

Advanced Therapeutic Dressings For Effective Wound Healing

Joshua Boateng^{1*#}, Ovidio Catanzano^{1#}

¹Department of Pharmaceutical, Chemical and Environmental Sciences, Faculty of Engineering and Science, University of Greenwich, Medway, Central Avenue, Chatham Maritime, Kent, UK, ME4 4TB

*Correspondence: Dr Joshua Boateng (J.S.Boateng@gre.ac.uk, joshboat40@gmail.com)

#Boateng and Catanzano are Joint First Authors

ABSTRACT

Advanced therapeutic dressings that take active part in wound healing to achieve rapid and complete healing of chronic wounds is of current research interest. There is a desire for novel strategies to achieve expeditious wound healing due to the enormous financial burden worldwide. This paper reviews the current state of wound healing and wound management products, with emphasis on the demand for more advanced forms of wound therapy and some of the current challenges and driving forces behind this demand. The paper reviews information mainly from peer reviewed literature and other publicly available sources such as the FDA. A major focus is the treatment of chronic wounds including amputations, diabetic and leg ulcers, pressure sores, surgical and traumatic wounds (e.g. accidents and burns) where patient immunity is low and the risk of infections and complications are high. The main dressings include medicated moist dressings, tissue engineered substitutes, biomaterials based biological dressings, biological and naturally derived dressings, medicated sutures and various combinations of the above classes. Finally, the review briefly discusses possible prospects of advanced wound healing including some of the emerging approaches such as hyperbaric oxygen, negative pressure wound therapy and laser wound healing, in routine clinical care.

1 INTRODUCTION

1.1 Overview

Wound healing is a global medical concern with several challenges including the increasing incidence of obesity and type II diabetes, an ageing population (especially in developed countries with low birth rates) and the requirement for more effective but also cost effective dressings.¹ Wound healing is a complex process involving several inter-related biological and molecular activities for achieving tissue regeneration. The main physiological events include coagulation, inflammation and removal of damaged matrix components, followed by cellular proliferation and migration, angiogenesis, matrix synthesis and deposition, re-epithelialization and remodeling.² These are generally classified into five major phases known as hemostasis, inflammation, proliferation, migration and remodeling/maturation.¹ Wound healing and the different phases involved have been extensively discussed in several reviews and textbooks and the reader is referred to these for detailed exposition on the molecular and physiological basis of the different stages of wound healing.¹⁻⁹

1.2 Wounds

A wound can be defined as an injury or disruption to anatomical structure and function resulting from simple or severe break in the skin and can extend to other tissues and structures such as subcutaneous tissue, muscles, tendons, nerves, vessels and even to the bone.^{1,9,10} Of all the body tissues, the skin is definitely the most exposed to damage and easily prone to injury, abrasions and burns due to trauma or surgery. The rapid restoration of homeostatic physiological conditions is a prerequisite for complete lesion repair, because a slow and incorrect repair can cause serious damages including the loss of skin, hair and glands, onset of infection, occurrence of skin diseases, injuries to the circulatory system and, in severe cases, death of the tissue.

Based on the nature of the repair process, wounds can be classified as acute or chronic wounds. Acute wounds are usually tissue injuries that heal completely, with minimal scarring, within the expected time frame, usually 8–12 weeks.¹¹ The primary causes of acute wounds include mechanical injuries due to external factors such as abrasions and tears, which are caused by frictional contact between the skin and hard surfaces. Mechanical injuries also include penetrating wounds caused by knives and gunshots and surgical wounds caused by

incisions, for example to remove tumors. Another category of acute wounds includes burns and chemical injuries, which arise from a variety of sources such as radiation, electricity, corrosive chemicals and thermal sources. Chronic wounds, on the other hand, arise from tissue injuries that heal slowly that have not healed in 12 weeks and often reoccur.⁵ Chronic wounds are often heavily contaminated and usually involve significant tissue loss that can affect vital structures such as bones, joints and nerves. Such wounds fail to heal due to repeated trauma to the injured area or underlying physiological conditions such as diabetes, persistent infections, poor primary treatment and other patient related factors.¹² These result in a disruption of the orderly sequence of events during the wound healing process.^{5,13,14} Furthermore, impaired wound healing can lead to an excessive production of exudates that can cause maceration of healthy skin tissue around the wound.¹⁵

Wounds are also characterized based on the number of skin layers and area of skin affected.¹⁶ Injury that affects the epidermal skin surface alone is referred to as a superficial wound, whilst injury involving both the epidermis and the deeper dermal layers, including blood vessels, sweat glands and hair follicles is referred to as partial thickness wound. Full thickness wounds occur when the underlying subcutaneous fat or deeper tissues are damaged in addition to the epidermis and dermal layers. Ferreira et al.¹⁷ have described both acute and chronic wounds that are difficult to heal as ‘complex wounds’ with unique characteristics which can be summarized as extensive loss of the integument which comprises skin, hair, and associated glands; infection (e.g. Fournier’s gangrene) which may result in tissue loss; tissue death or signs of circulation impairment and presence of underlying pathology.

Nawaz and Bentley,⁷ have described some of the factors that contribute towards retardation in wound healing (chronic wounds) which are summarized in table 1 below. Common chronic skin and soft tissue wounds can be divided into three major groups due to similarities in their pathogenesis. These are leg ulcers (of venous, ischemic or of traumatic origin), diabetic foot ulcers, and pressure ulcers.¹⁸ It also includes other hard-to-heal acute wounds such as wounds caused by cancer, pyoderma gangrenosum, immunologic and hematologic wounds,¹⁹ amputations, abdominal wounds, burns and skin grafts.²⁰ In recent years, other more serious forms of chronic wounds such as buruli ulcer, caused by bacterial infection which involves significant skin tissue loss, have been reported.^{21,22}

Table 1. Local and systemic factors that slow down wound healing.⁷

Local factors	Systemic factors
<ul style="list-style-type: none">• Inadequate blood supply	<ul style="list-style-type: none">• Shock
<ul style="list-style-type: none">• Wound dehiscence	<ul style="list-style-type: none">• Chronic renal and hepatic failure
<ul style="list-style-type: none">• Infection	<ul style="list-style-type: none">• Advancing physiological age
<ul style="list-style-type: none">• Excess local mobility, such as over a joint	<ul style="list-style-type: none">• Obesity
<ul style="list-style-type: none">• Poor surgical apposition or technique	<ul style="list-style-type: none">• Smoking
<ul style="list-style-type: none">• Increased skin tension	<ul style="list-style-type: none">• Chemotherapy and radiotherapy
<ul style="list-style-type: none">• Topical medicines	<ul style="list-style-type: none">• Diabetes mellitus
<ul style="list-style-type: none">• Poor venous drainage	<ul style="list-style-type: none">• Systemic malignancy
<ul style="list-style-type: none">• Presence of foreign body or foreign body reactions	<ul style="list-style-type: none">• Immuno suppressants, anticoagulants, cortico steroids
<ul style="list-style-type: none">• Hematoma	<ul style="list-style-type: none">• Vitamin and trace elements deficiency

Venous leg ulcers are triggered by malfunction of venous valves causing venous hypertension in the crural veins (veins supplying the leg), which increases the pressure in capillaries and results in edema. Venous pressure exceeding 45 mmHg certainly leads to development of a venous leg ulcer. Diabetic foot ulcer is triggered by monotonous load on the neuropathic and often ischemic foot while pressure ulcers are caused by sustained or repetitive load on often vulnerable areas such as the sciatic (spinal nerve roots), tuberculum, sacral area, heels, and shoulders in the immobilized patient.²³ Patients with chronic ulcers usually present with underlying complicated factors caused by immunological defects, dysfunction in diabetic fibroblasts and the effect of local infection or critical colonization and disruptive effects of bacteria in the form of increased cytokine cascades that prolong the inflammatory phase by continuous influx of polymorphonuclear neutrophils which release cytotoxic enzymes, free oxygen radicals, and inflammatory mediators. These factors are responsible for cellular dysfunction and damage to the host tissue,²⁴ which cause delays or stop completely, the wound healing process.²⁵ The physiological basis of chronic wound evolution is complex. Continuous migration of neutrophils into the wound area causes raised levels of the destructive proteins called matrix metallo-proteinases (MMPs) ²⁶⁻²⁸ including MMP-8 and neutrophil-derived elastase. This is in contrast to normal healing wounds in which excess levels of matrix metallo-proteinases MMPs are inhibited through the nonspecific proteinase inhibitor, α 2-

macroglobulin and the more specific tissue inhibitors of matrix metalloproteinases (TIMMP)²⁹. In chronic wounds, the ratio of the harmful MMP (to the protective TIMMP is raised, resulting in the degradation of extracellular matrix³⁰⁻³², changes in the cytokine profile, and reduced levels of proliferative factors required for effective healing.^{33,34} Table 2 summarizes the different types of chronic wounds commonly encountered in clinical management whilst figure 1 shows photographic representation of the four most common chronic wounds commonly reported.



Figure 1. (A) Arterial ulcer at the cross malleolus of the leg with sharp margins and a punched out appearance; (B) Venous stasis ulcer with irregular border and shallow base, (C) Diabetic foot ulcer with surrounding callus, severe ulcer caused by diabetic neuropathy and bony deformity; (D) Pressure ulcer in a paraplegic (impairment of motor or sensory function in the lower extremities) patient, causing full-thickness skin loss (Adapted from Fonder et al., 2008; with permission).³⁵

Table 2. The major chronic wounds commonly encountered in clinical wound therapy

Type of Ulcer	Description	Risks factors	Symptoms
Diabetic Ulcers	Diabetic foot ulcers (also known as neuropathic ulcers) are a major complication of diabetes mellitus. The most common cause is uncontrolled blood glucose (sugars) over a prolonged period of time. Two other disorders, diabetic neuropathy and peripheral vascular disease, can also contribute to ulcer formation.	<ul style="list-style-type: none"> • Uncontrolled blood sugars • Diabetic peripheral neuropathy • Peripheral vascular disease 	Diabetic ulcers usually present on the foot at an area of trauma or a weight-bearing surface. The wound bed is commonly dry and may have necrotic tissue or a foul odor. This kind of ulcer may be a small wound area on the outside but can hide an underlying abscess. The skin around the wound commonly has hyperkeratosis. These ulcers are generally painless due to altered sensation or neuropathy.
Pressure Ulcers	Pressure ulcers, also known as decubitus ulcers or bed sores, occur in people with conditions that limit or inhibit movement of body parts that are commonly subjected to pressure, such as the sacrum and heels. A pressure ulcer is an area of skin that deteriorates when the skin is exposed to prolonged pressure. This prolonged and unrelieved pressure restricts blood flow into the area and tissue damage or tissue death results.	<ul style="list-style-type: none"> • Patients confined to wheelchair or bed • Increased age • Mental or physical deficits that affect their ability to move • Chronic conditions that prevent areas of the body from receiving proper blood flow • Fragile skin (patient under steroidal therapy), urinary or fecal incontinence • Malnutrition 	A pressure ulcer generally starts as reddened area on the skin and, if the contributing pressure is unrelieved, the ulcer progresses to a blister, then an open sore, and finally a deep crater. This deterioration may occur rapidly. The most common places for pressure ulcers to form are over bones close to the skin, such as the sacrum, heels, elbows, hips, ankles, shoulders, back, and back of the head. Pressures sores are categorized from stage I (earliest signs) to stage IV (worst) according to severity and the treatments depend on the wound stage. Two additional stages can be used in case of severe wounds. They are “unstageable” and “suspected deep tissue injury”.
Venous ulcers	Venous ulcers, also known as vascular or stasis ulcers, develop as a consequence of venous insufficiency. The damaged valves allow blood to pool in the vein, and as the vein overfills, blood may leak out into the surrounding tissue leading to a breakdown of the tissue and development of a skin ulcer. Venous ulcers commonly occur on the sides of the leg, above the ankle and below the knee.	<ul style="list-style-type: none"> • Deep vein thrombosis • Obesity or poor nutrition • Pregnancies • A family history of varicose veins • Smoking and excessive alcohol use • The lack of physical activity • Aging • Work that requires prolonged standing more 	The first sign of a venous skin ulcer is skin that turns dark red or purple over the area where the blood is leaking out of the vein. The wound bed is often beefy red and may bleed easily. The ulcer may be painful. Necrotic tissue, slough (yellow, tan, grey, green, or brown) and/or eschar (tan, brown, or black), may also be present. The skin may also become thick, dry, and itchy. Venous ulcers are commonly slow to heal and often require lifetime modifications to prevent re-development.
Arterial ulcers	Arterial ulcers result from a complete or partial blockage in the arteries. They are almost always caused by atherosclerosis. In this pathology, cholesterol or other fatty plaques settle in the arteries causing obstructions which result in poor blood circulation. This poor circulation leads to tissue death and ulcer formation.	<ul style="list-style-type: none"> • Trauma • Limited joint mobility • Increased age • Diabetes mellitus • High blood pressure • Arteriosclerosis • Peripheral vascular disease 	Wounds commonly have minimal drainage and are often very painful. Pain is often relieved by dangling legs and increased when legs are elevated.

