical technique). In theory, NPWT promotes wound healing by reducing lateral tension on the wound edges, reducing seroma or hematoma formation and thus the risk of infection, and improving lymphatic drainage by decreasing tissue edema. Despite being shown to increase tissue perfusion in open abdominal wounds, an experimental study has shown that its effect on perfusion is minimal in incisional wounds.

Based on this theory, various studies have been conducted in different surgical disciplines to evaluate the benefits of this therapy related to wound infection [16–18]. Some of the first studies used existing NPWT devices designed for open wounds. Currently, small portable devices developed specifically for the treatment of closed incisions are marketed.

In clean surgeries such as cardiac or orthopedic surgery, NPWT has been shown to play an important role in the prevention of SSI. In these disciplines, asepsis marks the success of surgery, so proper wound management is essential to avoid contamination. Colli and Camara published a pilot study in ten patients in which a portable NWPT device was used over sternotomies with no reported complications of SSI [19]. Further trials such as those by Grauhan et al. demonstrated a lower incidence of SSI associated with NPWT use in median sternotomies in obese patients [20].

In the field of general surgery, regarding clean procedures, Olona et al. [21] pointed out a reduction of postoperative drain requirements to an average of 4 days and an absence of postoperative complications after large incisional hernia repairs managed with NPWT. NPWT has also been studied in colorectal surgery. Colorectal procedures are among the surgical interventions with the highest infection rates, especially if per-

formed emergently, or when fecal spillage or manipulation of the bowel occurs. Chadi et al. [22] evaluated the incidence of SSI in perineal wounds (after abdominoperineal resection) in a retrospective study. They reported fewer SSIs of perineal wounds associated with NPWT. Bonds and colleagues retrospectively reviewed the risk factors for SSI in colorectal surgery and determined that NPWT significantly reduced SSI in their series [23]. They used a cut strip of V.A.C. GranuFoam Dressing (KCI) attached to a wound vacuum pump, set at 75 mmHg continuous suction, over open colectomy incisions. The use of this device significantly reduced SSI.

Stoma creation and closure, following the same principles, are also at high risk of infection and could therefore benefit from this type of therapy. Regarding ileostomy reversal, Cantero and colleagues observed a lower rate of SSI associated with NPWT in a pilot study [24].

### 4.3 Contraindications to NPWT

NPWT is contraindicated in the presence of malignant disease because it may stimulate the proliferation of malignant cell [25]. Nor should it be used in the presence of non-enteric or unexplored fistulae. NPWT should also be avoided in the presence of untreated osteomyelitis [26, 27]. Devitalized tissue in the wound bed impairs wound healing and increases the risk of infection and therefore contraindicates the use of NPWT. All necrotic tissue should be debrided prior to NPWT.

Special caution should be taken in cases of friable or exposed blood vessels since direct negative pressure may cause trauma and bleeding [28]. Negative pressure can cause avulsion of the skin at the margins of the wound in patients with fragile skin (due to use of corticosteroids, age, or disorders of collagen formation). Patients with high risk of bleeding (patients who have received anticoagulants or platelet aggregation inhibitors) should be monitored. If fresh red blood is detected in the tube, NPWT should be discontinued and bleeding control is mandatory.

**Conclusions**

NPWT reduces SSI. The current knowledge shows that there is no indication of systematic use of negative-pressure wound therapy to all abdominal surgery incisions because of its high costs in comparison with that of standard dressings. It is indicated to prevent SSI in high-risk patients.

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# How to Manage the Open Abdomen

Arnulf Willms, Christoph Güsgen,
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## 1 Introduction

The open abdomen has become a standard technique in the management of critically ill patients with severe intra-abdominal conditions. There are three undisputed reasons for leaving the abdomen open. These are shown in Table 1.

In Western Europe, the leading indications for open abdomen management are in order of frequency:

1. Secondary peritonitis
2. Abdominal compartment syndrome (ACS)
3. Trauma

The mortality of open abdomen management varies between 10 and 45% [1–3]. Mortality, however, is associated more closely with the underlying disease than with the technique used [4]. The mortality of patients with peritonitis, for example, is higher than that of non-trauma patients and patients with abdominal compartment syndrome [4].

**Table 1** Reasons for leaving the abdomen open

| | |
|---|---|
| 1. | Source of infection difficult to control adequately/ perfusion difficult to assess |
| 2. | Need for a limited surgical intervention (damage control surgery) |
| 3. | Treatment and prevention of abdominal compartment syndrome (ACS) |

Although laparostomy (the peritoneal cavity is opened anteriorly and deliberately left open, often called "open abdomen") is a common technique, it is indicated only in rare cases. Since there is a lack of multicenter studies and patient populations are usually small and heterogeneous in the available literature, the level of evidence on which current guidelines for the management of the open abdomen are based is low. We here describe the best and probably most effective treatment modalities [1, 2]. The focus of attention is on optimizing factors of critical importance for the success of open abdomen management and on preventing complications and long-term adverse effects [3, 5–7].

Negative-pressure therapy has played a major role in current modifications of open abdomen management and has led to a paradigm shift. For this reason, negative-pressure techniques are widely used today [4].

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## 2 Abdominal Management

Abdominal management techniques should ideally be able to meet the following requirements [4]:

1. Provide optimum protection of the intestinal serosa in order to prevent the formation of fistulas.
2. Prevent fascial retraction in order to achieve high fascial closure rates and avoid further complex surgical procedures.
3. Ensure the continuous removal of exudate in order to prevent the formation of abscesses, reduce edema, and monitor fluid losses: When these requirements are met, a high level of patient care and comfort can be achieved.

The prevention of fistula formation and the closure of the fascia at the earliest possible stage lead to a decrease in morbidity and mortality rates associated with the procedure [4, 6].

Open abdomen management involves the use of three components that can be used alone or in combination (Fig. 1):

1. Negative-pressure therapy (suction)
2. Visceral protection (a plastic sheet that prevents adhesion of the viscera to the abdominal wall)
3. Fascial traction (which prevents fascial retraction and promotes reapproximation of the fascial edges until closure)

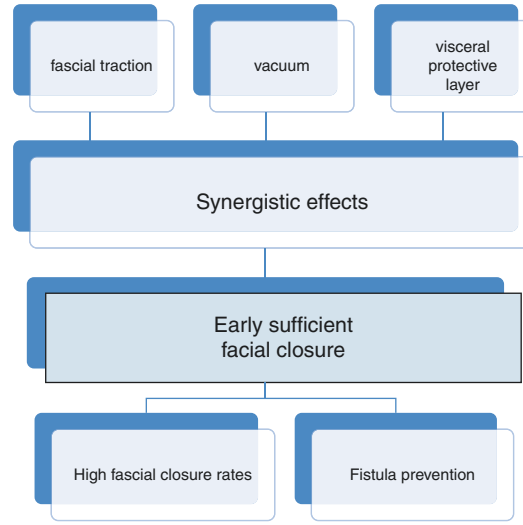


**Fig. 1** Three treatment components of open abdomen management

### 2.1 Negative-Pressure Therapy (Suction)

The application of negative pressure leads to adequate exudate removal, edema reduction, and improvement of intestinal microperfusion. The continuous removal of exudate prevents the accumulation of fluid and thus the formation of intra-abdominal abscesses [4, 6]. Compared with passive drainage, the active application of negative pressure allows a significantly larger volume of exudate to be removed [4]. A closed system facilitates the management of fluid and protects the open abdomen from the external environment and reduces the number of dressing changes [5]. On the whole, this technique improves wound healing and accelerates granulation tissue formation. Evidence-based recommendations regarding the level of negative pressure are unavailable. Negative pressure should not be so high that tissue damage is caused and should not be so low that exudate is not adequately removed. A mean pressure of 75 mmHg is applied.

For patients with polytrauma and coagulopathy, we recommend that the level of negative pressure be reduced to 25 mmHg. We prefer the application of continuous to intermittent negative pressure. The use of negative pressure for open abdomen management can thus be recommended [4].

## 2.2 Visceral Protection

Since serosal lesions were reported to be the starting point for and cause of intestinal fistulas, the management of the surface of the small intestine was identified as a key factor in the prevention of fistulas [8]. Compared with the application of rough material, the placement of an inert nonadhesive sheet in contact with the viscera is therefore likely to reduce the incidence of fistula formation to a minimum [5, 8, 9]. The use of an inert nonadhesive and usually perforated plastic sheet enables us to minimize the formation of adhesions of the viscera to the abdominal wall and to components of the abdominal dressing [9]. Initial concerns that negative-pressure therapy might promote fistula formation were dismissed by recent studies in which the application of an inert sheet was found to provide effective visceral protection [5]. This type of visceral protection reduces the risk of serosal lesions that would be caused during the removal of adhesions. All negative-pressure systems that are commercially available for open abdomen management include a visceral protective layer. If a visceral protective layer is used inappropriately or not at all, the risk of intestinal microtrauma during surgical revisions will likely increase [4]. On the whole, we recommend the application of a visceral protective layer in order to reduce the rate of fistula formation.

## 2.3 Fascial Traction

Since the rate of complications increases with the length of treatment, early fascial closure can improve outcome and minimize risks associated with the management technique. Fascial retraction was found to begin as early as about 3 days after surgery and results in a progressively larger gap in the fascia [1]. The literature suggests that early fascial closure should ideally be achieved within 10 days [7]. In recent years, a focus of attention has therefore been placed on developing techniques that ensure fascial traction. Alloplastic absorbable and nonabsorbable mesh, mattress sutures, artificial burr Velcro-like devices (Wittmann Patch™), and abdominal reapproximation anchor system (ABRA™) are used for the management of the open abdomen. These techniques allow delayed primary fascial closure rates as high as 90% to be achieved [3, 7].

In addition, delayed primary fascial closure prevents the formation of sometimes giant ventral hernias that require complex reconstructive procedures and are associated with considerable risks.

These three components of treatment can be combined in order to achieve synergistic effects that allow the primary objectives of open abdomen management to be achieved more rapidly and more effectively than if each component were used individually (Fig. 1).

Figure 2 presents the treatment algorithm that we use at our institution. It combines the three treatment components and ensures a standardized approach [1, 5].