1.3 The need for advanced dressings

Wound dressings are traditionally used to protect the wound site from contamination³⁶ but they can be exploited as platforms to deliver bioactive molecules to wound sites. The use of topical bioactive agents in the form of solutions, creams and ointments for drug delivery to the wound is not very effective as they rapidly absorb fluid, and in the process lose their rheological characteristics and become mobile.¹ For this reason, the use of solid wound dressings is preferred in the case of exudative wounds as they provide better exudate management and prolonged residence at the wound site. Unlike traditional dressings such as gauze and cotton wool that take no active part in the wound healing process, advanced dressings are designed to have biological activity either on its own or the release of bioactive constituents (drugs) incorporated within the dressing¹. The incorporated drugs can play an active role in the wound healing process either directly as cleansing or debriding agents for removing necrotic tissue, or indirectly as antimicrobial drugs, which prevent or treat infection or growth agents (factors) to aid tissue regeneration. In chronic wound management, where patients usually undergo long treatments and frequent dressing changes, a system that delivers drugs to a wound site in a controlled fashion can improve patient compliance and therapeutic outcomes. Bioadhesive, polymeric (synthetic, semi-synthetic or naturally derived) dressings are potentially useful in the treatment of local infections where it may be beneficial to achieve increased local concentrations of antibiotics while avoiding high systemic doses thus reducing patient exposure to an excess of drug beyond that required at the wound site.³⁷

Composite dressings comprising both synthetic and naturally occurring polymers have also been reported for controlled drug delivery to wound sites.¹ By controlling the degree of swelling, crosslinking density, and degradation rate, delivery kinetics can be tailored according to the desired drug release schedule.³⁸ Drug release from polymeric formulations is controlled by one or more physical processes including (a) hydration of the polymer by fluids, (b) swelling to form a gel, (c) diffusion of drug through the polymer matrix and (d) eventual degradation/erosion of the polymeric system.^{37,39,40} Upon contact of a dry polymeric dressing with a moist wound surface, wound exudate penetrates into the polymer matrix. This causes hydration and eventually swelling of the dressing to form a release system over the wound surface (figure 2). In certain wound dressings, the mechanism for drug release has been explained by the hydrolytic activity of enzymes present in the wound exudates⁴¹ or from bacteria in the case of infected wounds.⁴²

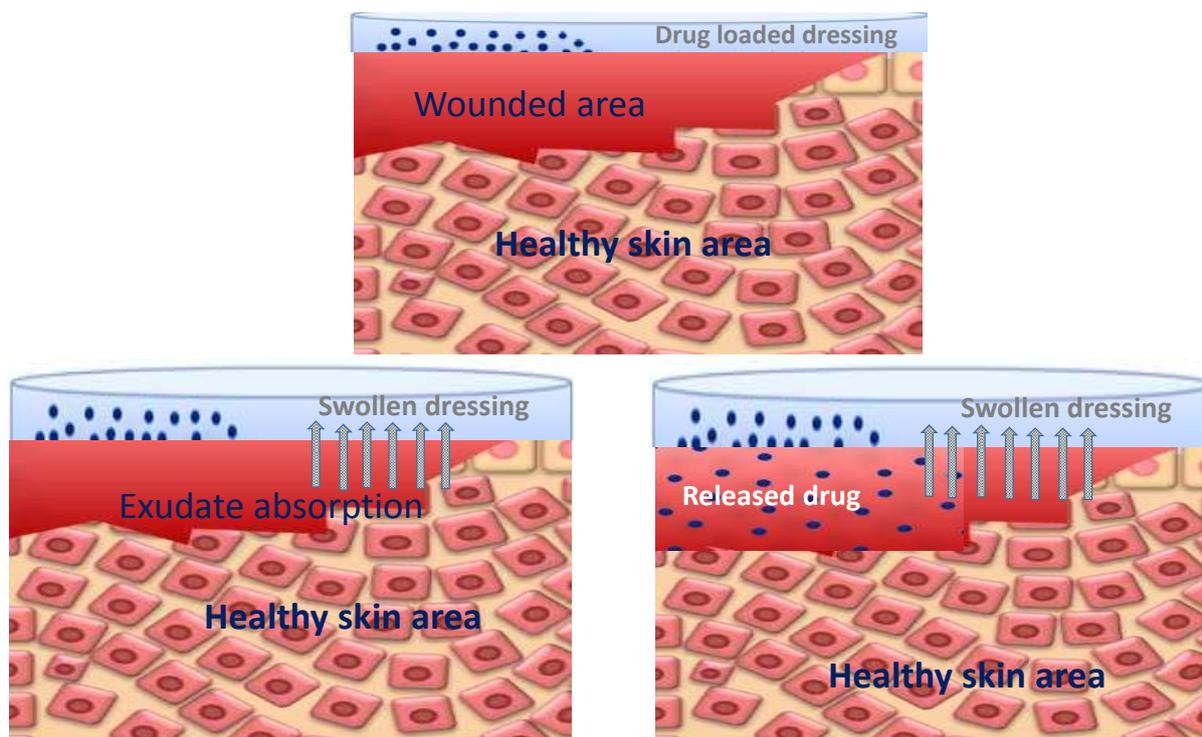


Figure 2. Schematic diagrams illustrating the movement of exudate into and drug release from swollen bioactive dressings during wound healing.

1.4 Dressing materials

Polymeric materials employed in the formulation of wound dressings can be broadly divided into natural inert, natural bioactive and synthetic polymers. A brief overview of these categories of polymers used in wound healing and associated references are summarized in table 3 and briefly discussed below. However, for a detailed description about the use of these materials in wound healing, the reader is referred to the recent review article by Mogosaanu et al.⁴³

Table 3. Summary of the different type of polymers used in commonly used dressings.

Natural	Carboxymethylcellulose ⁶⁹⁻⁷¹
	Bacterial cellulose ^{44,72-74}
	Silk fibroin ⁷⁵⁻⁷⁷
	Pectin ^{78,79}
	Carrageenan ⁸⁰⁻⁸²
Synthetic	Poly(ethylene oxide) ⁸⁰⁻⁸³
	Poly(vinyl alcohol) (PVA) ⁸⁴⁻⁸⁷
	Poly-L-lactic acid ⁸⁸⁻⁹⁰

	Poly(ethylene glycol) ^{61,91,92}
	Polyurethane ^{60,93,94}
Bioactive	Collagen ^{95,96}
	Gelatin ^{97,98}
	Hyaluronic acid ^{53,54,99,100}
	Chitosan ¹⁰¹⁻¹⁰⁴
	Sodium alginate ¹⁰⁵⁻¹⁰⁸

1.4.1 Natural inert polymers

Natural polymers can be obtained from plant, bacterial, fungal, or animal sources and are commonly used due to their biocompatibility and biodegradability. Bacterial cellulose is a pure natural exopolysaccharide produced by specific microbial genera. The good biocompatibility, hemocompatibility, mechanical strength, microporosity and biodegradability make this material one of the most trending natural polymeric materials used for wound care.⁴⁴ Bacterial cellulose is used especially as a healing scaffold/matrix for chronic wound dressings because it possesses many of the characteristics of an ideal wound dressing. It is known to promote autolytic debridement, reduce pain and accelerate granulation, ensuring effective wound healing.⁴⁵ Furthermore, therapeutically active wound dressings with modified cellulose can be prepared by co-immobilization with different active molecules such as enzymes, antioxidants, hormones, vitamins and antimicrobial drugs.⁴⁴ Silk fibroin is another natural biopolymer with a highly repetitive amino acid sequence, which leads to the formation of a biomaterial with remarkable mechanical and biological characteristics. The unique properties of biocompatibility, biodegradability, flexibility, adherence, and absorption of exudates with minimal inflammatory reaction make silk a very promising material for wound dressings.⁴⁶ Other examples of natural polymers employed in wound dressings include carrageenan, carboxymethylcellulose and pectin.

1.4.2 Natural bioactive polymers

Bioactive polymers are also commonly used due to their biocompatibility and biodegradability but more importantly, they have an active therapeutic effect on one or more stages of wound healing. Most of them form part of the natural body matrix or contain components that possess physiological activity as part of the natural wound healing process. The most common bioactive polymer dressing materials include collagen (and gelatine),

hyaluronic acid, chitosan and sodium alginate.

Sodium alginate probably has the largest number of applications in biomedical science and bioengineering due to its biocompatibility, bioresorption and ease of gelation. Alginate is typically used in the form of a hydrogel in biomedicine, including wound healing, drug delivery and tissue engineering applications.³⁸

The most common method to prepare hydrogels from an aqueous alginate solution is to combine with an ionic cross-linking agent such as divalent cations (e.g. Ca^{2+}). The interaction occurs between G-rich regions of adjacent polymer chains resulting in the formation of a bulk structure in a shape of an 'egg-box'.⁴⁷ The composition in the guluronic segments (molecular weight and M/G ratio) and the extent of cross-linking will largely affect the quality of the matrices formed. When hydrogels are made from alginate rich in guluronic acid residues, the resulting gels tend to be rigid, while more elastic gels are produced from alginates with low α -l-guluronic acid content.⁴⁸ The ability of calcium ions (Ca^{2+}) to form crosslinks with alginate makes calcium alginate dressings ideal materials as scaffolds for tissue engineering.⁴⁹

Alginate based absorbent wound dressings may be used on multiple wound types, including pressure, diabetic and venous ulcers and cavity, and some bleeding wounds. Indeed, the high water absorption limits wound secretions and minimizes bacterial contamination.⁵⁰ The wide acceptance of alginates in wound healing is also related to the positive clinical advantages shown in various studies. For example, a randomized, controlled trial involving patients with full-thickness pressure ulcers reported better clinical outcomes using alginate wound dressing when compared to topical treatment with a dextranomer paste.⁵¹

Hyaluronic acid is one of the principal components of the human connective tissues and has become recognized as an active participant in tissue repair processes, including wound healing.⁵² It is already used in some commercially available advanced dressings such as Hyalofill[®] (Anika Therapeutics, USA), Hyalomatrix[®] (Anika Therapeutics, USA) and Hyiodine[®] (Contipro Pharma, Czech Republic), which have demonstrated that the application of exogenous hyaluronic acid on wounds can exert positive effects on the wound-healing process and pain management.⁵³ Hyaluronic acid can be easily included within gauze, foams or creams for topical use and have a high capacity to retain water and provides a moist environment to protect the wounded tissue surface from dryness and promotes wound

healing.⁵⁴

Collagen gives the skin its tensile strength and like hyaluronic acid, forms part of the natural tissue matrix, is biodegradable and plays an active part in normal physiological wound healing and new tissue formation, which makes it an attractive choice from a tissue biocompatibility and a toxicological point of view.⁵⁵⁻⁵⁷ Chitosan has ideal wound healing properties including hemostasis and antibacterial activity.^{58,59} It is reported to be able to stimulate formation of granulation tissue followed by angiogenesis and deposition of collagen fibers to further improve repair of dermal and epidermal wounds.

1.4.3 Synthetic polymers

Synthetic polymers commonly employed in wound dressings include polyvinylalcohol (PVA), polyethylene oxide (PEO) and polyurethane. Their hydrophilic nature imparts important functional wound healing characteristics such as moisture absorption capacity and water vapor transmission which allows maintenance of a moist wound environment whilst avoiding collection of excess exudate. In addition, they are generally adhesive which allows prolonged residence as well as being biocompatible and possessing higher mechanical strength than the natural ones described above. Synthetic polymer dressings can be produced using various techniques, such as electrospinning and hydrogel synthesis.⁴³ Often synthetic materials are used in combination with natural or bioactive polymers to improve the mechanical properties of the final wound dressing, as in the case of electrospun polyurethane-dextran nanofiber mats⁶⁰ or poly(ethylene glycol)/chitosan,⁶¹ both of which are dressings with antibacterial activity due to the presence of ciprofloxacin hydrochloride.

1.4.3.1 Hydrogels

Hydrogels have been widely reported in the peer reviewed literature and in patents whilst several products are commercially available.⁶² A hydrogel can be described as a three-dimensional network of hydrophilic polymers⁶³. They can be prepared from various water soluble polymers with a wide range of chemical and physical properties. Hydrogels are capable of absorbing large volumes of water due to the presence of hydrophilic chains which allows them to swell extensively without changing their gelatinous nature. This property enables hydrogels to function as moist absorbent wound dressings⁶⁴. They can be used on dry, sloughy or necrotic wounds but usually need a secondary dressing to hold it close against the wound bed⁶⁵. These dressings are conventional for unusual shapes of wounds due to their jelly-like

nature. Hydrogels are non-particulate, non-toxic and non-adherent⁶⁶. They also assist in providing a moist environment to dehydrated tissue to prevent them from desiccation and absorb exudates from wounds. Gamma radiation crosslinking was employed by Rosiak and co^{67,68} to obtain sterile hydrogels used in wound care. The materials used included natural polymers such as gelatin and agar and synthetic polymers such as polyvinyl pyrrolidone and polyvinyl alcohol. Some of the most common hydrogel dressings currently available commercially include Intrasite™, Nu-gel™, Kikgel, Aqua-gel and Aquaform™.

2 TRADITIONAL AND IMPREGNATED DRESSINGS

Majority of dressings currently on the market, only take a passive part in the wound healing process. Traditional dressings include cotton, wool, natural or synthetic bandages and gauzes and may be used as primary or secondary dressings, or form part of a composite of several layers with each performing a specific function.¹ These were used commonly in the past and though now less widely used, they are still of some benefit in certain clinic settings for wound treatment. Traditional wound healing agents have been largely replaced for chronic wounds and burns by the more recent and advanced dressings they do not provide a moist environment for wound healing. However, sometimes, moist dressings showed no clinic advantages over treatment with traditional dressing (as for example in case of treatment of split-thickness skin graft donor sites¹⁰⁹) that can be preferred due to ease of use, ready accessibility in most clinics and surgical centers, lower treatment costs and better patient acceptance.

Traditional dressings can provide some bacterial protection, but it is lost when the outer surface of the dressing becomes moistened either by wound exudate or external fluids.¹¹⁰ Further, traditional dressings provide only little occlusion and allow evaporation of moisture, resulting in a dehydrated wound bed, and they tend to become more adherent to wounds as fluid production diminishes and are painful to remove.. An improvement of the properties of these dressings can be obtained by impregnating them with other materials or compounds to obtain a functional dressing. For example, paraffin (petrolatum) impregnated dressings, prevent sticking of the dressing to dry wound surface and are more occlusive and easier to remove from the skin and therefore avoids causing trauma and bleeding during dressing change. Gauze and bandage can also be functionalized with topical antimicrobials, which can prevent or reduce bacterial bioburden or reinfection especially during dressing changes.

Commonly used topical antiseptic agents include iodine-releasing agents (e.g. povidone iodine [PVP-I]), chlorine-releasing solutions (e.g. Dakin's and sodium hypochlorite solutions), hydrogen peroxide, chlorhexidine, silver-releasing agents, and acetic acid. These compounds can be used to either kill or control the growth of micro-organisms in wounds^{111,112} and generally are classified as antiseptics or antibiotics and characterized by low specificity to treat wound infection. Antiseptics, which are disinfectants that are used on intact skin and some open wounds to kill or inhibit microorganisms, tend to have multiple microbial targets, a broad antimicrobial spectrum, and residual anti-infective activity. However, they can be harmful to healthy tissues and cell components essential for effective wound healing such as fibroblasts, keratinocytes, and possibly leukocytes.¹¹³ Antibiotics are potent antimicrobial agents or chemicals with high specificity, which in dilute concentrations, inhibit or kill microorganisms. They usually act on one specific cell target, and are relatively non-toxic, however, they are more susceptible to loss of activity due to the development of bacterial resistance.¹¹³ These are discussed in further detail under the antimicrobial dressings section below. In terms of efficacy, acetic acid (1%) has limited activity but has been used with great success in the management of wounds heavily colonized with *Pseudomonas Aeruginosa*.^{114,115}

3 DRUG-CONTAINING (-DELIVERY-) DRESSINGS

3.1 Wound drug delivery

Different wound types require different dressing materials possessing different characteristics including fluid absorption, residence time on the wound and mechanical strength. A relatively new approach to wound healing involves the use of polymeric wound dressings to deliver various pharmacological agents that can take active part in one or more stages of the wound healing process. The activities of these compounds together with the physical characteristics of the dressing can enhance the wound healing rate, whilst eliminating some of the factors which can impair wound healing. Hydrogels, hydrocolloids, foams, films and wafers can be used to deliver a variety of compounds such as antimicrobials, anti-inflammatory agents, analgesics, growth factors, proteins, and supplements directly to the wound site, thus increasing the efficiency of the therapy.

3.2 Antimicrobial dressings

Many new wound dressings loaded with antimicrobial drugs were developed in the last 20 years, taking advantage of the properties of advanced dressing to actively kill bacteria and

/ or fungi present in infected wounds, reduce bacteria bio-burden and prevent reinfection during healing, wound inspection, surgical procedures or dressing change.

3.2.1 Wound infection

Infection occurs in wounds when one or more microorganisms (mainly bacteria and sometimes fungi) compete with the host natural immune system. Most open injuries are contaminated with different microbes, however, this usually has no clinical significance since they express no evidence of infection and heal as expected. Pathogenic bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and some *Proteus*, *Clostridium* and *Coliform* species are the most common causes of infection and most frequently cited as the reason for delayed wound healing.^{114,116-119} Inadequate control measures in the management of infected wounds can lead to cellulitis and ultimately bacteremia and septicemia, both of which can be fatal. Wound colonization describes the presence of multiplying micro-organisms on the surface of a wound, but with no immune response from the host,¹²⁰ and with no associated clinical signs and symptoms. The invasion of viable tissue by these microorganisms provokes a series of local and systemic host responses such as purulent discharge, painful spreading erythema or symptomatic cellulitis around a wound that can lead to soft tissue destruction.^{111,112} As reported by several authors, high microbial load has severe implications in delaying wound healing and the formation of a bacterial biofilms are one of the critical mediators of chronic wounds.^{114,121,122} It has been reported that approximately 75% of wounds caused by burns have a risk of infection through contamination by microorganisms from the sweat glands and hair follicles, gastrointestinal and upper respiratory tracts, and the presence *Pseudomonas aeruginosa* and *Staphylococcus aureus* significantly reduced skin graft healing.^{114,123-125} Chronic wounds are prone to infection due to the formation of high microbial bioburden and inability of leukocytes to deal with impaired migration, phagocytosis and intracellular killing of microorganisms.¹²⁶ Local tissue necrosis, hypoxia, ischemia and some immune deficiencies such as the one caused by human immunodeficiency virus (HIV) or chemotherapy are factors that promote wound infection.¹¹⁴

3.2.2 Antibiotic drugs

The use of antibiotic drugs for local wound application is gradually becoming popular, at least in the scientific literature, due to many factors, the most common being the lower amounts required when applied directly at the wound sites compared to systemic administration via injections or the gastrointestinal route. Different classes of antibiotics have been used in wound dressings for delivery to wound sites, and a selection of these are summarized in table 4. Treatment of wound infection requires a decrease in exogenous microbial bioburden which can be achieved using various approaches including topical and systemic broad-spectrum antimicrobial agents, debridement of devitalized tissue, appropriate dressing, maximization of immune resistance and provision of adequate nutrition.^{114,128,129} Combinations of antibiotics can be used to cover multidrug resistant microorganisms, however, clinical data supporting this strategy are limited.¹²⁷

Table 4. Different antibiotics and the type of dressings used to deliver them to infected wounds.

Delivery system	Drug	Author / Reference
Chitosan films	Minocycline	Aoyagi et al. ¹³⁸
Chitosan sponges	Vancomycin	Stinner et al. ¹³⁹
Polyox composite film	Streptomycin	Pawar et al. ⁸²
Polyox/carrageenan composite film	Streptomycin	Boateng et al. ⁸⁰
Polyox/carrageenan and polyox/sodium alginate wafers	Streptomycin	Pawar et al. ⁸¹
Wafers	Neomycin	Labovitiadi et al. ¹⁴⁰
Polysaccharide wafers	Chlorhexidine digluconate	Labovitiadi et al. ^{141,142}
Electrospun polyurethane-dextran nanofiber mats	Ciprofloxacin	Unnithan et al. ⁶⁰
Poly(ethylene glycol)/chitosan scaffold	Ciprofloxacin	Sinha et al. ⁶¹

However, the persistent emergence of antibiotic-resistant strains of pathogens, together with the reduced rate of new antibiotics coming through the drug discovery pipeline has resulted in the need for alternative treatments to manage wound infections more effectively. To overcome this problem, novel dressings containing non-antibiotic compounds (e.g. silver and plants) are continually developed and their use can enhance the antimicrobial activities of dressings, limiting the occurrence of antimicrobial resistance.¹³⁰⁻¹³⁷

3.2.3 Silver

Silver, and the newer silver nanoparticles (AgNPs) have been recognized as optimal candidates for overcoming pathologies previously treated with conventional antibiotics, because of their strong and broad-spectrum antimicrobial characteristics.

Various mechanisms have been proposed for silver's antibacterial action. The first proposed mechanism involves bacterial cell membrane enzyme protein deactivation by binding to thiol groups. These proteins are known to take part in membrane energy production and ion transport.¹⁴³ Davis and Etris¹⁴⁴ reported that silver is involved in catalytic oxidation reactions resulting in disulfide bond formation by catalyzing reactions between oxygen present in the cell and hydrogen from thiol groups, ultimately inhibiting cell function due to changes in protein structure. Other authors have reported the binding of silver to the 30S ribosomal subunit thereby preventing protein translation.¹⁴⁵ Another mechanism reported involves the entry of positively charged silver ions into the cell and denaturing DNA by 'locking' itself between purine and pyrimidine base pairs¹⁴³ though this has not been proved conclusively. For silver to exhibit antibacterial activity it needs to be in the ionized form and therefore unionized silver metal is non-active and only becomes active in the presence of moisture (exudate in the case of wounds).^{146,147}

New wound dressings have been developed that release silver to help prevent wound infections caused by both Gram-positive and Gram-negative bacteria both in vitro and in vivo.¹⁴⁸ In the past, the use of silver has been severely limited by the toxicity of its ions to humans, however, the development of nanotechnology has facilitated the production of nano-structured silver particles with a high surface area (and therefore a higher area-to-volume ratio) that demonstrates greater efficacy against bacteria and more importantly, less toxicity to humans.¹³¹

Novel composite scaffold dressing comprising β -chitin and AgNPs for wound healing showed bactericidal activity against *Escherichia coli* and *Staphylococcus aureus* in addition to good blood-clotting ability due to chitin.¹⁴⁹ In a related study, Bishweshwar and co-workers reported on nylon nanofibers incorporating AgNPs by an electrospinning method for wound healing.¹⁵⁰ Their results showed that the composite system exhibited antibacterial activity against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*. Silver loaded dressings have also been reported as effective against non-bacterial targets, including fungi.¹⁵¹ In a recent study, silver-containing activated carbon fibers compared with commercial silver dressings were investigated to determine the effects of different silver concentrations on

the dressing efficacies.¹⁵² that the “various silver-containing activated carbon fibers exhibited good antibacterial effects and biocompatibility in terms of cell viability and that silver concentration showed a minor influence on cell growth”. The authors concluded that silver-containing activated carbon fiber and other commercial silver dressings aided wound healing by promoting granulation and collagen deposition. Chitosan and polyvinyl pyrrolidone based film dressing containing silver oxide has been functionally evaluated for potential wound healing properties, compared to cotton, pure chitosan and other chitosan based dressing.¹⁵³ The results showed better performance of the composite chitosan-PVP-silver oxide dressing compared to the other materials.