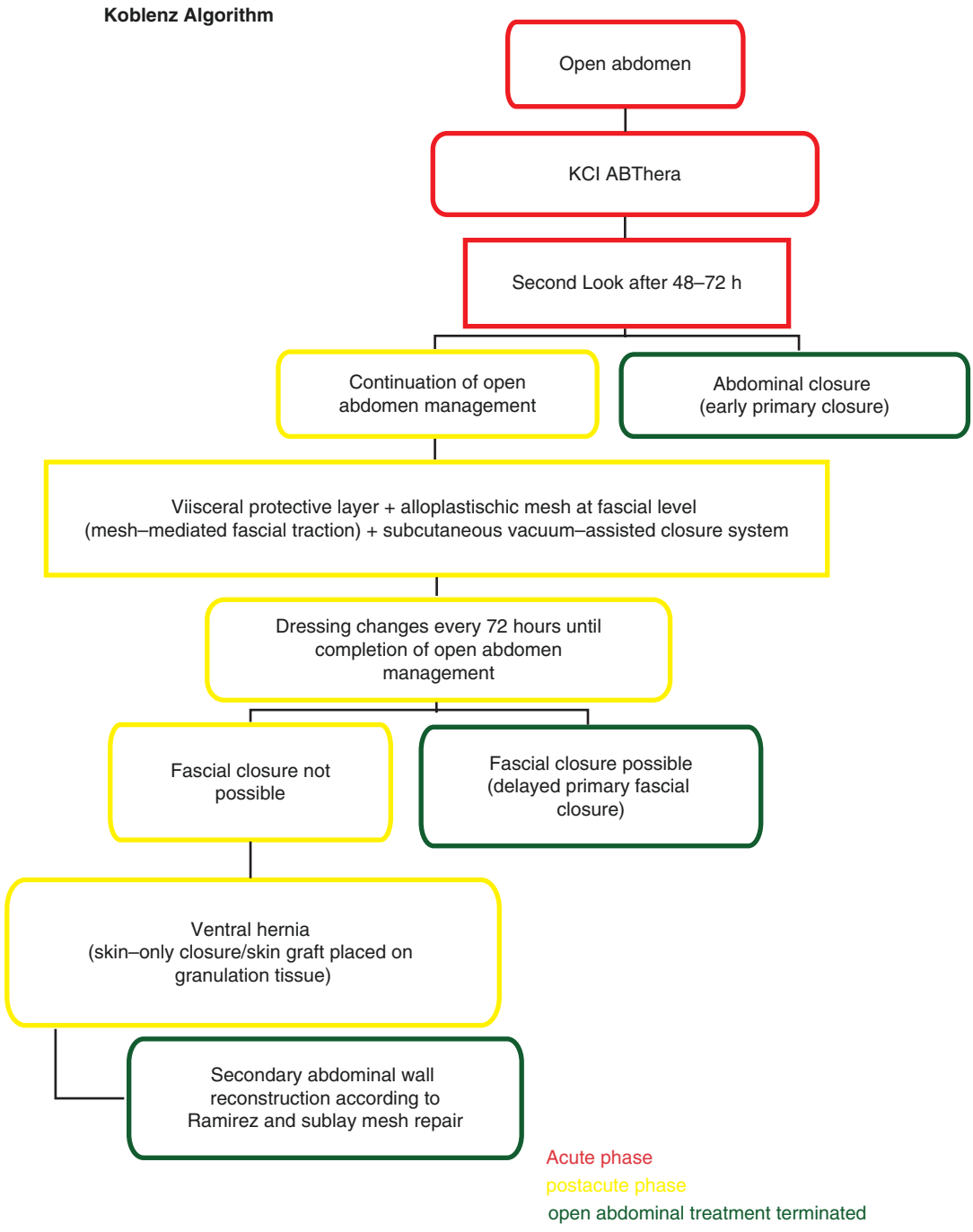


**Fig. 2** Koblenz algorithm for treating the open abdomen

## 3 Practical Advice on How to Perform the Surgical Procedure

The open abdomen technique described here involves the placement of a visceral protective layer, the use of subcutaneous foam, and the application of negative pressure. A number of different commercial systems are available for this purpose. Particular attention must be paid to completely covering the viscera with the visceral protective layer in order to avoid contact between the subcutaneous foam and intestinal serosa and between the abdominal wall and the viscera.

Depending on systemic parameters of the patient and expected local intra-abdominal situation, the first revision and the first dressing change should be performed after 48–72 h. If early primary closure is inadvisable or impossible, fascial traction should be initiated. An inexpensive stable mesh should be placed as a temporary inlay between the visceral protective layer and the subcutaneous foam (VAWCM = vacuum-assisted wound closure and mesh-mediated fascial traction) (Fig. 3) [1]. The objective of mesh placement is to prevent fascial retraction and to achieve high rates of delayed primary closure. In a first step, a polyethylene sheet is cut to size and inserted into the abdominal cavity in such a way that the viscera are completely covered. The purpose of this layer is to prevent any contact between the viscera and the mesh, which is inserted in a next step. The mesh is then sutured to the fascial edges with robust sutures under appropriate tension in a continuous and circular manner (Fig. 4). Subcutaneous foam is placed on the mesh and continuous negative pressure of 75–100 mmHg is applied. The level of negative pressure is reduced in patients with an increased risk of bleeding and can be as low as 25 mmHg. At the second dressing change, a longitudinal incision is made to open the mesh in the middle. The protective layer is removed through the opening and the revision is performed. As far as the volume of the intra-abdominal contents and intra-abdominal pressure permit, the edges of the mesh are excised and the mesh is resutured with a running #0 Vicryl suture under moderate tension (Fig. 5). This technique

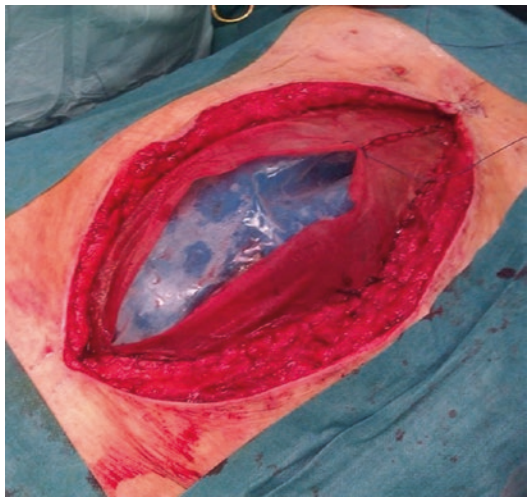

**Fig. 3** Fascial traction with an Polyglactin 910 mesh (day 3)

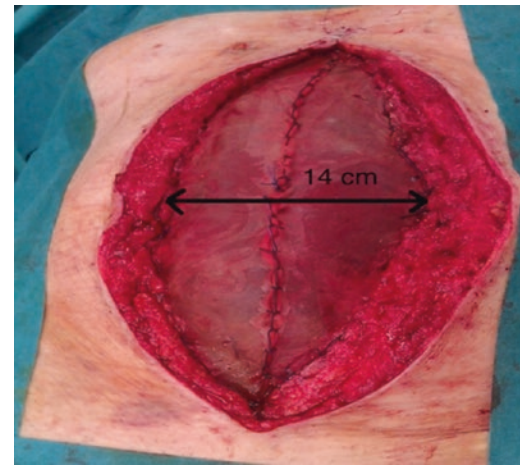


**Fig. 4** Fascial gap at day 3

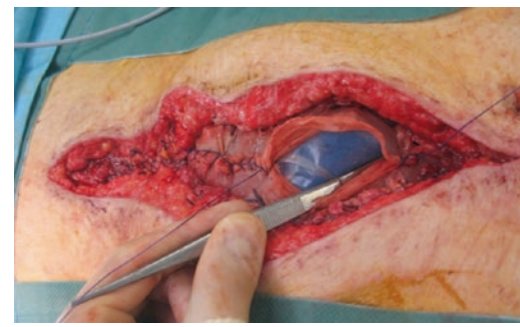

**Fig. 5** Performing fascial traction by resuturing the mesh after partial resection (day 9)

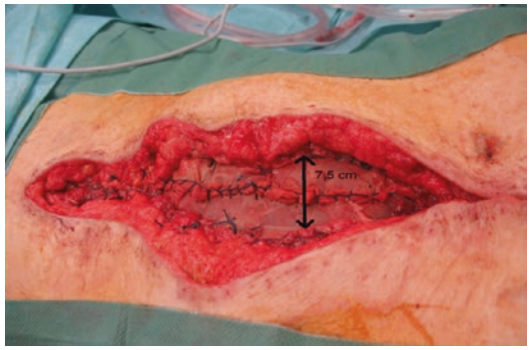


**Fig. 6** Facial gap at day 9

enables the surgeon to reduce the fascial gap progressively with every revision procedure (Figs. 4 and 6). When the fascial edges can be reapproximated and open abdominal management can be completed, the mesh is removed and delayed primary fascial closure is performed. The fascial edges are united using slowly absorbable, interrupted sutures. In the case of unfavorable fascial conditions, strips of mesh may be left in an onlay position to augment the fascia and support the suture line. Mesh into which tissue has grown can be left in place as well. If there is macroscopic evidence of compromised local wound healing, a subcutaneous vacuum-assisted closure system should be placed. If there is evidence of a clean wound, the skin can be closed.

## 4 Information About Potential Risks

1. Injuries to adjacent structures:
   a. Vessels (postprocedural bleeding, hematoma)
   b. Organs (intestines, bladder)
2. Incisional hernia formation
3. Wound healing problems
4. Subsequent procedures
5. Formation of a giant ventral hernia as a result of the failure to achieve fascial closure
6. Small-bowel fistula
7. Split-thickness skin grafting
8. Enterostomy

## 5 Central Registry

Standardized multicenter data should be collected in order to improve the clinical results of open abdomen management and to obtain data of an appropriate level of evidence [3, 6].

A central registry provides a suitable basis for data collection [10].

For this reason, an Open Abdomen Registry was initiated by the Surgical Working Group for Military and Emergency Surgery (CAMIN) of the German Society for General and Visceral Surgery (DGAV) and implemented in cooperation with the European Registry of Abdominal Wall Hernias (EuraHS) [10]. The registry is available as an online database at www.eurahs.eu. The objective of the registry is to collect information about the most important parameters of all patients undergoing open abdomen management in order to obtain robust data on open abdomen treatment.

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# Negative-Pressure Wound Therapy

Roberto Cirocchi, Andrea Boccolini,
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## 1 Introduction

In our society characteristics and population health needs are constantly changing. The change in lifestyle, introduction of screening, and innovation of medical and surgical therapies have allowed greater survival chance in frail patients and in patients undergoing more invasive surgeries. It led to a change in the incidence of disease and in postoperative outcomes (with a few number of complications).