Commercially, there are many dressings which are just upgrades of existing polymer based moist wound dressings, loaded with silver either in pure form, as salts or as nanoparticles for treating and / or preventing infection in various wound types. The different silver loaded dressings currently available on the market are summarized in table 5 below. Most of these have been reported in the peer reviewed scientific literature and shown in most cases to have antibacterial activity both in vitro^{154,155} 135,136 and in vivo.^{156,157}

Table 5. Commercially available wound care products containing silver.¹⁵⁸

FORMULATION	PRODUCT NAME	MANUFACTURER	SILVER FORM
Fibrous / cloths, others	Silverseal	Derma Sciences	Silver oxide
	Tegaderm Ag Mesh Dressing with Silver	3M	Silver sulfate
	Urgotul SSD	Laboratoies Urgo	Silver sulfadiazine
	Vliwaktiv Ag, Absorbent Activated Charcoal	Lohmann and Rauscher	Silver
	Vliwaktiv Ag, Activated Charcoal Rope with Silver	Lohmann and Rauscher	Silver
Films / meshes	Acticoat 7	Smith and Nephew	Elemental silver
	Arglaes film	Medline	Silver
	Restore Contact Layer with Silver	Hollister Wound Care LLC	Silver chloride
Foams	Acticoat Moisture Control	Smith and Nephew	Elemental silver
	Allevyn Ag	Smith and Nephew	Silver sulfadiazine
	Biatain Ag	Coloplast	Silver
	Mepilex Ag	Molnlycke	Silver
	Optifoam Ag Adhesive	Medline	Ionic silver
	Optifoam Ag Non-adhesive	Medline	Ionic silver
	PolyMem Silver Island	Ferris Mfg. Corp.	Elemental silver
	PolyWic Silver	Ferris Mfg. Corp.	Elemental silver
	Restore non-adherent foam with silver	Hollister Wound Care LLC	Silver
	Silverlon Negative Pressure	Argentum Medical, LLC	Ionic silver

	SilverSite	Centurion	Silver alginate
	UrgoCell Silver/Cellosorb Ag	Urgo Medical	Silver salts
	V.A.C GranuFoam Silver	KCI	Silver
Gauze	Urgotul SSD/S.Ag	Urgo Medical	Silver sulfadiazine
Hydrocolloid	Contreet Hydrocolloid	Coloplast	Silver
	SILVERSEAL Hydrocolloid	DermaSciences	Silver
	SureSkin	EuroMed	Silver zeolite
Hydrofiber	Aquacel Ag	ConvaTec	Ionic silver
Hydrogel	Elta Silvergel	Elta	Silver
	ExcelGinate Ag	MPM	Silver
	Gentell Ag Hydrogel Wound Dressing	Gentell	Silver sulfadiazine
	Silvasorb Gel	Medline	Ionic silver
	SilverMed Antimicrobial Silver	MPM	Silver
	SILVERSEAL	DermaSciences	Silver oxide
	Silver-Sept Antimicrobial Gel	Anacapa Tech Inc	Silver salt
Powder	Arglaes Powder	Medline	Silver
Wash	SilverMed Antimicrobial Wound Cleanser	MPM	Silver microparticles

3.2.4 Antimicrobial peptides and bacteriolytic enzymes

Infections caused by multi-drug resistant organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), extended spectrum beta-lactamase (ESBL), vancomycin-resistant *Enterococcus* (VRE) and multidrug-resistant *Acinetobacter baumannii* (MRAB) can lead to increased patient morbidity and mortality and increase of the cost of treatment due to prolonged hospitalization. Antimicrobial peptides (AMPs) are recognized as promising candidates to overcome infections caused by resistant bacteria. These therapeutic agents are widely synthesized in nature by microorganisms, plants and animals (both invertebrates and vertebrates) as components of their natural defences against invading pathogens. AMPs are active against a broad spectrum of microorganisms, including multidrug-resistant strains such as MRSA, VRSA, ESBL, VRE and multidrug-resistant *Acinetobacter baumannii* due to the fact that they have a low propensity for developing microbial resistance making them very efficient at treating infection^{159, 140}. This activity is attributed to a rapid mechanism of action and the ability to discriminate between host and microbial cells (cell selectivity) making them promising candidates for clinical applications and potential alternatives to conventional antibiotics. More than 2,000 antimicrobial AMPs have been reported with differences in their sequence and structure, and

they all are generally low molecular weight (10–50 amino acids) peptides and have at least two positive charges.¹⁶⁰

AMPs are widely used to functionalize biomaterial surfaces which impart to it anti-biofilm properties and their immobilization within wound dressings is just one of the applications in the biomedical field.¹⁶¹ Chemically and physically cross-linked natural and synthetic hydrogels are probably the most versatile platforms for the delivery of drugs and peptides to mitigate biofilm formation. In particular, when hydrogels are used to simultaneously co-deliver antimicrobial polymers/peptides and conventional antimicrobial agents, a strong synergistic effect can be achieved.¹⁶² Biodegradable antimicrobial polymers or peptide-loaded gels are more attractive than gels loaded with antibiotics or metal (e.g. silver) nanoparticles since bacteria easily develop resistance to antibiotics, and the non-degradability of metal nanoparticles can result in toxicity. Good results were also obtained when AMPs were included in freeze-dried wafers, polyelectrolyte multilayers or cotton gauzes.^{163,164}

The use of bacteriolytic enzymes can be another promising strategy for the treatment and prevention of drug resistant organisms and biofilm establishment. The biopolymers involved in cell attachment are the main target of such enzymes, leading to an inhibition of biofilm formation or promoting detachment of established biofilms. Several enzymes have been shown to exhibit this anti-biofilm activity and are currently extensively studied for preventing bacterial colonization on surfaces if incorporated into anti-biofilm coatings.¹⁶¹ Recently, Miao et al. proposed the use of these molecules to produce a functional wound dressing with antimicrobial activity against a drug resistant bacterial strain.¹⁶⁵ Lysostaphin, a cell lytic endopeptidase derived from bacteriophages, was immobilized onto biocompatible polymeric fibers generated by electrospinning to obtain an anti-infective bandage. The resulting dressing was tested in an in vitro skin model, and showed good activity against *Staphylococcus aureus* and a low toxicity toward keratinocytes, suggesting a possible application of these materials as antimicrobial wound dressings. Other hydrolytic enzymes derived from bacteriophages have been proposed as promising and potent antibacterial therapeutics even against MRSA and VRSA strains, and for this reason they can become an interesting future therapeutic tool as first line antibiotics in the battle against resistant bacteria strains.¹⁶⁶

3.2.5 Poly(hexamethylene) biguanide hydrochloride (PHMB)

PHMB is a low molecular weight polymer with structure (figure 3) related to chlorhexidine. It is an antimicrobial agent with broad spectrum activity against several Gram-positive and Gram-negative bacteria, fungi and yeast and reported to be particularly active against the difficult to control *Pseudomonas* species. Due to its water solubility, it is used in water-based products, which are most susceptible to microbial growth. As a preservative, PHMB is used in cosmetics, personal care products, fabric softeners, contact lens solutions and hand washes. Moreover, PHMB has also been used to prevent microbial contamination in wound irrigation and sterile dressings and has been reported for use in reducing bloodstream infection caused by catheter use.¹⁶⁷ In a study comparing electrospun poly(lactide) (PLA) nanofibers loaded with either PHMB or chlorhexidine, it was shown that the nanofibers became smoother and their diameter smaller with increasing amount of PHMB with a resultant increase in surface roughness and hydrophobicity of the scaffold.¹³⁷ The PHMB-loaded PLA scaffolds showed antibacterial properties by inhibiting adhesion and bacterial growth, and at the same time exhibited biocompatible characteristics that allowed cell adhesion and proliferation of fibroblasts and epithelial cells *in vitro*.¹³⁷ In a randomized clinical trial, comparing the effectiveness of bio-cellulose dressing containing PHMB with silver sulfadiazine cream, in partial thickness burns, the former showed faster and better reduction in pain compared to the silver sulfadiazine cream. This suggests that PHMB reduced the duration of inflammation by controlling infection.¹⁶⁸ Dilamian et al.¹⁶⁹ prepared composite electrospun membranes using chitosan and polyethylene oxide incorporating PHMB to impart antimicrobial properties for use as a medical biomaterial. The effect of PHMB on the electrospinnability and antimicrobial properties of chitosan/PEO nanofibers were studied together with viscosity of the solutions and nanofiber morphology. The results showed that PHMB in chitosan/PEO solutions resulted in decreased zero-shear rate viscosity up to 20%, whilst increasing PHMB from 0.5 mM to 1 mM led to formation of thinner fibers. The drug loaded fibers showed activity against *Escherichia coli* and *Staphylococcus aureus* with a burst release of PHMB from the materials in the first hour.¹⁶⁹

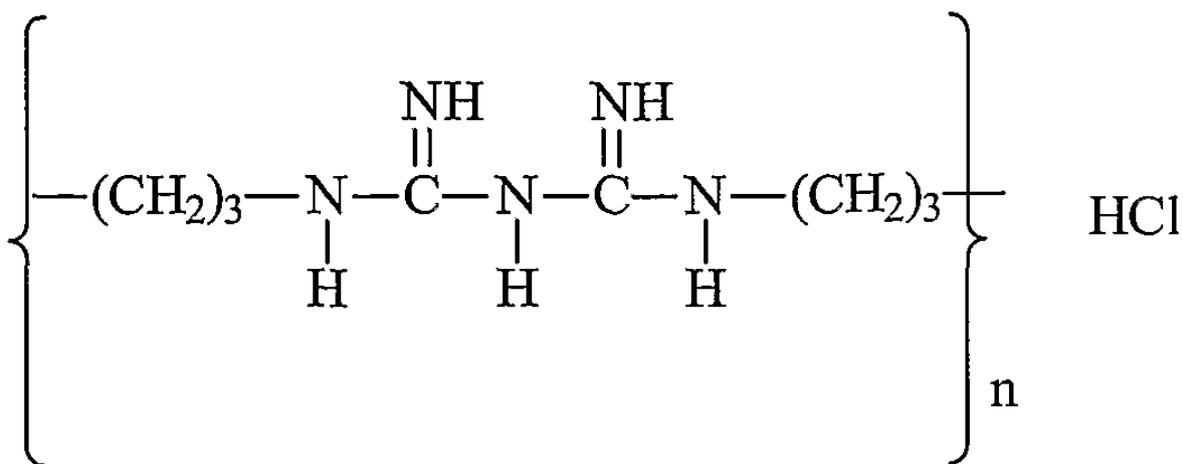


Figure 3 Chemical structure of PHMB

3.3 Anti-inflammatory and analgesic dressings

Wound healing begins with an acute inflammatory phase within a few hours after injury with release of exudate rich in proteins. This causes vasodilation through the release of histamine and serotonin, which allows phagocytes to enter the wound and engulf dead cells. As a result of this inflammatory phase a wound clot is formed, to stop bleeding, and give strength and support to the injured tissue. However, this inflammatory phase is also characterized by swelling and pain, which can be severe in certain wound types. In chronic wounds, the wound is stuck in a continuous cycle of inflammation and patients can be in constant pain, which can be very debilitating. Pain also occurs either due to repeated tissue insults caused by physical trauma, but most common wound pain is probably due to dressing change, especially in the case of dry wounds, debriding, and wound cleansing. In addition, wound infection can contribute to wound pain by triggering a continuous inflammatory response. The response against the infecting microorganisms causes the release of inflammatory mediators and stimulates the production of enzymes and free radicals, which can cause tissue damage.¹⁷⁰ Furthermore, the pain-related stress reduces the immune response to infection and stimulates pro-inflammatory cytokine production in wounds.¹⁷¹ For these reasons the treatment of pain and infection should be prioritized on an equal basis.

Wound pain can be classified into two types: nociceptive and neuropathic pain. Nociceptive pain is an appropriate physiological response to a painful stimulus, and occurs as a result of tissue damage. This type of pain is usually time limited, but when the wounds are slow to heal, the prolonged inflammatory response may cause heightened sensitivity in both the wound (primary hyperalgesia) and in the surrounding skin (secondary hyperalgesia).¹⁷¹ Neuropathic pain is an inappropriate response caused by a primary lesion or dysfunction in the

nervous system. Nerve damage is the commonest cause of the primary lesion, which may be due to trauma, infection, metabolic disorder or cancer. Neuropathic pain is a major factor in the development of chronic pain.¹⁷² Reduction of pain is the highest treatment priority from the patient's perspective, especially in the case of a chronic wound. An appropriate wound management can significantly improve a patient's quality of life and may indirectly promote healing by improving appetite and sleep.¹⁷³ In skin transplants to help wound regeneration, the wound created is extremely painful since the layer of skin harvested touches the painful nerve endings and therefore requires pain management at the secondary wound site.

Topical treatment using pharmacological agents is an effective and safe approach to manage wound pain. Medicated dressings can perform the two essential functions (i) the treatment of the cause (e.g. wound infection) and (ii) the management of the actual wound pain. The treatment of wound infection, by reducing bacterial load and thereby reducing the inflammatory stimulus to the nervous system, should result in a reduction in pain. Antimicrobial drugs, however, may take some days to have a significant effect on pain. Therefore, to obtain rapid pain relief, dressings loaded with drugs, such as local anesthetics (e.g. lidocaine), or NSAIDs can be very useful to reduce wound pain during wear time and at dressing change. In particular, ibuprofen has excellent local effects on superficial wounds, without detectable systemic levels¹⁷⁴ and provided clinically relevant pain relief for patients with exuding, painful venous ulcers.¹⁷⁵⁻¹⁷⁸ In a multi-center randomized controlled trial, Arapoglou and co-workers examined the analgesic effect (over 5 days) of foam dressings loaded with ibuprofen (112.5 mg) compared to local best practice wound management in various wound types (arterial, venous and mixed arterial-venous ulcers, vasculitis and traumatic ulcers).¹⁷⁵ They showed that the ibuprofen releasing foam dressing produced significantly higher analgesic effect than the local best practice group based on patient scores. They concluded that local pain relief by ibuprofen is possible in the most common painful exuding, chronic and acute wounds and therefore a safer alternative to systemic drug administration.¹⁷⁵ Romanelli et al. showed that the commercial ibuprofen containing foam dressing (Biatain Ibu, Coloplast, Denmark) provided better pain relief for painful exuding wounds compared to patients treated with local best practice wound management.¹⁷⁸

Another option to induce efficient analgesia in patients with severe skin wounds is the topical application of opioids. Opioid receptors are up regulated during inflammation and in addition to its analgesic functions, they can also directly modulate the inflammatory process and wound healing.^{179,180} Topical opioid treatment can be used to achieve local analgesia and

increase wound healing, reducing the severe adverse effects of systemic administration. Furthermore, wound dressings can be properly engineered to ensure a slow release, increasing the safety and extending the interval between regular dressing changes.¹⁸¹

4 ADVANCED DRESSINGS CONTAINING BIOLOGICAL AGENTS

4.1 Growth factors

The use of growth factors to promote wound healing has always been considered one of the possible therapeutic approaches to overcome the problem of difficult to heal (chronic) wounds. Growth factors (GFs) are a class of biomacromolecules locally secreted by the extracellular matrix (ECM), capable of regulating biological processes by transferring signals between cells and their local environment, regulating proliferation, migration and differentiation of cells.^{182,183} Interactions between the ECM, GFs, and cells are fundamental to all phases of wound healing and abnormalities in those interactions usually lead to chronic wounds.¹⁸⁴ In an exhaustive review, Barrientos et al.¹⁸⁵ summarized the action and the therapeutic effects of various GFs in the clinical management of non-healing wounds. Four GFs have shown the greatest potential for wound healing in randomized controlled trials: granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).¹⁸⁶ The local application of the GFs on the wound site is essential to exert a therapeutic action on wounds, but the need for continuous local injection makes this formulation difficult to use in clinical practice. The formulation of GFs in a topical delivery system (cream, gel or ointment) directly administered to the wound surface could facilitate their therapeutic application in the clinical management of non-healing wounds. However, to date, only REGRANEX[®] Gel (Becaplermin 0.01%, Smith & Nephew, UK) has been approved by the FDA for the treatment of diabetic foot ulcers.^{187,188} Despite the ability of Becaplermin to accelerate wound closure and significantly reduce amputations,¹⁸⁹⁻¹⁹² its use is expensive, requires frequent dressing changes and is associated with an increased risk of cancer.¹⁸⁸

Polymeric wound dressings were successfully developed for incorporation of free GFs using biocompatible biomaterials such as gelatin,^{193,194} dextran,¹⁹⁵ collagen¹⁹⁶ or chitosan.¹⁹⁷ Micro and nano encapsulation are often necessary to protect GFs during the formulation and production phases and to achieve a long-term exposure, a characteristic required for the

delivery of GFs to chronic wounds. Furthermore, as reported by Ulubayram et al.¹⁹⁴, incorporating GFs into a wound dressing either in free form or loaded within microspheres, (to provide sustained release) have shown greater effects in wound healing than only free GFs. Electrospun nanofibers is another very popular approach to develop novel multifunctional platforms by integrating controlled release strategies within scaffolding materials, which are able to control and regulate the wound healing process.¹⁹⁸ Different fabrication techniques have been used for the development of GFs-loaded electrospun fibers. GFs can be incorporated into the nanofibers¹⁹⁹ or conjugated onto the fibers surface²⁰⁰ and different release characteristics are obtained, depending on the loading method. An interesting hybrid approach was proposed by Kulkarni et al. which used a layer-by-layer assembly technique, to obtain a dressing able to preserve the bioactivity of encapsulated EGF whilst allowing the tuning of EGF release for an extended period, depending upon the number of layers deposited onto the surface.²⁰¹

Wound healing is one of the most complex mechanisms in the human body where multiple cellular pathways are simultaneously activated by different molecules. For this reason, the delivery of a single GF might be insufficient and a combined action of different GFs was shown to improve the reparative processes in the wounded skin of diabetic mice better than single-agent treatment.²⁰² Furthermore, the local concentration and the spatio-temporal gradients can be crucial for a successful treatment and combining different preparation techniques provides the possibility of simulating the natural conditions involved in the wound healing process. Using a combination of encapsulated and free GFs, it is possible to engineer a multiple release system with a controlled, sequential release of GFs mimicking the physiological action sequence and providing the most effective outcome. Multiple GFs including bFGF, EGF, VEGF and PDGF were encapsulated in collagen and hyaluronic acid based electrospun nanofibers loaded with gelatin nano-capsules by Lai et al. for sequential release of the GFs on the wound site¹⁹⁶ GFs encapsulated either in nanofibers or in nanoparticles are released over 1 month by gradual degradation of nanofibers/nanoparticles simulating the temporal release of regulatory factors in the normal wound healing process. The initial delivery of bFGF and EGF bio-mimics the early stage of the wound healing process, whereas slow controlled release of VEGF and PDGF-BB imitates the late stage of skin reconstruction promoting re-epithelialization, dermal reconstruction and formation of mature vasculature as confirmed by *in vivo* studies on streptozotocin-induced diabetic rats.¹⁹⁶

Platelets can constitute a natural potential source of multiple GFs and proteins involved

in tissue regeneration. For this reason topical treatments with platelet derivatives have increasingly been described as capable of accelerating wound healing and to aid in tissue repair.²⁰³ Platelet lysate (PL) is a hemo-derivative obtained through platelet destruction by freeze-thawing and was shown to have activities of different cell types involved in wound healing.²⁰⁴ The possibilities to use allogeneic PL, which minimizes individual variability, represents an advantage compared to patient derivatives such as platelet-rich plasma (PRP) or platelet-rich fibrin (PRF). Different controlled-release systems have been developed to provide sustained delivery of PL to the wound, including sponge-like dressings,²⁰⁵ mucoadhesive gels²⁰⁶ and eyedrops.²⁰⁷ Recently a powdered alginate formulation was proposed for the combined delivery of PL and vancomycin hydrochloride in chronic skin ulcers.²⁰⁸ The alginate particles released the active drugs and also absorbed wound exudates to form a gel and at the same time enhance fibroblast proliferation.²⁰⁸

4.2 Nucleic acids

The local delivery of GFs presents some challenges and there has been limited success of clinical trials. The combined effects of physical inhibition and biological degradation cause significant loss of drug activity which minimizes their therapeutic efficacy. The introduction and expression of exogenous DNA into a host cell to achieve a permanent insertion (known as gene therapy) or transient transformation (gene medicine) has great potential in the treatment of wounds, stimulating the cells themselves to produce the GFs directly onto the wound site.²⁰⁹ Such an approach could avoid the degradation of GFs on the wound site and achieve a temporary expression of these factors until wound closure. One of the first attempts to use a plasmid DNA coding for interleukin 8 (IL-8) genes in wound healing was by Hengge et al. by injecting naked genes into the skin which resulted in a significant recruitment of dermal neutrophils.²¹⁰ However, naked DNA constructs injected into the skin has been proven to have a low transfection efficiency due to their fragility in the extracellular environment, large size and electrical charge. The transfection efficiency can be enhanced using a gene-activated matrix (GAM) that, allows better control over the duration of transgene expression and promotes new tissue formation in a more effective way. A controlled release from a matrix can maintain the right level of the vector over time, providing repeated opportunities for transfection/transduction and extending transgene expression. For this reason, the design parameters of gene-loaded scaffolds (e.g. material, architecture, vector incorporation, biochemical cue presentation) are very important and directly affect the transgene expression and tissue repair.²¹¹ Biodegradable carriers loaded with adenoviral vectors have been

investigated for gene transfer in different animal wound healing models showing an increased granulation tissue formation, vascularization and re-epithelialization compared to controls treated with carriers alone or carriers containing a reporter gene vector.²¹²⁻²¹⁴ However, the limited loading capacity, the high costs of production and the safety risk restrict their application range. Synthetic DNA delivery systems, known as non-viral vectors, have the advantage to deliver genes to target cells without the potential for recombination with wild type viruses and possible cellular damage due to repeated exposure to the viral vectors.²¹⁵ Typically, these non-viral vectors are complexes of naked plasmid DNA (pDNA) with cationic polymers (polyplex), lipid (lipoplex) or inorganic particles. These synthetic constructs have a lower risk of toxicity and offer the possibility of using a wider range of DNAs with different sizes, but at the expense of lower transfection efficiency compared to viral vectors. The transfection rate and the consequent success of the therapy, depends on the degradation rate of biomaterials and the cellular infiltration into the scaffolds. The control of these two parameters allows a modulation of the therapeutic action over a long period of time, making this system very attractive for wound dressing application. Hydrogels containing pDNA coding for TGF-beta1²¹⁶ and VEGF²¹⁷ have already been shown to promote wound healing in mouse wound models. Electrospun nanofibers can be easily engineered to obtain scaffolds for delivery of nucleic acids due to their high surface area, high porosity and interconnected pores beneficial for cell adhesion/proliferation and oxygen/nutrient transfer.¹⁹⁸ The blending of DNA with an electrospinning solution did not give satisfactory results due to improper encapsulation and transfection efficiency¹³² but the development of other techniques, such as the incorporation of DNA-loaded particles into nanofibers, core-shell nanofibers, or surface modification, helped to overcome the low transfection efficiency of naked DNA-loaded nanofibers.¹⁹⁸ Saraf et al., formulated a fiber mesh scaffold containing a non-viral gene delivery vector polyethyleneimine-hyaluronic acid complex (r-PEI-HA) and pDNA within the sheath and core of the fiber, respectively.²¹⁸ They showed that the release rate and the transfection efficiency could be tuned by changing parameters such as concentration of pDNA and molecular weight of the core polymer.

Small interfering RNAs (siRNA) are small pieces of double-stranded mRNA that can inhibit gene expression and prevent the production of specific proteins.²¹⁹ The use of siRNA in wound healing could provide a gene-specific silencing of inflammatory or other specific proteins directly involved in chronic wounds. However, for an effective siRNA wound therapy, it is necessary to protect and deliver the nucleic acid directly into the cytoplasm, a process

complicated by the very short half-life in vivo and by the difficult cellular internalization.²¹⁹ Research in this field is very attractive and many biomaterials and nanoparticles (NPs) are constantly developed and optimized to create efficient delivery systems for siRNA.²²⁰ Biodegradable scaffold injected or implanted directly at the wound site have already been investigated and demonstrated the ability to achieve a high level of gene silencing efficiency and tunability in vivo.²²¹ However, despite the enormous potential of these technologies in wound healing, only few attempts^{222,223} have been made to develop dressings or medical implants for localized and sustained siRNA delivery to the wound.

4.3 Stem cells

In recent years, there has been increasing evidence showing that the paracrine effect of stem cells can play an important role in wound healing, in particular regulating the levels of cytokines and GFs around the wound site.²²⁴⁻²²⁶ Compared to many differentiated cell phenotypes, stem cells are potentially permanent residents of the wound site and naturally modulate the healing response in acute and chronic wounds, synthesizing and delivering multiple GFs. The use of biomaterial scaffolds loaded with stem cells can provide a local delivery of GFs, and at the same time, strengthen the action of the stem cells which creates a favorable environment to promote cell adhesion, proliferation, migration and differentiation. Different cell types and methods can be used in the stem cell therapy of wound healing and Branski et al. have provided a detailed outline of these technologies.²²⁷ Bone marrow-derived stem cells (BMSCs) are probably the most studied marrow-derived stem cells (MSCs) and several clinical studies have demonstrated their usefulness in wound healing.^{228,229} However, bone marrow harvesting is an invasive, and painful procedure and some pathologic conditions (e.g. severe burn trauma, sepsis, silver sulfadiazine toxicity or old age) can reduce the BMSCs availability.²²⁷

Adipose-derived stem cells (ADSCs) are considered an interesting alternative to BMSCs for wound healing application because they express a similar array of cytokines and GFs and can be easily isolated from sections of whole fat (biopsy) or lipo-aspirate, which means a less aggressive and painful harvesting procedure. The biggest challenge in the use of MSCs is to keep the cells in contact with the wound bed and keep them viable in the hostile wound microenvironment. In situ forming injectable hydrogel dressings have been successfully applied for the delivery of large volumes of cells or biomolecules as they allow the retention of the cells at the injection site, therefore increasing efficiency. Furthermore, the relative ease

of loading living cells into those systems and the conformability to complex tissue or implant shapes make hydrogels a very popular scaffold for cell encapsulation.²³⁰ BMSCs²³¹ and ADSCs²³² loaded thermo-responsive hydrogels have already been tested in wound models and showed potential as a bioactive wound dressing. A new interesting application of ADSCs is as filler in biodegradable sutures to provide a local pro-regenerative effect at the injured site. The simultaneous release of key molecules involved in the different phases of wound healing in association with the mechanical wound fixation, represents a promising tool to promote wound healing.