Among the diseases the presence of which is constantly increasing there are wounds, which frequently complicate the quality of life of these patients. The wound represented a considerable economic interest from the industry that has introduced many advanced medications and devices. It has achieved a considerable interest from researchers and clinicians, who have made a significant number of studies. Despite the large number of publications on the subject very few randomized trials have been published, which reflects in very low quality of the available evidence.

The causes of the absence of randomized studies are many; the most important is represented by the classical difficulties to randomize surgical patients, which in this case also have disabilities and therefore difficulties in acquiring the consent to randomization. Many randomized trials have been registered and started, but only few have been published, which results in significant publication bias.

The wound is a very heterogeneous disease with different states of gravity, which ranges from mild to severe forms. Severe forms are those that require a higher consumption of resources; they are due to a deterioration in the quality of life and increased morbidity and mortality. Various methods for treatment of these complex wounds have been described in the literature, but negative-pressure wound therapy (NPWT) have gained considerable popularity during the last two decades.

## 2 Negative-Pressure Wound Therapy (NPWT)

The application of NPWT in treatment of the wound is very heterogeneous, making it very difficult to make a proper review of the literature;

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so in order to facilitate the analysis of the literature the different studies were grouped into three different categories according to what has already been carried out in the reviews of the Cochrane Wounds Group:

1. Negative-pressure wound therapy for treating surgical wound healing by secondary intention
2. Negative-pressure wound therapy for treating leg ulcers
3. Negative-pressure wound therapy for treating pressure ulcers

The Cochrane Wounds Group made further revisions on negative-pressure wound therapy for closed surgical incisions [1], on negative-pressure wound therapy for partial-thickness burns, and on negative-pressure wound therapy for skin grafts and surgical wound healing by primary intention [2–5]. We did not analyze these topics because in these cases the use of NPWT is prophylactic, not therapeutic. These new arrangements for revision proposed by the Cochrane replace the old method of revision in which only one analysis was carried out of topical negative pressure for treating chronic wounds.

Purpose of our review of the literature is to make a research and critical analysis of the literature revisions in which the wounds were treated with NPWT, this in order to assess whether there is clinical evidence of superiority over conventional NPWT wound dressing techniques.

## 3 Methods

The objective of this study was to compare negative-pressure wound therapy (NPWT) versus surgical wound healing by secondary intention (SWHSI). Randomized controlled trials (RCTs) were identified through a systematic review of published literature (full article, thesis, or abstract).

We analyzed all studies with patients presenting with pressure ulcers in any location.

The types of interventions were NPWT in experimental group versus SWHSI in control group (surgical debridement, enzyme or chemical necrosectomy).

The primary outcomes of interest were summarized in efficacy (ulcer healing, reduction of ulcer volume, local improvement in ulcer characteristics), and the secondary outcomes in the socioeconomic advantages (consumption of health resources).

## 4 Study Selection

A systematic literature search, in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) standards [6], was conducted using the PubMed and Scopus search engine up until the May 1, 2017, including the terms "negative-pressure wound therapy" and "pressure ulcers" or "wound healing." No language, publication date, and restrictions were imposed. All titles and abstracts of the considered studies were analyzed to select only the studies that reported the PICO [7]. When multiple articles were published from a single study group and overlapping study periods were reported, only the most recent article was considered so as to avoid duplication of data. The PubMed function "related articles" was used to enlarge each search, and the reference list of all potentially eligible studies was analyzed. To minimize retrieval bias, a manual search method including the Science Citation Index Expanded, Scopus, and Google Scholar databases was performed. After this initial process, the full-text papers were independently screened by two authors for eligibility. The final decision on eligibility was reached by consensus between the two screening authors. Data were extracted by two authors based on an intention-to-treat principle. Any disagreement was resolved through discussion with a reassessment of the data and/or by involving a third author.

The methodological quality assessment of the included studies was evaluated with the instructions and the items given in the Cochrane Handbook for Systematic Reviews of Interventions (sequence generation and allocation concealment for selection bias, blinding of participants or personnel for performance bias, blinding of outcome assessors for detection bias, incomplete outcome data for attrition bias, and selective reporting bias).

## 5 Results

The PRISMA flow diagram for systematic reviews is presented in Fig. 1. We identified 3.963 publications using the literature search strategy described above. After excluding the records following the review of the titles and abstracts, abstracts eligible for full-text evaluation remained, and in full-text assessment we identified nine publications that fulfilled the inclusion criteria: six publications reported data about pressure ulcers [8–13], two publications reported data about the surgical wound healing by secondary intention (SWHSI) [14, 15], and one publication reported data about the leg ulcers [16].

### 5.1 Negative-Pressure Wound Therapy for Treating Surgical Wound Healing by Secondary Intention

The systematic review includes two trials by Monson et al. in 2014 [14] and Biter et al. in 2014 [15] that were performed in Sweden and the Netherlands (Table 1). The Monson et al.'s study [14] evaluated NPWT with an alginate dressing for the treatment of infected open groin wounds that followed from arterial surgery (Table 2); the Biter et al.'s [15] study compared NPWT to open wound surgical technique in the excision of pilonidal sinus.

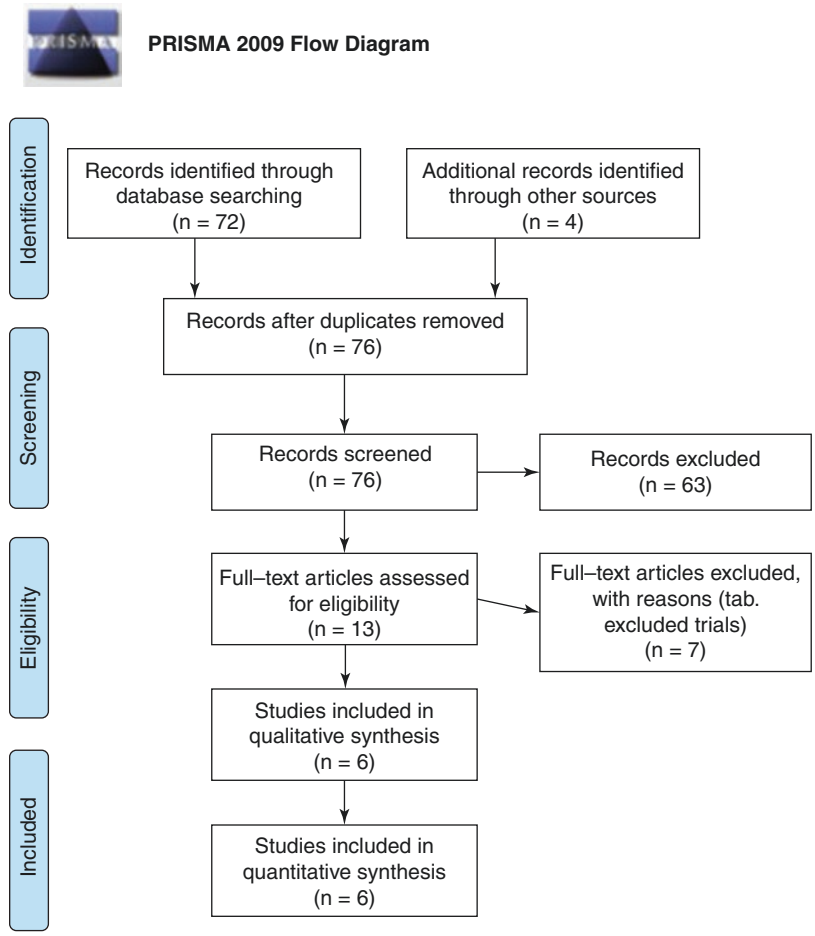


**Fig. 1** PRISMA 2009 flow diagram

**Table 1** Trials reported different types of wound and outcome, so the reported outcomes were not comparable

| | Monsen et al. [14] | Biter et al. [15] |
|---|---|---|
| Type of study | RCT | RCT |
| Nation where the trial was performed | Sweden | The Netherlands |
| Type of procedure | Vascular surgery | Excision of pilonidal sinus disease |
| Location of the incision | Groin | Sacral |
| Infection of wound | Deep infected wounds (Szilagyi grade III) | None |
| Patients who underwent NPWT | 10 | 24 |
| Patients who underwent alginate (Sorbalgon) therapy | 10 | 25 |

**Table 2** Outcomes: VAC vs. alginate treatment

| Monsen et al. [14] | |
|---|---|
| Time to full skin epithelialization | VAC (median, 57 days) vs. alginate (median, 104 days) group (*P* 0.026) |
| Wound treatment time | Hospital in the VAC group compared with the alginate group (*P* 0.034) |
| Wound surface area, $cm^2$ | VAC 13 (7.6–37.6) vs. alginate group 20.5 (4.6–44.5) |
| Wound depth, cm | VAC 4 (2.3–8.5) vs. alginate group 6 (2.5–13.5) |
| Reduction of C-reactive protein | 7 days (11 vs. 20, *P* 0.68) |
| | 14 days (7 vs. 13, *P* 0.46) |
| | 21 days (6 vs. 9, *P* 0.81) |
| Amputation (follow-up period of 14 months, range 2.2–51) | 3 vs. 2 |
| Mortality (follow-up period of 14 months, range 2.2–51) | 2 vs. 5 (*P* 0.35). |

**Table 3** Outcomes: VAC vs. control treatment

| Biter et al. [15] | |
|---|---|
| Complete wound healing | 84 days in the vacuum therapy group vs. 93 days in control patients ($p = 0.44$) |
| Visual analog scale score | No difference |
| Wound size ratio at day 14 (i.e., wound healing rate) | Significantly lower in the vacuum therapy group (0.30 vs. 0.57, $p = 0.02$) |
| Time to resume daily activities | 27 days in the patients undergoing vacuum therapy and 29 days in the control patients ($p = 0.92$) |
| Recurrence within 6 months after wound closure | Difference in wound infection rate and disease recurrence between both groups 3 (13%) vs. 1 (%) ($p = 0.30$) |

The trials are very heterogeneous and it is not possible to perform a meta-analysis.

All the included trials reported different types of wound and outcome, so the reported outcomes were not comparable. In the treatment of infected groin wound and pilonidal sinus disease the use of VAC therapy does not show any advantage than conventional treatment (Table 3).