5 DRESSINGS CONTAINING NATURALLY DERIVED AGENTS

5.1 Naturally occurring plant compounds

The development of new wound management products based on traditional or alternative medicine has become very popular in recent years. Before the advent of modern medicine, people of all continents used medicines from natural sources and nowadays the perception towards traditional medicine has also changed. Natural products, including the β -glucans, aloe, honey, cocoa, essential oils and oak bark extracts are already used in wound healing.²³³ However, the lack of standard methods to evaluate their composition has made it more difficult to determine the true efficacy of these products for wound healing.

5.1.1 Aloe vera

Aloe vera (*Aloe barbadensis*) preparations have been used for centuries to treat wounds and burns and its wound healing properties have always attracted the interest of the scientific community. Aloe vera gel is an extremely complicated mixture of natural products, but the biological activity is principally attributed to polysaccharides and glycoproteins (e.g. lectins) present in the leaf pulp.²³⁴ Acemannan, the main polysaccharide present in aloe vera gel, seems to play an important role in the wound healing process by inhibiting bacterial growth and stimulating macrophage activity.²³⁵ Furthermore, the anti-septic and antimicrobial activity are also related to the presence of natural antiseptic agents such as lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulfur, which have inhibitory activity against fungi, bacteria and viruses.²³⁶ Several authors have already proposed the use of aloe vera as alternative to synthetic drugs to develop active wound dressing materials useful for wound healing

applications.²³⁷⁻²³⁹

5.1.2 Other plant extracts

Table 6 summarizes the use of other herbal medicines useful in wound care. Plant extracts from *Chamomilla recutita*,²⁴⁰ *Hamamelis virginiana*,²⁴¹ *Polisiphonia lanosa* seaweed,²⁴² *Acacia arabica* and *Moringa oleifera*,²⁴³ are already being employed in the development of advanced wound dressings. Recently, a collagen sponge containing an extract of *Macrotyloma uniflorum*, generally utilized as cattle feed, was developed by Muthukumar and coworkers.²⁴⁴ The plant extract imparts antimicrobial activities to the sponge and at the same time, increased the tensile strength and the stability against collagenase enzyme.

Table 6. Extracts from different plants useful in wound healing. Adapted from Dorai et al.^{244,245}

Herbal medicine	Properties
Aspilia Africana	Hemostatic properties on wounds, inhibits the growth of microbial organism, accelerates wound healing, treatment of rheumatic pain, bee and scorpions stings, remove corneal opacity and foreign bodies from the eyes
Bridelia ferruginea, Parkia biglobosa Jacq	Increased the proliferation of dermal fibroblast
Elaeis Guineensis leaf extract	Improve tissue regeneration
Cedrus libani, Abies cilicica subsp cilicica	Improved wound healing and anti-inflammatories properties
Carapa Guineensis leaves	Increased rate of wound contraction, skin breaking straight and hydroxyproline content
Combination of Yasha Bhasma, shoea robusta and flax seed oil	Increased wound contraction, higher collagen content and better skin breaking straight
Hippophae rhamniodes L	Improve wound healing
Carica papaya latex	Increased wound contraction and epithelialization rate
Methanol extract of Heliotropium indicum Linn. leaves	Improve wound healing
Rafflesia hasselti, buds and flower extract	Improve wound healing rate and wound contraction
Melaleuca alternifolia	Antimicrobial, antiseptic, antiviral, antifungal and anti-inflammatory properties

Essential oils are the volatile products of secondary metabolism of plants and can be

obtained from plant flowers, seeds, leaves, fruits and roots most commonly via distillation, expression or solvent extraction. Approximately 3,000 essential oils are known, of which around 300 are commercially important.¹³⁶ Some of these, such as thyme oil, oregano, bay, lavender, peppermint, cinnamon, tea tree, rosemary, eucalyptus and lemongrass have been found to exhibit antimicrobial properties, but only lemongrass, oregano and bay essential oil showed antimicrobial activity at concentrations $\ll 2\%$ (v/v).²⁴⁶ Liakos et al.²⁴⁷ tested the antimicrobial and antifungal properties of nine different essential oils at three different concentrations incorporated in a sodium alginate-based film. The loaded films showed the capacity of inhibiting bacterial and fungal growth depending on the essential oil type and concentration and can be suitable to use as novel antimicrobial wound dressing. Several other studies have been conducted on the antimicrobial activity of essential oils in wound dressing systems. Thyme oil was successfully incorporated into chitosan films to obtain antibacterial and permeable films for wound healing applications.²⁴⁹ Thyme oil showed good antimicrobial effects on both Gram-negative and Gram-positive microorganisms and its efficacy as safe and effective source of natural antioxidant and antimicrobial agents was confirmed also by their incorporation into gelatin films²⁵⁰ and N-carboxybutylchitosan /agarose foam using supercritical carbon dioxide.²⁵¹ Eugenol and limonene were doped in nanofluid-based magnetite and used to fabricate modified wound dressings with antimicrobial properties.²⁵² *Garcinia mangostana* extracts were incorporated into electrospun chitosan based nanofiber mats that showed the ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*.²⁵³ However, essential oils, due to their hydrophobicity, tend to have a poor dispersion and eventual phase separation can occur either in solution or in the final dried film. To avoid these phenomena and improve the dispersion and the stability of the essential oils, the use of a surfactants is often required. A different approach was used by Catanzano et al.²⁴⁸ who proposed a microemulsion as carrier to obtain a homogeneous distribution of tea tree oil in an alginate hydrogel.

5.2 Honey

in which nectar is collected and stored in beehives. Over centuries, honey, produced by the pollination bioactivity of industrious honeybees (*Apis mellifera*), has been valued for its biomedical activity in treating various types of wounds including burns, diabetic ulcers, pressure ulcers and leg ulcers.²⁵⁴ Different ancient Sumerian and Greek manuscripts mentioned the use of honey as a drug against wounds such as ulcers.²⁵⁵ Even as far back as World War I, Russian soldiers used honey to prevent wound infection as well as to accelerate healing of their

wounds. The Germans also used honey in combination with cod liver oil to treat ulcers, burns, fistulas and boils.²⁵⁶ A broad spectrum of wounds are reported to be responsive to honey, including scratches, boils, amputation, leg ulcers, burns, chill blains, burst abdominal wound, cracked nipples, fistulas, diabetic, malignant, leprosy, traumatic, cervical, varicose and sickle cell ulcers, septic wounds, surgical wound or wounds of abdominal wall and perineum²⁵⁷.²³⁸ The pharmacological activities of honey^{258,259} relevant for wound healing include, antimicrobial, deodorizing, debriding, osmotic, anti-inflammatory and antioxidant actions which are known to enhance the rate of wound healing.²⁶⁰ Various studies have demonstrated the antimicrobial effectiveness of honey in killing challenging wound-infecting bacteria²⁶¹ with significant increase in randomized clinical trials using honey to treat wounds.²⁵⁹ In its natural state, honey contains major and minor ingredients which account for its biomedical actions in the treatment of various wounds including burns and ulcers²⁵⁴ and these ingredients vary in their physico-chemical properties depending on the plant species on which the bees feed as well as the climatic and variations in general vegetation.²⁶² The main ingredients in honey are carbohydrates of which 95% are sugars, (mainly glucose and fructose) which form the building blocks for other more complex sugars present in quite small quantities. These sugars form during a chain of enzymatic (invertase, diastase, glucose oxidase and catalase) reactions occurring within the honeybee during the ripening of honey or by chemical action in the concentrated honey.²⁶³ Honey also contains various organic acids, such as gluconic acid, which make up just 0.5% of the total solids with pH ranging from 3 to 4.5. Other acids in honey include formic, acetic, butyric, lactic, oxalic, succinic and tartaric acids.²⁶³ Another group of important constituents of honey are polyphenols which account for the natural antioxidants properties. Among these polyphenols, catechin, quercetin and taxifolin have been reported to have the highest anti-oxidation effects.²⁶⁴

5.2.1 Antimicrobial activity

The antibacterial activity of honey is reported against over 60 bacteria species including aerobes and anaerobes, Gram-negative, Gram-positive and some fungi. These include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Escherichia coli*, , coagulase negative *Staphylococci*, *Acinetobacter baumannii* *Stenotrophomonas maltophilia*, MRSA and VRE²⁵⁷⁻²⁵⁹.²³⁸⁻²⁴⁰ Furthermore, honey plays an important role in preventing biofilm formation.²⁶⁵ The high sugar content of honey was previously considered as the main antibacterial agent due to the osmotic action of sugars which deprive bacterial cells of water

vital for growth.²⁶⁶ However, dilution in water increased the antimicrobial efficiency of honey and further research later identified hydrogen peroxide as the main antimicrobial agent^{267, 248}. The antimicrobial properties of honey is attributed to the cumulative action of high sugar content, acidity (low pH),²⁶⁶ hydrogen peroxide^{268,269} and some phytochemicals, including flavonoids and phenolic acids. The flavonoid, pinocembrin has been identified as an antimicrobial factor²⁷⁰ possibly resulting from the ability of flavonoids to form complexes with soluble proteins and cell walls of bacteria. Phenolic acids such as methyl syringate are reported to possess antibacterial activity, however they only account for about 4% of the non-peroxide antibacterial activity of diluted honey.²⁷¹ Furthermore, freshly extracted honey from the comb is known to have high levels of lysozyme which possesses antimicrobial action.²⁷² Other important chemical factors such as volatiles, organic acids, lysozyme, beeswax, nectar, pollen and propolis are important for the antibacterial properties of honey.²⁷³

Though there is no conclusive evidence of benefit in medical use of honey,²⁵⁵ honey dressings, gels and the pure liquid have been gaining in popularity, fueled by scientific reports on their medical benefits and occasional news accounts of the dramatic recovery of a patient with chronic wound. The mostly low quality of the evidence and the heterogeneous nature of the patient populations make it difficult to draw overall conclusions about the effects of honey as a topical treatment for wounds. However, from data collected in a recent Cochrane review, honey appears to heal partial thickness burns more quickly than conventional (polyurethane film, paraffin gauze, tobramycin-impregnated gauze, sterile linen) treatment whilst infected post-operative wounds healed more quickly than antiseptics and gauze.²⁷⁴ Honey dressings are available in various commercial preparations such as honey gel ointment, honey impregnated tulle dressings, honey impregnated calcium alginate dressings, and honey-based sheet hydrogel dressings (table 7).^{130,258,275,276}

Table 7. Commercially available honey products used in wound healing

Commercial name	Company	Forms	Honey type
MGO™ Manuka Honey	Manuka Health New Zealand Ltd	Pure Honey	Manuka honey
Manuka Fill®	Links Medical	Sterile Manuka Honey	Manuka honey
Manuka IG®	Links Medical	Honey impregnated gauze	Manuka honey
Surgihoney™	H&R Healthcare	Pure Honey	Bio-engineered honey
TheraHoney®	Medline	Sheets, ribbon, gel	Manuka honey
Medihoney®	Derma Sciences	Hydrogel colloidal sheet, Honey colloid Dressing, calcium alginate dressings, gel and paste	Manuka honey
Activon®	Advancis Medical	Knitted viscose mesh dressing, pure honey	Manuka honey
Algivon®	Advancis Medical	Alginate ribbon and dressing	Manuka honey
Actilite®	Advancis Medical	Composite foam/silicone dressings, non-adherent viscose net dressing,	Manuka honey

Manuka honey is probably the most widely known honey used over centuries as a wound dressing. It is a mono-floral honey produced in New Zealand and Australia from the nectar of the mānuka tree (*Leptospermum scoparium*), plant which is endemic in parts of Australia and New Zealand. Manuka honey has been reported to exhibit antibacterial activity against a broad spectrum of bacteria including *Staphylococcus aureus* (including MRSA), *Pseudomonas aeruginosa* and VRE.²⁷⁷ The antibacterial properties of Manuka honey are principally, but not exclusively, due to methylglyoxal.²⁷⁸ Medihoney® Dressing (Derma Sciences, USA) was the first wound dressings based on active Manuka Honey to receive FDA approval for clinical use. According to the FDA, Medihoney® dressings are indicated for the management of light to moderately exuding wounds such as diabetic foot ulcers, venous or arterial leg ulcers, partial or full thickness pressure ulcers/sores, first and second partial thickness burns, traumatic and surgical wounds.