## 5.2 Negative-Pressure Wound Therapy for Treating Pressure Ulcers

The systematic review included six trials [8–13] that were performed in Austria, India, the Netherlands, Switzerland, the UK, and the USA (Table 4). In three studies, the authors described

**Table 4** Characteristics of the included studies for pressure ulcers

| | Type of study | Nation where the trial was performed | Etiology of immobilization | Location of ulcers | Patients who underwent NPWT | Patients who underwent conventional treatment |
|---|---|---|---|---|---|---|
| Dwivedi et al. [8] | RCT | India | Traumatic paraplegia | Sacral | 21 | 23 |
| Ashby et al. [11] | RCT | UK | NR | Heel trochanter, sacrum, gluteal, and ischial | 6 | 6 |
| De Laat et al. [13] | RCT | The Netherlands | Traumatic paraplegia | NR | 6 | 6 |
| Wild et al. [10] | RCT | Austria | NR | Sacral | 5 | 5 |
| Wanner et al. [9] | RCT | Switzerland | Traumatic paraplegia | Sacral | 11 | 11 |
| Ford et al. [12] | RCT | USA | NR | Ischial, sacral, lateral malleolar. Trochanter and calcaneal | 20 | 21 |

the etiology of immobilization and reported a traumatic paraplegia [8, 9, 13]. The location of ulcer was reported in five studies and it was exclusively sacral in the paper of Dwivedi et al. [8], Wanner et al. [9], and Wild et al. [10]; differently Ashby et al. [11] and Ford et al. [12] reported a mix of locations: sacral, ischial, lateral malleolar, and trochanter. In the trials, the grade of ulcers was reported according to different systems; the pressure ulcers were referred to as grades by the European Pressure Ulcer Advisory Panel [17], by Wagner et al.'s scale [18], or Daniel et al.'s suggestion [19]. Ford et al. [12] did not use a grading system and reported only that the ulcers have a full-thickness tissue loss.

In the studies included in this systemic review all patients have ulcers with a full-thickness tissue loss. In the review 141 patients were enrolled: 62 underwent NPWT and 79 other conventional treatments.

All the included trials reported different types of outcomes, so the reported outcomes were not comparable. The analyzed outcomes are extremely numerous and are categorized into some different groups: healing of ulcer, reduction of ulcer volume, local improvement in ulcer characteristics, and consumption of health resources.

**Table 5** Ulcer or wound healing

| | Ulcer healed/time to healing | Wound healing |
|---|---|---|
| Ashby et al. [11] | Only one pressure ulcer healed (NPWT group) during follow-up (time to healing 79 days) | |
| De Laat et al. [13] | | Statistically significantly faster wound healing in the topical negative pressure group |

Only two trials evaluated the healing of ulcer: Ashby et al. [11] and de Laat et al. [13]. In the first study only one pressure ulcer (16.6%) healed in NPWT group (79 days) [11]; in the other study the authors performed a subgroup analysis of patients with pressure ulcers, but they didn't describe the results and reported only the conclusion ("statistically significant faster wound healing in the topical negative pressure group") [13] (Table 5).

Three trials described a better reduction of ulcer volume in patients who underwent NPWT [8, 9, 12]. Dwivedi et al. [8] reported the length

**Table 6** Reduction of volume of the ulcer

| | Length | Width | Reduction in ulcer volume (%) | Mean (SD) time to reach 50% of the initial volume |
|---|---|---|---|---|
| Dwivedi et al. [8] | Significantly ($p < 0.01$) decreased in NPWT. At weeks 1, 2, and 3, depth was significantly ($p < 0.05$) higher in NPWT group, whereas at week 9 a significant reduction ($p = 0.01$) was observed | | | |
| Wanner et al. [9] | | | | 27 (10) days in the vacuum-assisted group and 28 (7) in the traditional group |
| Ford et al. [12] | | | 42.1% with HP and 51.8% with VAC($p$_0.46) | |

and width of ulcer; differently Wanner et al. [9] reported the mean (SD) time to reach 50% of the initial volume and Ford et al. [12] reported the mean percent reduction in ulcer volume. In the first study the length and width of ulcer decreased significantly ($p < 0.01$) in NPWT group vs. standard care group at week 9 [8]; in the other two studies the authors did not report significant difference between the two groups: in Wanner et al. [9] 27 days in NPWT group and 28 in the traditional treatment and 51.8% with NPWT in Ford et al. [12] and 42.1% with traditional treatment (Table 6).

Very heterogeneous was the authors' choice of the characteristics for the local evaluation of ulcer improvement. These characteristics were macroscopically evaluated or through a biopsy with a histologic examination. In the macroscopic examination the presence of the granulation tissue was the most important favorable prognostic sign. The evaluation of this tissue was performed in different modalities: newly formed granulation tissue and wound contracture (measured the volume instead of the area of the usually undermined wounds) as reported by Wanner et al. [9], the absolute and relative proportion of wound surface granulation tissue as reported by Wild [10] or the conversion of slough into red granulation tissue as reported by Dwivedi et al. [8]. Conversely the presence of wound discharge, fibrin, or necrosis was a poor prognostic sign: absolute and relative proportion of fibrin tissue at the wound base or absolute and relative proportion of necrosis.

The results were heterogeneous in the included trials: only few significantly better results were reported in patients who underwent NPWT (lower exudates in NPWT group at weeks 4 and 9 and higher conversion of slough into red granulation tissue, increase in surface granulation); differently the other evaluation didn't report any advantage in macroscopic (newly formed granulation tissue and wound contracture, absolute and relative proportion of fibrin tissue at the wound base and of necrosis) or biopsy evaluation (mean number of PMNs and lymphocytes per high-power field and mean number of capillaries per high-power field) (Table 7).

The cost analysis was performed only by Wanner et al. [9] but they did not report any data but only the conclusion about NPWT "cheaper than the traditional dressings"; other studies reported the indirect costs as the discharge from hospital, the mean number of treatment visits per week, or the median number of dressing changes per day. In all outcomes, there was an advantage in NPWT group. Dwivedi et al. [8] reported that the hospital stay was significantly lower ($p = 0.001$) in NPWT at week 2. Ashby et al. [11] reported a lower mean number of treatment visits per week in patients who underwent NPWT (3.1 vs. 5.7) and finally Wild et al. [10] reported only that lower dressing changes resulted in patients who underwent NPWT (Table 8).

**Table 7** Different outcomes in each study: few significantly better results were reported in patients who underwent NPWT

| | Exudates | Conversion of slough into red granulation tissue | Mean number of PMNs and lymphocytes per high-power field | Mean number of capillaries per high-power field | Improved biopsy-proven osteomyelitis underlying the ulcers | Newly formed granulation tissue and wound contracture (measured the volume instead of the area of the usually undermined wounds) | Absolute and relative proportion of wound surface granulation tissue | Absolute and relative proportion of fibrin tissue at the wound base | Absolute and relative proportion of necrosis |
|---|---|---|---|---|---|---|---|---|---|
| Dwivedi et al. [8] | Significantly ($p = 0.001$) lower in NPWT group at weeks 4 and 9 | Significantly higher in NPWT group ($p = 0.001$) | | | | | | | |
| Wild et al. [10] | | | | | | | Increase in surface granulation tissue of 54% was observed in the V.A.C. group and a reduction in the Redon group (*P* 0.001) | Increase in fibrin tissue at the wound base of 21.8%, whereas in the V.A.C group, a 27% reduction was observed (*P* 0.035) | Reduced in the V.A.C. group, but this difference did not reach significance |
| Wanner et al. [9] | | | | | | Equally effective | | | |
| Ford et al. [12] | | | Decreased in the V.A.C. group and increased in the HP group | The mean number of capillaries per high-power field was greater in the V.A.C. group | Improved with V.A.C. | | | | |

**Table 8** Measurable costs and benefits

| | Discharge from hospital | Mean number of treatment visits per week | Analysis of costs | Quality of life | Median number of dressing changes per day |
|---|---|---|---|---|---|
| Dwivedi et al. [8] | Significantly ($p = 0.001$) lower in NPWT at week 2 | | | | |
| Ashby et al. [11] | | 3.1 (NPWT) and 5.7 (SC); 6/6 NPWT and 1/6 SC participants withdrew from their allocated trial treatment | | | |
| Wanner et al. [9] | | | It is cheaper than the traditional dressings | Better | |

**Table 9** Vuerstaek et al.'s 2006 [2] study for NPWT in leg ulcers

| Type of study | Nation where the trial was performed | Type of ulcers | Location of the incision | Infection of wound | Patients who underwent NPWT | Patients who underwent conventional wound therapy |
|---|---|---|---|---|---|---|
| RCT | The Netherlands/ Belgium | Arteriolosclerotic, venous or combined venous/arterial leg ulcers | Leg | Deep infected wounds (Szilagyi grade III) | 30 | 30 |

The quality of life was evaluated only by Wanner et al. [9] and it was better in patients who underwent NPWT, but they reported only the conclusion and did not report the data. In the surgical wound healing by secondary intention (SWHSI) all the studies described the type of procedure performed and the locations of the incision.

### 5.3 Negative-Pressure Wound Therapy for Treating Leg Ulcers

Characteristics of studies: The systematic review includes only one trial by Vuerstaek et al. (2006) [20] that was performed in the Netherlands and Belgium (Table 9). The study evaluated NPWT versus wound dressings in patients with chronic leg ulcers. The median time to complete healing was 29 days in the NPWT group compared with 45 days in the control group ($P < 0.0001$).

## 6 Discussion

Despite the considerable amount of studies on the subject, there were few RCT. Patient characteristics, treatment methods and outcomes were very different. For these reasons, it was impossible to perform a meta-analysis. This issue has been addressed by the systematic review carried out by Peinemann [21], which resulted in a large number of unpublished RCTs. In fact, a lack of access to unpublished study represents a high risk of bias: on 28 RCTs present in literature, only 13 were published.

In 2015 three Cochrane reviews on the subject were published, but none of them had led to significant results.

1. Systematic Review

   "Negative pressure wound therapy for treating surgical wounds healing by secondary intention" [22]. Dumville et al. have

included only two RCTs and concluded: "There is currently no rigorous RCT evidence available regarding the clinical effectiveness of NPWT in the treatment of surgical wounds healing by secondary intention as defined in this review. The potential benefits and harms of using this treatment for this wound type remain largely uncertain."

2. Systematic Review

   "Negative pressure wound therapy for treating pressure ulcers" [23]. Dumville et al. have included only two RCTs and concluded: "There is currently no rigorous RCT evidence available regarding the effects of NPWT compared with alternatives for the treatment of pressure ulcers. High uncertainty remains about the potential benefits or harms, or both, of using this treatment for pressure ulcer management."

3. Systematic Review

   "Negative pressure wound therapy for treating leg ulcers" [16]. Dumville et al. have included only one RCT and concluded: "There is limited rigorous RCT evidence available concerning the clinical effectiveness of NPWT in the treatment of leg ulcers. There is some evidence that the treatment may reduce time to healing as part of a treatment that includes a punch skin graft transplant, however, the applicability of this finding may be limited by the very specific context in which NPWT was evaluated. There is no RCT evidence on the effectiveness of NPWT as a primary treatment for leg ulcers."

Our updated systematic reviews of the literature did not lead to any new conclusions. Our result is still a result of the bias of publications. In fact, from 2008 to now, very few articles have been published. It follows that at present our conclusions resemble those written by Peinemann and Sauerland in 2011 [24]: "Although NPWT may have a positive effect on wound healing, there is no proof that it is either superior or inferior to conventional wound treatment. Further RCTs of good methodological quality are required."

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# Use of Negative-Pressure Wound Therapy on Malignant Wounds

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## 1 Introduction

Malignant wounds occur when cancerous cells invade the epithelium and the supporting blood and lymph vessels, resulting in loss of vascularity, leading to tissue necrosis. They can be related to a primary cancer or secondary to cancer metastasis or seeding of malignant cells during surgery [1]. These wounds present a number of challenging management issues such as malodour, exudate, bleeding, and pain, and may be further complicated by excessive necrotic tissue, fistula or sinus formation, and extensive wound infection and/or hemorrhage [2, 3]. Current management is limited to control of symptoms and to improve quality of life; wound closure, while ideal, is typically not the goal of management as malignant wounds very rarely heal [4]. Furthermore, incidence of malignant wounds cannot be overlooked. It is estimated in international studies to be between 5 and 10% in patients treated for cancer [5]. A survey conducted in three different geographical regions of Switzerland reported the presence of malignant wounds to be 6.6% in patients with metastatic cancer [6].

The therapeutic potential of negative-pressure wound therapy (NPWT) is well documented in a wide variety of clinical scenarios. Despite the broadening clinical usage of NPWT, malignancy is often regarded as an absolute contraindication due to the risk of cancer cell proliferation. This belief is derived from studies on normal tissues where cellular differentiation, migration, and angiogenesis were observed after the application of NPWT [7]. It is important to note that no literature directly supports the hypothesis that NPWT expedites oncological progression. On the contrary, NPWT may in fact placate many of the difficult-to-manage symptoms of malignant wounds, as demonstrated in palliative literature [8, 9]. We present one patient with refractory metastatic colon cancer who developed a nonhealing malignant wound that was successfully closed with the help of NPWT in order to be eligible for further treatment. A staged reconstruction was performed utilizing two novel types of NPWT: NPWT with instillation of topical wound solutions with a dwell time (NPWTi-d) and closed-incision negative-pressure therapy (ciNPT). Skin closure remained intact on follow-up and the patient resumed oncologic treatment.

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## 2 Case Presentation

A previously healthy 44-year-old male was transferred to our institution for management of T4N0M1 adenocarcinoma of the colon which has reoccurred despite surgical debulking, multiregimen chemotherapy (FOLFOX, FOLFIRI), and local radiation. The patient presented with periumbilical pain and scant spontaneous purulent drainage from the surgical incision site. No attempt at definitive resection was undertaken because of the extensive nature of the recurrence and the presence of a significant soft-tissue infection in the area. NPWT was initially applied to his open wound but discontinued 2 days after discharge by a local wound care facility. Following recovery, the patient received further radiation therapy to the abdomen and was treated with regorafenib. During this period, his tumor burden remained stable but the abdominal wound persisted. Primary closure was then attempted but failed and the wound was managed with moist-to-dry dressings for approximately 1 year.

At that time, plastic surgery was consulted for wound closure with the primary intention of securing eligibility in a clinical trial for treatment of metastatic disease. The patient was ineligible for the trial while having an open wound. Examination of the abdomen revealed a chronic malignant wound measuring 10 cm by 7 cm in the epigastrium with a clean, granulating base despite gross tumor association (Fig. 1). The patient agreed to proceed with a staged reconstruction of the defect. Intraoperatively, the wound (Fig. 2) was debrided and irrigated with antibiotic solution to further remove biofilm from the wound bed. NPWTi-d was applied to the open wound and set to cycle every 4 h with instillation of Clorpactin followed by a 10-min dwell time. Pathological examination of the lateral and deep skin margins revealed necrotic squamous epithelium and soft tissue involved by poorly differentiated adenocarcinoma, consistent with colonic primary. Final reconstruction of the defect was performed 3 days later. Use of an advancement flap from the lateral chest wall allowed for primary closure. After skin closure, ciNPT was applied to offset any additional tension. The patient was discharged 4 days later with ciNPT in place for 1 week.

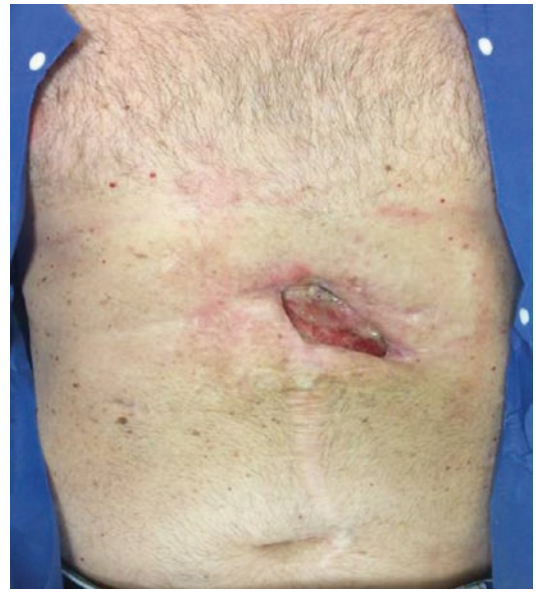

**Fig. 1** 44-year-old male with refractory metastatic colon cancer presents with an abdominal wound with gross tumor association. Plastic surgery was consulted for wound closure with the primary intention of securing patient's eligibility in a clinical trial for treatment of metastatic disease

At 1 month postoperatively, the patient's wound had healed and he was successfully enrolled in the clinical trial (Fig. 2). Despite a recovery complicated by a seroma that was treated with an intra-abdominal drain and antibiotics, skin closure remained intact at serial follow-ups (Fig. 3). The patient expired 6 months after wound closure due to progression of his metastatic disease. He was able to live without wound recurrence during that period.

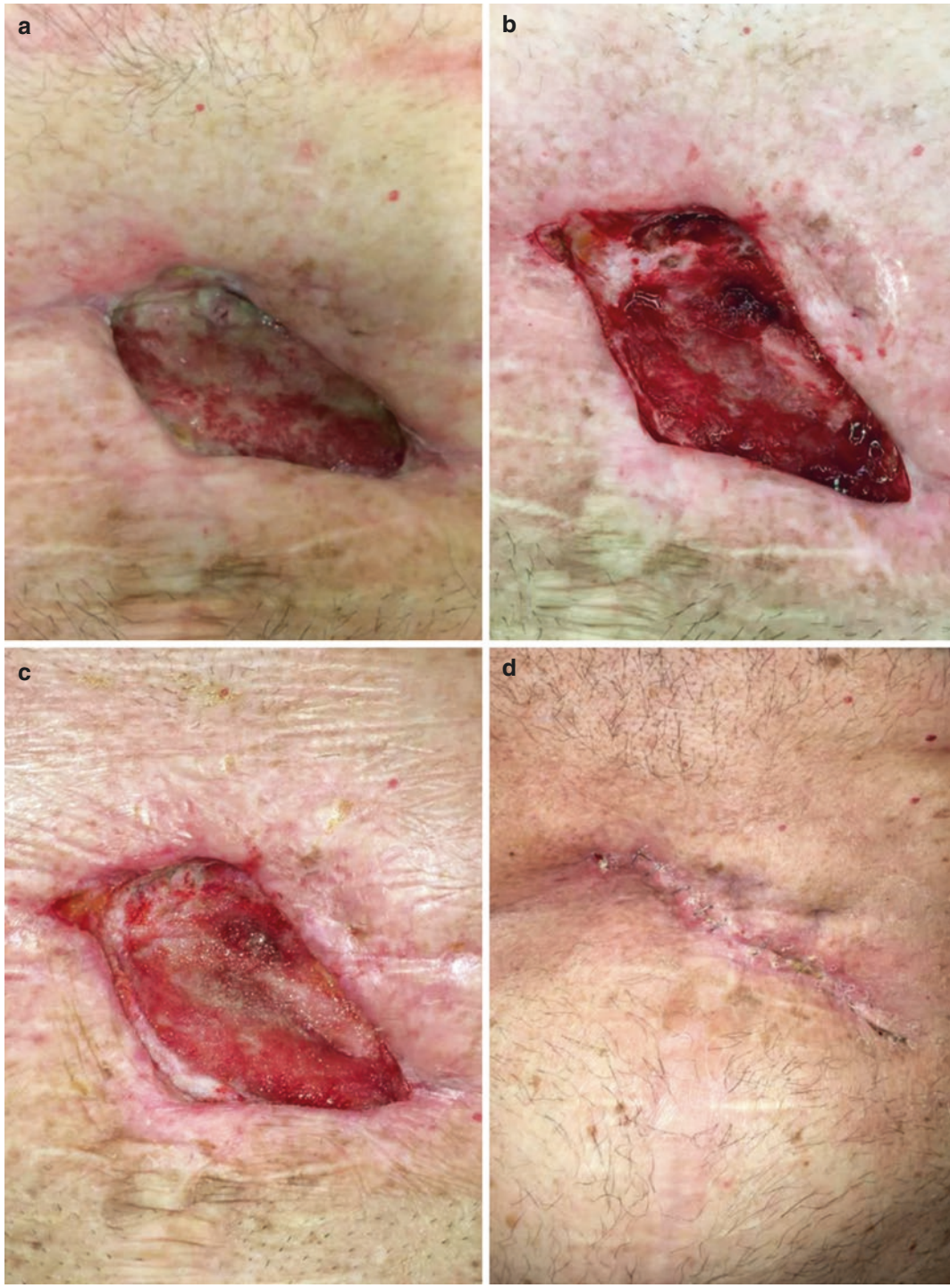


**Fig. 2** Staged reconstruction of the wound. (**a**) Preoperative mid-abdominal wound measuring 10 cm by 7 cm with a granulating base and gross tumor association. (**b**) Status post-extensive wound debridement and antiseptic irrigation. (**c**) Abdominal wound after the application of NPWTi-d for 3 days ready for closure. (**d**) One month postoperatively, skin closure remained intact and staples were removed at this time