A high-standardized synthetic antibacterial honey was developed by H&R Healthcare using a proprietary manufacturing process to produce precise levels of antimicrobial potency

through steady delivery of oxygen free radicals. Surgihoney[®] is a licensed sterile product based on natural, organic honey from a variety of sources, which has been developed for wound care and as a prophylactic dressing for wounds. The antimicrobial activities mediated by hydrogen peroxide²⁷⁹ make Surgihoney[®] active against both Gram-positive and Gram-negative bacterial at very low concentration.²⁸⁰

Due to their natural origin and the high purity, honey dressings have few contraindications, however, they should be avoided in patients with a known history of allergy to either honey or bee venom. It was also reported that patients with diabetes should have their blood sugar monitored as they may be at higher risk of hyperglycemia due to the high sugar content of honey.²⁷⁵

Propolis (honeybee glue) is another natural substance produced by honeybee useful in wound healing.²⁸¹ It is a resinous mixture of botanical balsams and resins with digestive enzymes of bees used principally as a sealant in the hive. In traditional medicine, propolis is widely used for the treatment of various ailments including ulcer and wound healing. The presence of biologically active ingredients such as flavonoids, phenolic acids, terpenes, benzoic acids, amino acids and vitamins, impart to propolis an antioxidant, antimicrobial and immunomodulatory action with a resultant acceleration of wound healing.^{281,282} Collagen-based films containing hydro-alcoholic extracts of two different varieties of propolis were studied by de Almeida et al. on dermal burn healing in a rodent model.²⁸³ These films significantly decreased the inflammatory severity improving the biological events associated with burn healing and seems to be a promising new dressing for wound occlusion and tissue repair.²⁸³

6 MEDICATED SUTURES

Sutures are biomaterial devices (natural or synthetic), usually used for mechanical wound closure to hold tissues together following surgery or trauma. Suturing is one of the most ancient wound healing techniques and although other methods for mechanical wound closure, such as staples, tape, and adhesive, have been developed over the years, sutures are still the most widely used materials.²⁸⁴ Sutures are generally categorized according to the type of material (natural or synthetic), the lifetime of the material in the body (absorbable or non-absorbable) and the form in which they were made (braided, twisted, and monofilament). Each type of suture has different characteristics, properties and surgical application, as reported by Pillai and Sharma.²⁸⁴ Despite the differences in materials and performance, the main goal of sutures is the approximation of the epithelial portion of the wound, maintaining the tensile strength across the wound until tissue tensile strength is adequate. To exert this action, sutures are in direct contact with the wound, and for this reason can represent a useful scaffold for local delivery of active molecules to the wound.

Despite the significant advances in aseptic principles of surgery and the ongoing improvement of minimal invasive surgery, surgical site infections (SSIs) are still the major source of prolonged illness and death in surgical patients.²⁸⁵ SSIs occur when pathogenic organisms (usually members of the Staphylococci family) proliferate in surgical wounds, resulting in the impeding of wound healing, separation of the wound edges (dehiscence), and increase in the risk of abscess in deeper wound tissues. At least 5% of patients undergoing surgery develop SSIs which increases the duration of hospitalization by 20-fold and results in a greater risk of readmission and higher healthcare costs.²⁸⁶ Sutures can be a source of surgical wound contamination because of their non-shedding surface to which bacteria can adhere, form biofilms and potentiate SSIs. The presence of foreign materials in a wound enhances the susceptibility of surrounding tissues to infection and in the presence of sutures only 100 colony-forming units (CFU)/mg are necessary to produce infection.²⁸⁷ Bacteria can also contaminate the suture itself making local mechanisms of wound decontamination become ineffective.

To reduce bacterial adherence and colonization of suture materials, sutures impregnated or coated with antibacterial agents have been developed. Suture materials, especially braided or twisted sutures, are frequently coated to facilitate their handling properties and the incorporation of antibiotic drugs or silver ions is one of the approaches adopted to impart antimicrobial activity. Ideally, an antimicrobial-impregnated suture should prevent bacterial adhesion and biofilms formation using antiseptics drugs with a rapid, potent and broad

microbiocidal spectrum, long-lasting effects and no risk of developing antimicrobial resistance. Furthermore, they should be biocompatible with medical products, not impair healing processes and be well tolerated in wounds with no toxicity or systemic absorption. Even though the development of an antibacterial surgical suture has been under consideration since the early 1980s, the first commercial antimicrobial suture, Polyglactin 910 suture loaded with triclosan (Vicryl Plus[®]), was only approved for clinical use by the FDA in 2002. Different polymeric triclosan-coated sutures are actually on the market, but clinical studies are still unclear about the real effectiveness of these antibacterial sutures.²⁸⁶ The main disadvantage of triclosan is that its widespread use in non-medical products such as cosmetics, soaps and detergents, has resulted in a rise in triclosan-resistant bacteria.

The enormous market potential of this device makes research into anti-microbial surgical sutures very attractive and as a result, new potential alternatives to triclosan are currently under investigation. A suitable alternative to overcome triclosan bacterial resistance is chlorhexidine, a wide spectrum antimicrobial agent principally used as oral antiseptics. Chlorhexidine coated sutures were recently successfully developed using different fatty acids as coating material to achieve a high anti-microbial efficacy and biocompatibility.²⁸⁸ In addition silver,²⁸⁹ and AgNPs,²⁹⁰ have been proposed for suture coating, showing an anti-inflammatory and antimicrobial activities suitable for potential clinical application.

This new generation of suture materials when used to deliver GFs, enzymes or other biomacromolecules directly to the wound site, can result in significant improvement beyond the currently employed surgical procedures. Several studies have demonstrated the possibility of incorporating GFs into polymeric bioadsorbable coating materials. Bigalke et al. investigated a poly(L-lactide) (PLLA) coating on a commercially available suture for the delivery of VEGF.²⁹¹ The authors obtained a well-tuned VEGF release from the suture wire, which resulted in an increased vascularization and consequent wound healing enhancement. Other GFs, such as IGF-1 or growth differentiation factor-5, have been investigated and observed to promote healing in rat models of anastomoses²⁹² and tendon repair²⁹³ respectively. An innovative approach for GFs release from a suture wire was proposed by Reckhenrich et al. who prepared a surgical suture filled with adipose-derived stem cells (ADSCs) to provide pro-regenerative features and allowed the treatment and the fixation of the wound in one single step.²⁹⁴ The incorporation of ADSCs into the inner core of the suture did not affect their viability and the cells remained attached to the suture materials after implantation, constantly releasing cytokine and GFs. However, the low mechanical properties of this ADSCs-loaded suture (due to the filling procedure), restrict their use only to elastic tissues.

Tissue degradation is a problem that often occurs at the repair site, resulting in increased risk of post-operative leakage. Implantation of a foreign material into the tissues invariably evokes a reaction, characterized by an elevated production of MMPs, an enzyme that degrades the extracellular matrix, allowing the suture to cut through the tissue and thus contributes to repair-site elongation and gap formation. Medicated sutures coated with doxycycline, an MMP inhibitor, were used to improve the suture-holding capacity in tendon repair procedure during early repair of collagenous tissues.²⁹⁵

Though coating has been shown to be an easy procedure to prepare drug-loaded sutures, such fabrication procedures can have negative effects on the suture's mechanical strength, especially at the site of the knot, which is essential for effective wound closure. Moreover, it has been shown that suture coatings can lead to physical disruption of the bioactive reagent during the mechanically bearing suturing process.²⁹⁶ To overcome these limitations, new strategies have been developed. For example, Lee et al.²⁹⁷ prepared a composite surgical dressing by assembling together a drug loaded biocompatible polymeric sheet with a surgical suture material, which enabled controlled delivery of an analgesic drug, and is already in clinical use. The drug loaded suture showed good biocompatibility and mechanical properties comparable to those of the original surgical suture and by modifying only the polymeric sheet, it is possible to tune the drug release for up to six days, effectively relieving the pain at the surgical site during the period of wound healing. Drug-eluting electrospun fibers have been proposed for the local delivery of antibiotics²⁹⁸ and local anesthetics²⁹⁹ but their weak mechanical properties and difficulty of scaling up, make these sutures difficult to be applied in clinical settings.

Extrusion processes are usually employed for the large-scale synthetic production of sutures because they allow a precise and controlled manufacturing process resulting in uniform and reproducible properties. However, the high temperature required to melt the polymers can degrade the bioactive molecules, limiting the application of this process in the biomedical field. To protect the drugs from degradation, inclusion of active drugs into an organic or inorganic microstructure that can be dispersed in the polymeric matrix during the extrusion phase, has been proposed.³⁰⁰ Medicated sutures containing an anti-inflammatory agent loaded into an inorganic layered material has already been developed, showing the potential of this approach.³⁰¹

7 TISSUE ENGINEERED SKIN SUBSTITUTES

For wounds where there has been excessive skin loss or damage, in which both epidermal and dermal skin layers are lost, wound healing using only dressing materials or delivery of active agents alone is not viable. Therefore, alternative solutions using either artificial or bioengineered skin substitutes are required to allow the necessary regeneration and replacement of lost tissue. According to Mansbridge, tissue engineered skin substitutes, function effectively due largely to the ability of fibroblasts and keratinocytes to spontaneously form three dimensional structures similar to skin, though other cell types have been included which allow a wide range of properties naturally displayed by normal intact skin.³⁰² Limova³⁰³ in 2010, made the following poignant summary about these highly advanced wound healing products: “extensive skin loss and chronic wounds present a significant challenge to the clinician. With increased understanding of wound healing, cell biology and cell culture techniques, various synthetic dressings and bioengineered skin substitutes have been developed. These materials can protect the wound, increase healing, provide overall wound coverage and improve patient care. The ideal skin substitute may soon become a reality”. Since this observation, several advances have been made in this field and skin substitutes represent a significant improvement over modern moist dressings and advanced drug delivery dressings. In addition, they also provide a more convenient alternative to the harvesting and use of skin grafts from healthy areas of the body as these are very painful and self-defeating because of the need to create a wound elsewhere in the body.

Unlike dressing or direct regenerative approaches, tissue engineered skin substitutes comprise fabricated biomaterial polymer matrix (such as collagen) which acts as scaffolds for engineered skin substrates which grow to actively replace lost tissue. The scaffolds possess mechanical and anatomic characteristics ideally approaching that of the tissue (normal dermis) which they are to replace.³⁰⁴ The scaffold materials gradually degrade within the body, leaving behind a matrix of connective tissue with the appropriate structural and mechanical properties. Hartmann Fritsch et al³⁰⁵ have reported on reinforced collagen hydrogels as dermal-epidermal skin substitutes in rats. Their results showed that the skin substitutes developed into a homogeneous and well-stratified epidermis over the entire surface of the grafts, with a continuous basement membrane and dermo-epidermal junction. An antibacterial scaffold was prepared by electrospinning of a solution comprising dextran, polyurethane and ciprofloxacin HCl (CIP HCl) drug.³⁰⁶ The results showed favorable interaction between fibroblast cells and the scaffolds, in particular the ciprofloxacin loaded matrices.³⁰⁶ Jin et al³⁰⁷ also showed the potential of electrospun nanofibers containing polycaprolactone and the plant extract of *Memecylon edule* as substrates for skin tissue engineering in burn wounds.

Several tissue engineered skin substitutes are available on the market but these have been previously reviewed,¹ and the reader is referred to this review for relevant references and more detailed discussion. However, there has been several published literature on the subject including newer models and advanced characterization of these wound healing systems, most driven by recent advances in tissue regeneration approaches including plastic surgery.

Michael et al.³⁰⁸ proposed a mice model for the functional characterization and testing of skin substitutes using the dorsal skin fold chamber of mice. They inserted commercial dermal construct, (Matriderm[®], MedSkin Solutions Dr. Suwelack AG, Germany) covered with collagen gel, into full thickness wounds in the skin fold chambers and showed good integration into the nearby healthy skin and wound epithelialization within 11 days. They suggested that such a model could be useful in situations where a lack of sufficient areas for obtaining split thickness skin grafts becomes an issue.³⁰⁸ Martin et al.³⁰⁹ investigated the effect of tissue-engineered biological dressing matrices loaded with human in vitro-differentiated adipocytes and ADSCs by evaluating re-epithelialization, granulation tissue formation and

neovascularization of full-thickness cutaneous wounds in fluorescent epidermis of a mouse model.³⁰⁹ It was demonstrated that the tissue engineered treated wounds showed significantly faster wound closure than control wounds without the dressing application over an 18-day period. They also showed by non-invasive imaging of GFP-expressing keratinocytes, that the rate at which the wounds re-epithelialized were similar for both groups with the treated wounds exhibiting thicker collagen enriched granulation tissues. It was concluded from this study that composite engineered substitutes comprising both adipocytes and ADSCs have potential to stimulate cutaneous wound healing when applied as temporary dressings. Table 8 summarizes other reported uses of tissue engineered skin substitutes for treating various types of wounds including chronic wounds.