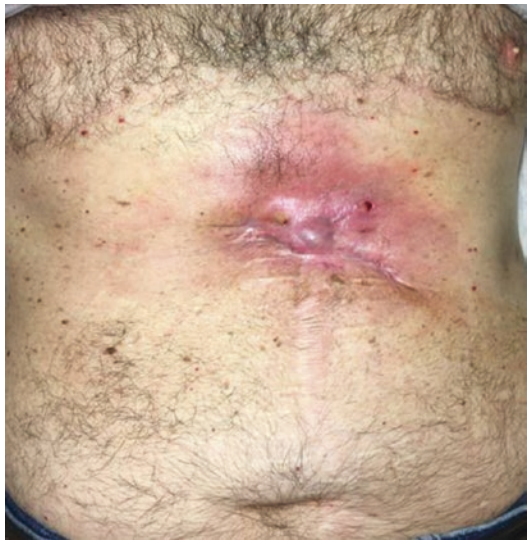

**Fig. 3** Patient's abdominal wound 3 months postoperatively. Skin closure remained intact and the patient successfully enrolled in a clinical trial

## 3 Discussion

Variations of NPWT, such as NPWTi-d and ciNPT, have shown promise in select situations. Instillation therapy combines the benefits of NPWT with cyclical cleansing of the wound bed in order to remove exudates and optimize tissue for closure. Benefits of NPWTi-d include shorter time to final surgical procedure, faster wound closure, and reduced hospital stay [10, 11]. Studies of high-risk, primarily closed surgical incisions have demonstrated reduced risk of infection, decreased development of postoperative seromas, and reduced rate of wound dehiscence for incisions treated with ciNPT [12, 13]. The benefit of ciNPT may be related to increased perfusion near the incision and improved lymphatic drainage [14].

The mechanisms by which NPWT accelerates wound healing are multifaceted and likely include facilitation of wound contraction by external suction, microcellular changes at the foam dressing-tissue interface, removal of excess fluid, and optimization of wound environment [7]. Angiogenesis is thought to be mediated by wound-site hypoxia and a subsequent stimulation of hypoxia-inducible factor-1α-VEGF pathway [15, 16]. Similarly, mechanical force of NPWT results in cell deformation which has shown to regulate cellular proliferation and migration [17, 18]. For these reasons, the same mechanisms by which NPWT promotes wound healing are argued to facilitate tumorigenesis.

One area of malignant wound care where NPWT has shown promise is in palliative settings, where the theoretical risk of increased malignant growth is a lesser concern. The earliest report described a patient with metastatic sigmoid carcinoma burdened by the malodorous pus from her malignant wound and the need for painful daily dressing changes [8]. A subsequent case series validated the use of NPWT in this clinical scenario. All patients reported decreased odor and exudates compared to conventional dressings, and fewer dressing changes that reduced pain and encouraged resumption of social activity. This study concluded that application of NPWT resulted in improved quality of life and earlier discharge to home [9].

Our case presents a unique application of NPWT in the curative setting as a mean to bridge the patient to further treatment. Our objective differs from that of palliative literature in that wound closure was the primary goal so the patient would be eligible to enroll in a clinical trial that may offer potentially life-prolonging treatments. However, malignant wounds rarely heal without assistance particularly in the setting of gross tumor association, extensive history of chemoradiation, high biofilm burden, and unsuccessful prior attempts at primary closure. For this patient, the addition of NPWT offered the best chance at wound closure. Initial application of NPWTi-d aims to decrease microbial biofilm, and ciNPT subsequently helps to offset tension on the incision with the added benefit of increased blood flow to promote long-term wound healing. Closure of an open abdominal wound in a cancer patient has added physiological and psychological benefits. An abdominal wound may result in fluid and protein loss, exposure of bowel leading to fistula, ventral hernia due to abdominal muscle retraction, and increased risk of abdominal wall or intra-abdominal infections. These complica-

tions may serve to worsen the patient's prognosis on top of an already bleak situation. Also, the psychological morbidity associated with the sight of an open wound has been shown to negatively alter the immune system and result in delayed wound healing [19]. In these respects, we believed that the use of NPWT in this patient was justified.

Despite the lack of evidence to support that NPWT promotes local malignant growth and early morbidity or mortality associated with malignancy, the belief that NPWT is contraindicated in malignant wounds prevails among many physicians and wound care providers. Our case highlights the problematic nature of this dogmatic approach to malignant wound care. Following the application of NPWT by the surgical oncologist, NPWT was prematurely removed at a local wound care facility 2 days later. The early termination of NPWT likely prevented wound healing. Not only did this prolong patient suffering but also potentially delayed enrollment in a clinical trial by several months.

Admittedly, there is robust evidence to suggest that NPWT promotes cellular division and angiogenesis in normal tissue; however it remains unclear what effect NPWT has on malignant tissue. Unfortunately for our patient, progression of disease was unknown after wound closure in the absence of subsequent imaging. If NPWT is found to play a role in tumorigenesis, the magnitude and time course of this effect should be delineated. Short-term application of NPWT may be acceptable and may not significantly alter tumor burden. Additionally, it is unknown if systemic chemotherapy or immunotherapy impacts any potential tumorigenic consequences of NPWT. It is possible that adjuvant systemic therapy may attenuate any tumorigenic effect from NPWT.

## Conclusions

The perception that NPWT is absolutely contraindicated in malignant wounds should be called into question. NPWT has shown to be effective in controlling malodor, exudate, pain, and bleeding in the palliative setting when the potential for malignant growth is of less concern. Our experience demonstrated the successful closure of a malignant wound using a combination of novel NPWTs. There may be a potential role for NPWT in cancer patients who plan to receive further oncologic treatment. The use of NPWT in malignant wounds should be individualized on a case-by-case basis with careful consideration of its risks and benefits, the patient's expectation, and future treatment plan.

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# Combined Approach to Severe Fournier's Gangrene with Negative Pressure Wound Therapy, Dermal Regeneration, and Split-Thickness Skin Graft

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## 1 Introduction

Fournier's gangrene (FG) is an expansive and necrotic acute bacterial infection of soft tissue of the perineum, the scrotum, and the lower part of the trunk [1]. In half the cases, it is a polymicrobial infection. The most important germs are *Escherichia coli*, *Streptococcus* spp., *Staphylococcus aureus*, *Enterococcus* spp., and *Pseudomonas aeruginosa* [2, 3]. The main predisposing factors include diabetes mellitus, severe obesity, prolonged hospitalization, hematologic illnesses, LES, immunosuppression, drug use, alcoholism, poor personal hygiene, and malnutrition [1–12]. Sometimes it may occur as a result of surgical sclerotherapy and hemorrhoidectomy and hemorrhoids [11]. Fournier's gangrene mortality is 20–50%. Clinically, the most specific sign of Fournier's syndrome is acute pain [10]. The gangrene begins as one small wound adjacent to the entry point of the microorganism and advancing to the fascia [9]. At first there is a proliferation of anaerobic microorganisms favored by low oxygen in the tissue necrotic [1, 2]. At the inspection it is presented with pale, shiny, edema-like skin. There is palpation touch cracking, cyanosis, vesicle formation, and hardening of the skin until it reaches the true gangrene. The pain appears very early and then tends to gradually disappear for the cutaneous nerve damage. Systemic symptoms are fever, tachycardia, and hypotension. The laboratory diagnosis is characterized by leukocytosis (WBC > 15,000), impaired balance hydroelectrolyte and base acid, clotting disorders, platelet reduction, hypocalcemia, hypoalbuminemia, anemia, and increased onset and creatinine [1–9]. The bacteriological diagnosis is crucial to setting up targeted antibiotic therapy. CT scan and Nuclear magnetic risonace are the only diagnostic exams to establish the extent and severity of lesions [1]. The therapeutic management of Fournier's gangrene therefore requires a multidisciplinary approach that involves the anestesiologists, radiologists, urologists, infectious disease specialists, general surgeons, and plastic surgeons. Depending on the degree of systemic impairment of the organism and the possible presence of comorbidity (e.g., diabetes, obesity), there are four classes of severity: (1) no comorbidity and systemic deterioration, (2) presence of comorbidities that may delay healing, (3) presence of SIRS (fever, tachypnea, tachycardia, and/or hypotension), and (4) severe sepsis, with a risk of survival. FG may involve, in isolation or in combination, superficial layers

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(dermis, subcutaneous cells) (necrotizing cellulite), the fascial plane (necrotizing fasciitis (NF)), or the muscular logs (necrotizing myositis). Specifically, NF is characterized by a rapid evolution of necrosis of peripheral and fascial structures, sustained by generally polymicrobial infections [5–18].

## 2 Etiology and Classification

From the microbiological point of view, three types of FG are classified. Bacterial classification is clinically important, as different germs can cause different clinical manifestations, affect populations of dissimilar patients, and have variable therapeutic implications [1–9].

### 2.1 Type 1

Polymicrobial infections are supported by a variety of Gram-positive germs (*Staphylococcus* spp., *Enterococcus* spp.) and Gram-negative (*E. coli*), as well as *Clostridium*. It affects the torso and the perineum. Patients are generally elderly, suffering from comorbidity (e.g., diabetes); rarely the history is positive for trauma. Clostridium infections (*Clostridium perfringens*, *C. septicum*, *C. sordellii*), known as gangrene, are rare but heavily burdened by the rapid evolution of clinical manifestations. They may become symptomatic within a few hours of the inoculum of the pathogen and typically manifest with disproportionate pain with respect to the extent of the lesions. Edema and the presence of gases in soft tissues may not be immediately apparent, as well as *Clostridium* isolation in media may be difficult. They are typical infections of drug abuse.

### 2.2 Type 2

Monomicrobial infections are supported by β-hemolytic Group A streptococci (GAS), isolated or in association with *Staphylococcus*. They mainly deal with young people without comorbidity, with a positive history of trauma, surgery, or drug abuse IV. The severity of type 2 is due, in addition to the release of the inflammatory mediators mentioned above, also to the release of exotoxins by that can reduce the bactericidal power of neutrophils, limit phagocytosis, and damage the structure of hyaluronic acid of the connective tissue.

### 2.3 Type 3

Monomicrobial infections are due to Gram-positive or Gram-negative germs such as *Vibrio*, *Clostridia*, *Eikenella*, and *Aeromonas*. The infection can be contracted through continuous trapping solutions or by ingestion of contaminated pathogenic crustaceans. Clinical progression is rapidly evolving, with signs of systemic toxicity early, even in the absence of clinically evident skin lesions.

In the immunocompromised patient (e.g., in cortisone, neoplastic, immunodeficiency syndrome, immunosuppressive therapy), clinical manifestations of FG may be atypical but particularly aggressive. Typically leukopenia, hypoglycemia, and hypotension are present. Mortality is twice that of immunocompetent subjects. Pathogens can also be represented by mycetes [6–8].

## 3 Diagnosis

The diagnosis of FG is initially clinical, mediated by the detection of signs and symptoms such as constant intense pain blisters, skin necrosis, subcutaneous emphysema, edema surrounding erythematous areas, skin anesthesia, signs of systemic toxicity (fever, organ damage), and progress of manifestations despite antibiotics that typically evolve within a few hours of their onset [13–21]. Some of these (pain disproportionate to the extent of skin manifestations, hard edema, erythema, vesicles, emphysema, skin pallor) are considered "strong signs." Their absence, however, does not rule out the diagnosis of FG [5]. Laboratory data alterations may be nonspecific. Countless scores were elaborated to facilitate the

**Table 1** LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis)

| Parameter | Value | Score |
|---|---|---|
| PCR (mg/dL) | <150 | 0 |
| | >150 | 4 |
| White Globes ($mm^3$) | <15 | 0 |
| | 15–25 | 1 |
| | >25 | 2 |
| Hemoglobin (g/dL) | <13.5 | 0 |
| | 11–13.5 | 1 |
| | <11 | 2 |
| Sodium (mmol/L) | >135 | 0 |
| | <135 | 2 |
| Cretinine (μmol/L) | <141 | 0 |
| | >141 | 2 |
| Glucose (mmol/L) | <10 | 0 |
| | >10 | 1 |

diagnosis of FG [20]. The most commonly used is the LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score (Table 1). A score of LRINEC ≥6 has been shown to have a positive predictive value (PPV) of 92% and a negative predictive value (NPV) of 96%. However, it must be interpreted with caution, since the parameters used for calculation can also be modified by other causes of systemic inflammation of the body. The only LRINEC score is not enough for the diagnosis of FG [1–19].

### 3.1 Traditional Radiology

Common x-rays of body segments with signs of potential FG clinicians may reveal unspecific pads, such as soft tissue thickening or radiopathic areas. However, they may be normal, even in the form of advanced FG. It should also be considered that the presence of air in soft tissues is a rare find and virtually absent if the pathogen is a compulsory aerobic.

### 3.2 Ultrasound

Ultrasonic diagnostics represents the point-of-care method for the patient's bed if the latter is severely unstable so that it cannot be subjected to more thorough investigations through computerized tomography or magnetic resonance imaging. It has been shown that ultrasound has a sensitivity of 88.2%, 93.3% specificity. Ultrasound thickening of the subcutaneous and the presence of fluid film >4 mm thick along the fascial plane are considered ultrasound signs of FG.

### 3.3 Computerized Tomography (CT)

CT has 100% sensitivity and 81% specificity, for FG diagnosis. CT pathognomonic signs are represented by inhomogeneity of the adipose tissue, presence of collagen and gas in the soft tissues along the fascia planes, thickening of the bands, and lack of contrast impregnation of the same.

Magnetic Resonance (RMN). RMN has 100% sensitivity, 86% specificity, and 94% accuracy for FG diagnosis. The limit of this method can be represented by an overestimation of the degree of involvement of the beam structures. Pathognomonic signs are thickening >3 mm of the fascial plane and reduction of signal intensity in the T2-weighed sequences.

### 3.4 Biopsy/Finger Test

The fascial tissue biopsy analyzed at the freezer can be a method for rapid diagnosis of FG. Another technique is the finger test, conducted in local anesthesia, by an incision of about 2 cm of skin and subcutaneous. If the introduction of the finger beneath the subcutaneous causes an easy disengagement of the same from the band, as well as the discharge of fluid (dishwater fluid) and the absence of bleeding from the cruelty structures, the test is positive and the diagnosis of FG certain.

## 4 Principles of Treatment

Fournier's gangrene is a condition at risk for the patient's survival due to the rapid extension of the lesions and the resulting systemic compromise. The rapid evolution of clinical manifestations is attributable to the production of toxins and

inflammatory molecules by pathogens, which can cause tissue damage, ischemia, and necrosis [1–21]. In particular, the effect of such toxins is stimulation of macrophages with the consequent production of TNF-α, IL-1, and IL-6. At systemic level, the effect of such cytokines is the activation of the body's inflammatory response (SIRS), which in the most serious forms may evolve in sepsis, multi-organ failure (MOF), until death [16]. At the tissue level, degranulation of neutrophils and the release of oxygen radicals result in endothelial damage, which combined with the activation of T cells by the superantigens released from the bacteria and the activation of the complement induce microcirculation thrombosis with further ischemia and tissue damage. A delay in diagnosis and treatment significantly increases mortality [18]. Fournier's gangrene (FG) is a surgical emergency.

In accordance with WSES guidelines (World Society of Emergency Surgery), the overwhelming of treatment consists of a large debridement of necrotic tissues, together with adequate antibiotic therapy and support for vital functions (recommendation 14, Grade 1C) [16]. Surgical incision must include the area affected by the infectious process (the surface of the same may be very wide) and be extended to meet healthy and bloody tissue. Samples of fluid and fragments of tissue to be subjected to culture examination should be taken during surgical intervention. Surgical revision should be scheduled every 24–48 h, until complete reclamation of devitalized tissues [16]. In the patient with FG, unable to be sure at the start of responsible pathogens, early spectrum empirical antibiotic therapy should be initiated early to cover Gram-negative, Gram-positive, aerobic, and anaerobic germs (recommendation 20, Grade 1C) [16]. A cover against methicillin-resistant staphylococci (MRSA) should be provided. If there is evidence of Gram-positive germs, antibiotic therapy should be enhanced with anti-ribosomal drugs (clindamycin/linezolid) [16–18]. If there is evidence of Gram-negative germs, it is necessary to introduce tetracyclines (recommendation 18, Grade 1C) [16]. Antibiogram must be obtained quickly in order to set targeted antibiotic therapy by de-escalating the drugs within the first 24–48 h (recommendation 21, Grade 1C).

It is essential to obtain a diversion of the fecal transit. This can be done by packing a colostomy or resorting to rectal probes that can protect the soft tissues from further contamination [14–21]. The dressings of the surgical site, pending final closure, must be made with nonadhesive materials, possibly with antiseptic substances. Once complete necrotic tissue removal is achieved, negative pressure dressing systems can be applied, which facilitate removal of secretions by promoting tissue granulation [15–21]. In the case of small-size masked surfaces, the final closure can be done by direct suture of the wound margins. In case of major defects, it is necessary to use skin grafts, flaps, or skin substitutes [21]. Nowadays extensive debridement can be primarily repaired with dermal substitutes, thus avoiding multistaged debridement [18–21]. In addition, incorporation of the dermal substitute can be accelerated by subatmospheric pressure, with improved take rate and fewer complications, especially when used in concave and circumferential areas of the body such as the perineum [17]. Moreover, the VAC (vacuum-assisted closure) device evacuates wound secretions and blood, thus lowering the risk of seroma, hematoma, and infection and shortening the time necessary for engraftment. Functional and cosmetic results have been superior to those obtained with skin grafts alone, which may result in impaired knee motility [20]. Indeed split-thickness skin grafts alone offer poor cosmetic and functional outcome, but on the other hand, they have good take since they allow drainage, thus preventing seroma, hematoma, and infection especially when meshed. On this matter timely application of the VAC therapy and subsequent use of dermal regeneration template play a crucial role to prevent irregular surface both to graft take and aesthetic result and complications as infection or hematoma [18]. In this background the use of dermal regeneration template avoids the contour disfigurement at the junction between healthy tissue and the grafted area attributed to the discrepancy of thickness of the subcutaneous layer around the junction following debulking [19].

Bladder, rectum, and testicles are usually spared since equipped by proper vascular supply different from that supplying the perineum. Indeed the tissue involved by FG receives branches from the pudendal plexus, while testicles are supplied by the testicular arteries from the abdominal aorta. Despite the different blood supply, testicles are involved in 21% of cases managed by monolateral orchiectomy. If, during surgical debridement, one or both testicles are involved by necrosis, explorative laparotomy is indicated to investigate possible thrombosis of the testicular arteries. In cases where testicles are spared, it is necessary to preserve them in the subcutaneous abdominal tissue waiting for complete wound healing and further delayed reconstruction [1–16].

## 5 Adjuvant Therapy

In the patient with FG, hemodynamic and metabolic support must be aggressive and premature in order to limit the effects of the inflammatory response of the body (recommendation 22, Grade 1A) [16]. The loss of fluids from the areas under attack may be of considerable magnitude, as well as the relative hypovolemia for vasodilatation induced by inflammation mediators. In the early stages of resuscitation, the use of crystalloids is recommended. The hemodynamic targets are represented by a mean arterial pressure (MAP) >65 mmHg, a central venous pressure (CVP) of 8–12 mmHg in combination with a central venous central blood oxygen saturation ($ScvO_2$) >70%, and an output urine >0.5 mL/kg/h [16]. In the patient with FG from *Staphylococcus* or *Streptococcus*, sepsis or septic shock is indicated by intravenous administration of immunoglobulins (recommendation 23, Grade 2C), as these seem to be able to bind the toxins produced by the pathogens and limit the inflammatory response [16–19]. Nutritional support must be initiated early (recommendation 24, Grade 2C), in order to counteract the catabolism triggered by the inflammatory response of the organism [16]. The recommended caloric intake is 25 kcal/kg/day for the first week to increase to 30–35 kcal/kg/day in the following weeks. Administration of enteral nutrients (EN) is preferred, reserving parenteral nutrition in case of contraindications or impossibility to perform EN. Pain control due to FG or accentuated during surgical maneuvers and dressings must be controlled by the administration of opioids in combination with nonsteroidal anti-inflammatory drugs as well as anxiolytics [1–7].

### Conclusions

Vacuum-assisted closure and the dermal regeneration template represent useful tools in reconstruction of the perineum following extensive debulking and can be considered as a valid alternative to immediate skin grafting to cover large defects, yielding excellent aesthetic and functional results. It is possible to obtain durable skin replacement of good pliability, which is not the case for split-thickness skin grafts alone, which are less durable and may result in significant contractures that may interfere with joint motility. Compared with direct grafting, this approach provides improved cosmetic results and less contracture, sparing the patient from additional scarring during graft harvesting. This reconstructive scale should be considered for the treatment of extensive debulking for patients suffering FG [18–21].

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# Negative Pressure Wound Therapy to Decrease Surgical Nosocomial Events in Colorectal Resections

Mei Lucy Yang and Michael Ott

## 1 Introduction

Surgical site infections (SSIs) cause significant morbidity to the colorectal surgery patient population. Infection rates are higher given the potential of contamination with gastrointestinal bacteria. SSIs are associated with further morbidity including prolonged hospital stay and higher risk for incisional hernias [1]. Rates of SSI in surgery range from 3 to 38%, with colorectal surgery at the higher end with reports of SSIs up to 45% [1–10]. The Surgical Care Improvement Project aimed to reduce SSI incidence and recommended a number of prophylactic measures including prophylactic IV antibiotics within 1 h of skin incision, appropriate antibiotic selection, discontinuing antibiotics within 24 h after surgery, appropriate hair clipping, perioperative normothermia, and strict perioperative glucose control in diabetic patients [11]. Other trials have demonstrated moderate improvements with the use of subcutaneous drains or wound protectors [1]. Laparoscopic surgery has been shown to decrease SSI incidence, but a large proportion of colorectal patients still require open laparotomies [1]. Therefore, despite all these prophylactic measures, SSI remains to be a pertinent issue that needs to be addressed.

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## 2 Negative Pressure Wound Therapy

Negative pressure wound therapy (NPWT) is used to accelerate wound healing in large open wounds or infected wounds by secondary intention. NPWT consists of a sterile sponge placed within the wound and attached to an external negative pressure device; the sterile sponge can also be placed outside a closed wound in incisional NPWT. The pressure applied causes a vacuum effect and removes fluid soaked in by the sponge. It also transmits mechanical forces to draw the surrounding tissue closer together. The sponge allows for equal transmission of pressure and force throughout the dressing. Currently, NPWT is indicated for open abdominal wounds, sternal wounds, soft tissue defects, skin graft fixation, fasciotomy wounds after compartment release, and burns [4, 12].

### 2.1 Mechanism of Action

There are a number of proposed mechanisms of action of NPWT. The first and most obvious benefit of this vacuum dressing is that it seals the incision in a sterile environment and prevents contamination [9, 13, 14]. The negative pressure

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allows consistent fluid removal, which decreases seroma/hematoma formation and pooling of fluid or blood that could become a culture medium for bacteria [9, 13]. Furthermore, studies have shown that the dressing and suction decreases lateral tissue tension and helps with tissue apposition [9, 14]. There is also preliminary evidence that NPWT increases blood flow to the tissue directly below [13]. This indicates better perfusion which stimulates healing and tissue granulation [13].

## 2.2 Incisional Negative Pressure Wound Therapy (iNPWT)

Incisional negative pressure wound therapy (iNPWT)—the utilization of NPWT as a mechanism to decrease SSI after wound closure is a relatively new concept. Incisional NPWT first emerged in the orthopedic trauma literature and was described by Gomoll et al. [12] in a case series of orthopedic patients at high risk for wound infection. In this series, they observed that iNPWT made a substantial difference in the postoperative wound care and zero out of 35 patients developed wound infections (follow-up 3 months). Since then, a number of surgeon investigators have also studied the effect of iNPWT on SSI in different patient populations. In 2013, Bonds et al. [1], Blackham et al. [3], and Matatov et al. [15] independently published three separate retrospective reviews analyzing the difference in SSI of patients who had standard dressing versus iNPWT. The patient population included general surgery patients undergoing open colectomies; surgical oncology patients undergoing laparotomy for colorectal, pancreatic, or peritoneal surface malignancies; and vascular patients with groin incisions [1, 3, 15]. All three studies demonstrated significantly decreased incidences of SSI in the iNPWT group (12.5% vs. 29.3%, $P = 0.036$; 6.7% vs. 19.5%, $P = 0.015$; 3% vs. 30%, $P = 0.0011$). In 2014, Chadi et al. [16] demonstrated significantly decreased SSI rate in patients who underwent abdominal perineal resection with iNPWT (15% vs. 41%, $P = 0.04$). In 2016, Swanson et al. [17] conducted a systematic review and meta-analysis on the effect of iNPWT after ventral hernia repair (VHR). They identified five observational comparative studies that analyzed rates of SSI, wound dehiscence, seroma formation, and hernia recurrence in VHR patients with standard dressing vs. iNPWT. Not only was there a significantly lower incidence of SSI in the iNPWT group (11.8% vs. 27%, $P < 0.0001$), iNPWT was also associated with less wound dehiscence (4.3% vs. 19.7%, $P = 0.001$) and lower hernia recurrence (2.4% vs. 10.1%, $P = 0.01$) [17].

## 2.3 Current Evidence

There are only two published randomized controlled trials (RCT) looking at the effect of iNPWT, both published in 2017. The studies demonstrate opposing findings. O'Leary et al. [7] conducted an open-label RCT of adult patients undergoing either elective or emergency laparotomy. Wound classes I (clean), II (clean-contaminated), and III (contaminated) were included in this trial. They had a small number of participants—24 in the treatment group, 25 in the control group. Their primary outcome was 30-day SSI rate, which was significantly lower in the treatment group (8.3% vs. 32%, $P = 0.043$) [7]. Contrarily, Shen et al. [9] conducted a phase II RCT of surgical oncology patients undergoing laparotomy for bowel resection, pancreatectomy, or HIPEC for peritoneal surface malignancy. Only wound class II (clean-contaminated) patients were included. A total of 132 patients were analyzed in the treatment group and 133 in the placebo group. In this study, they found absolutely no difference in overall SSI, superficial SSI, deep SSI, or organ/space infection (15.9% vs. 15.8%, $P > 0.99$; 12.9% vs. 12.8%, $P > 0.99$; 3.0% vs. 3.0%, $P > 0.99$; 3.8% vs. 5.3%, $P = 0.77$) [9]. From this trial, the evidence does not support routine iNPWT, at least for class II wounds. There are notable differences between these two trials—the patient population, wound class, and power of study. Any of which may contribute to the difference in end results.

**Table 1** Patient-related risk factors for wound complications [14]

| Patient-related risk factors |
|---|
| Diabetes mellitus[a] |
| ASA score ≥ 3 |
| Advanced age |
| Obesity BMI > 30[a] |
| Active tobacco use[a] |
| Hypoalbuminemia |
| Corticosteroid usage |
| Active alcoholism |
| Male |
| Chronic renal insufficiency |
| Chronic obstructive pulmonary disease |
| Hematoma |

[a]Indicates most commonly noted risk factors seen in literature

**Table 2** Surgery-related risk factors for wound complications [14]

| Surgery-related risk factors |
|---|
| High tension incision |
| Repeated incisions |
| Extensive undermining |
| Traumatized soft tissue |
| Edema |
| Contamination |
| Emergency procedure |
| Mechanically unfavorable site |
| Prolonged operation time[a] |
| Postsurgical radiation |

[a]Indicates most commonly noted risk factors seen in literature

There is one more RCT by Chadi et al. [18] that is currently undergoing. The patient population is colorectal surgery patients undergoing colorectal resections via laparotomy. Primary outcome is the incidence of SSI within 30 days of surgery. The study plans to recruit 300 patients total, 150 in the control group and 150 in the therapeutic group. Because all the patients are undergoing colorectal resection, the wound class is at least II. Results from this trial have yet to be published; however, it may help shed a new light on the recent contradicting results of the previous RCTs.

In 2017, Willy et al. [14] published international multidisciplinary consensus recommendations on iNPWT. Twelve international experts attended a multidisciplinary consensus meeting and developed consensus recommendations after detailed literature review. In this document, they identified 12 patient-related risk factors (Table 1) and 10 surgery-related risk factors (Table 2) for wound complications. Currently, there are no guidelines or rules designating how many risk factors are needed before one should consider using iNPWT. Both the authors and the international multidisciplinary consensus recommend that surgeons use their clinical judgment and experience. If a patient has one or more risk factors listed below, surgeons should consider using iNPWT as a prophylactic measure to decrease chances of wound complications. There has not been any cost-benefit analysis conducted on iNPWT to this date.

## 2.4 Technique

Pre- and perioperative principles for preventing SSIs still apply. Patients should receive preoperative antibiotics within 1 h of surgery. Additional doses of antibiotics should be given if the operation extends beyond the half-life of the initial antibiotic. The abdomen should be thoroughly prepped with 2% chlorhexidine before initiation of surgery. The skin can be closed with staplers or subcutaneous absorbable sutures. The incision should be cleaned and dried thoroughly while maintaining sterile technique. At this point, if the surgeons are in the practice of double gloving, the authors recommend taking off the outer gloves and proceed with the clean inner gloves. A piece of Adaptic (Johnson & Johnson Wound Management) or any other nonadhesive but permeable dressing should be placed over the wound. This is to cover the skin directly underneath the sponge to prevent dermal irritation from the appliance. A piece of sponge should be cut to precisely just cover the incision, with approximately 1 in. of foam on either side of the incision. The sponge is then secured in place with occlusive adhesive dressing. It is important to ensure that the adhesive dressing is completely stuck to the skin and that there is no leak. This is why the skin must be dried well with sterile gauze before application. Finally, make a cut over the sponge and attach the suction tubing.

The vacuum can be set to either 75 or 125 mmHg to work effectively [1, 3, 12, 15–17, 19]. There has

not been evidence to suggest adverse effects from either setting. It is the authors' practice to set the vacuum to 125 mmHg to maximize the effect of iNPWT. If there is evidence of skin irritation, blistering, maceration, necrosis, or pain, then suction can be turned down to 75 mmHg or the dressing can be taken off. In studies analyzing complications of iNPWT, only two patients experienced blistering of the skin due to adhesives, which resolved after NPWT removal [19]. There were no reports of pain or discomfort related to iNPWT at continuously high pressures [20]. In fact, the iNPWT dressing lowered patient anxiety and decreased pain and discomfort of frequent dressing changes.

There have not been any studies to demonstrate the optimal length of time to leave the iNPWT dressing. Historically, it has been left on from a range of 4–7 days [1, 3, 10, 12, 15–17]. The authors' current practice is to leave the dressing on for 5 days or until the day of discharge, whichever is first.

### Conclusions

Incisional NPWT is likely beneficial in decreasing SSI in high-risk colorectal surgery patients undergoing bowel resection. Patients with additional risk factors for wound infection such as diabetes, chronic smoking status, immunocompromised, and obesity should be considered for iNPWT. Overall, iNPWT is very low risk to the patient, and most evidence suggests lower rates of infection. Further investigations are warranted to assess the cost-benefit of iNPWT, optimal vacuum setting, and optimal duration of dressing placement.

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