Table 8. Selected tissue engineered substitutes reported in the literature for application to different wound types including chronic wounds.

Matrix	Construct source	Author/Reference
Collagen	Human dermis	Netchiporouk et al. ³¹⁰
Collagen-elastin	Human subcutaneous adipose tissue	Keck et al. ³¹¹
Synthetic electrospun polylactide (PLA)	Finely minced split thickness human skin	Sharma et al. ³¹²
Collagen	Living skin substitute	Wahab et al. ³¹³
EGF incorporated gelatin microspheres	Bone-marrow-derived mesenchymal stem cells (BM-MSCs)	Huang et al. ³¹⁴
3D fibrin / collagen type 1-hydrogels	Human dermo-epidermal skin substitutes (DESS)	Klar et al. ³¹⁵

8 ADVANCED WOUND HEALING THERAPIES

8.1 Oxygen-associated therapies

A significant number of recent research investigations have demonstrated the importance of oxygen in the field of chronic wound healing.^{316,317} Oxygen plays an essential role in support of cellular processes and infection control, and it is commonly accepted that inadequate cellular oxygenation and perfusion leads to impaired wound healing, triggering wound maceration and delayed healing.³¹⁸ Chronic wounds, in particular diabetic ulcers, usually have a compromised circulation due to a disruption of the blood flow or edema, which decrease or prevents oxygen delivery to healing cells.

Hyperbaric oxygen therapy was originally designed for use in decompression illness in deep sea divers and been used as an adjunct in wound healing for 40 years.³¹⁹ This treatment involves placing the patient in a sealed chamber where 100% oxygen is pressurized to between 1.5 and 3 atmospheres absolute (ATA) for 60 to 120 minutes over a course of multiple treatments. Hyperbaric oxygen significantly increases the oxygen saturation of plasma, raising the partial pressure (PaO₂) available to tissues, which in turn causes vasoconstriction. This vasoconstriction on the arterial end reduces capillary pressure, which promotes fluid absorption into the venous system thereby reducing edema, as well as causing an increase in hyper-oxygenated plasma to the tissues. Tissue repair processes such as collagen elongation and deposition and bacterial killing by macrophages are dependent upon oxygen, therefore

increased levels in wound areas that already have impaired perfusion, serve to facilitate wound healing. The application of hyperbaric oxygen is particularly advantageous in patients with diabetic foot ulcers where it is associated with significantly higher rates of wound healing and could significantly reduce the risk of major amputation.^{316,320,321} In addition to immediate assistance in healing, hyperbaric oxygen also has a role in long-term wound improvement, perhaps due to the realization of the full effects of neovascularization.³²²

Topical wound oxygen therapy is an alternative method of administering oxygen to a wound, where 100% humidified, pressurized oxygen is directly applied to the surface of an open, ischemic wound in order to increase the local oxygen levels in the tissue. This route of administration involves injecting pure oxygen into a portable inflatable bag, which encases the wound area. Topical oxygen therapy, used as an adjunct to other therapies, has been shown to be effective for wound healing,^{323,324} and the low costs, greater portability, and reduced risks of oxygen toxicity make this approach more beneficial than hyperbaric oxygen.³¹⁶ However, both these therapeutic approaches are time consuming and inconvenient for the patient due the required immobility during treatment.

The use of a therapeutic wound dressing to deliver oxygen directly to the cells may be an interesting strategy as it is more cost effective, portable and presents the possibility of promoting more rapid wound healing. Topically delivered dissolved oxygen has no deleterious effects and stimulates beneficial effects even on intact, non-wounded skin.³²⁵ Furthermore, these dressings maintain some of the properties of an ideal¹ wound dressing providing all the desirable useful features to promote effective wound healing. Different approaches have been proposed to obtain local oxygen release from wound dressings. Oxygen can be stored inside the dressing between an occlusive upper layer and a lower permeable film, which allows the dressing to supersaturate the wound fluid with regenerative oxygen for days. These “oxygen reservoir dressings” are foam based dressings containing oxygen micro-bubbles which begin to “dissolve” when the foam is moistened with exudate, and once dissolved, oxygen can easily travel according to the oxygen gradient across poorly perfused tissue. Transcutaneous dissolved oxygen was demonstrated to promote wound healing and limit necrosis, thus decreasing the healing time and the pain at donor sites.^{326,327}

Oxyzyme[®] dressing (Crawford Healthcare Ltd, UK) is an enzyme-activated hydrogel dressing developed to support the wound healing process by releasing oxygen and also impeding microbial growth due to the release of iodine. The dressing is a two component advanced hydrogel containing glucose oxidase to generate hydrogen peroxide and a halide iodide to generate hypiodite which leads to iodine production. When the dressing is removed

from its airtight package and the two layers are brought into contact with each other, the oxidase enzyme within the top layer is ready to start its reaction with oxygen. The enzyme activation generates a flow of hydrogen peroxide in the dressing. When applied on the wound, the hydrogen peroxide is converted to water and dissolved oxygen by serum catalase in the wound.³²⁸ The wound bed becomes rich in locally available oxygen, with all of its associated benefits, to work in harmony with the antimicrobial effects of the iodine and various other optimizing effects of the dressing. A similar product (Iodozyme[®] Crawford Healthcare Ltd, UK) has been developed for patients with chronic infection or bacterial bioburden using the same principle and differs only in the amount of iodine produced. Both dressings have lower levels of iodine if compared with other iodine based dressings, but have similar antimicrobial properties.³²⁸

Table 9. Commercially available topically delivered dissolved oxygen dressing.

Commercial name	Company	Form	Oxygen delivery system	References
Oxyzyme [®]	Crawford Healthcare Ltd, UK	2 part sterile hydrogel dressing	Enzyme-activated in situ oxygen production	Moffatt et al. ³²⁸
OxyBand [®]	OxyBand Technologies Inc, USA	Self-contained multiple layers hydrocolloid dressing. The top layer is a waterproof barrier film	Oxygen pre-filled wound dressing	Lairt et al. ³²⁶
Oxygenesys [®]	Halyard Health Inc, USA	Adsorbent foam dressing	Oxygen pre-filled wound dressing	Kellar et al. ^{325,327}

8.2 Negative pressure wound therapy

Negative-pressure wound therapy (NPWT) also known as topical negative-pressure therapy or vacuum-assisted closure has become an integral part of modern wound care practice and is used routinely in hospitals throughout the world, where it is estimated that 300 million acute wounds are treated globally each year.³²⁹⁻³³¹ Morykwas et al. first reported on this NPWT using an open-cell foam dressing with the application of a controlled sub-atmospheric pressure for the treatment of acute and chronic wounds.^{332,333} NPWT promotes wound healing by applying a vacuum through a special sealed dressing. The continued vacuum draws out fluid from the wound and increases blood flow to the area.

Preclinical and clinical studies have confirmed that NPWT provides a moist wound

healing environment, drains exudate, reduces tissue edema, contracts wound edges, mechanically stimulates the wound bed, alters blood flow in and around the wound edges, and stimulates angiogenesis and the formation of granulation tissue.³³²⁻³³⁴ The beneficial effects of NPWT on wounds are mediated by multiple mechanisms, which together contribute to the observed clinical effects. However, little is known about the influence of different NPWT settings on their biological activity in the wound.

The dressings used for the technique include open-cell foam dressings and gauze with a pore range of 400-600 µm cut to fit the wound surface and sealed with an occlusive dressing intended to contain the vacuum at the wound site. The open-cell polyurethane foam dressing enables equal distribution of the negative pressure over the entire wound bed, and also allows exudate to flow freely for collection and removal in the canister. The foam can be used to pack open cavity wounds and can also be cut to size to fill underlying areas. The pore size of the NPWT dressing foam dressings are larger than other foam dressings to maximize tissue growth.³³² The first device for NPWT introduced on the market was the V.A.C.[®] Therapy System (KCI, USA) and until 2003, was the only commercially available system. With the affirmation of the method, different devices were introduced with the main difference between them being the type of dressing used to fill the wound (foam or gauze).

NPWT can be used to achieve a variety of treatment goals, but cannot replace surgical procedures. The therapeutic efficacy depends on the patient and the characteristics of the wound,³²⁹ and usually may allow a wound to progress to the point at which a less invasive procedure is possible.³³⁵ NPWT can also be used in cases of infected wounds, as an adjuvant to an appropriate systemic antibiotic therapy. The application of negative pressure creates a hypoxic environment at the wound bed/dressing interface reducing the bacterial count at the wound bed up to 1,000 times after four days of treatment.³³⁶ Since its mode of action is not selective, NPWT is effective against difficult infections such as MRSA and drug-resistant bacterial strains. Commercially, a foam dressing coated with silver (GranuFoam[™] KCI, USA) was developed to impart additional antimicrobial properties.³³⁷

8.3 Physical therapies in wound healing

8.3.1 Electrical stimulation

Electrical stimulation (ES) is believed to aid in wound healing for the treatment of both acute and chronic wounds by imitating the natural electrical current that occurs in injured skin. The body naturally creates and uses electrical energy that aids in the recruitment of cells

necessary for healing through a process called galvanotaxis or electrotaxis.³³⁸ The undamaged skin contains an electro-potential of 30 mV to 100 mV between the stratum corneum and the dermis, however, when the epithelial cells break down due to injury, this difference in potential is lost. This loss in potential is the earliest indicator stimulus signal to initiate cell migration and re-epithelialization, and many epithelial cells including human keratinocytes have the ability to detect electric fields and respond with directed migration.³³⁹ In addition, other cell types such as neutrophils, macrophages and fibroblasts seem to be sensitive to ES, increasing the migration rate.³⁴⁰ Some experiments indicate that when the electric field is removed, the wound healing rate is 25% slower.³³⁸

The clinical evidence for the application of different types of ES to enhance cutaneous wound healing has recently been summarized by Ud-Din and Bayat.³⁴¹ ES has been shown to have beneficial effects on the different phases of cutaneous wound healing in both chronic and acute wounds, concluding that the application of an electric potential on the wounded skin results in a significant improvement in wound area reduction or accelerated wound healing compared to the standard methods of care as well as improved local perfusion.³⁴¹ Additionally, ES has action against bacterial infection, a major cause of impaired wound healing.³⁴² Usually, the ES is applied using an external device by placing the electrodes on the skin, and often, directly onto the wound. Several different modalities of ES have been described for each wound type with varying voltages, currents, electrical waveforms, modes and length of time of application, and no device-related complications or adverse effects have been reported in the existing literature, indicating that the therapy is safe and easy to use.³⁴¹

Bioelectric dressings (BED) are emerging as a useful method of delivering ES to the wound site. This device combines the beneficial wound repair characteristics of both an occlusive dressing and an electrical gradient, and simultaneously utilizes two separate mechanisms that have been shown to aid wound healing. One of the first BEDs introduced on the market was PosiFect[®]RD (Biofisica UK Ltd), which contains a miniature electrical circuit that delivers a micro-current derived from two lithium non-rechargeable coin cell batteries to the wound bed for a minimum of 48 hours. This device has been demonstrated to have potentially multiple positive effects on all phases of wound healing, in particular in treating chronic wounds that have previously been non-responsive to treatment.³⁴³ A new bioelectric bandage based on the PROSIT[™] technology was approved by the FDA to treat partial and full-thickness wounds. Its dressing form, Procellera[®] (Vomaris Wound Care Inc., USA) is a woven metallic BED (figure 4A) activated by wound exudate, thereby generating a sustained electric stimulation of 2 - 10 mV produced by micro-batteries of silver (Ag) and zinc (Zn) metals,

which are inside a woven material (figure 4B). After its application, the wound is covered with an overlying dressing to keep the wound moist and the dressing active for up to 30 days. The application of an electric field generated by Ag/Zn BED increases keratinocyte migration, a critical event in wound re-epithelialization, via redox-dependent processes,³⁴⁴ resulting in faster wound epithelialization and improved scar appearance.^{345,346} In addition, it showed antimicrobial properties against antibiotic-sensitive strains and multiple antibiotic-resistant strains of wound pathogens,³⁴⁷ even when these bacterial strains formed a polymicrobial biofilm.³⁴⁸ Procellera[®] can be easily cut to the size of the wound and conforms to irregular surfaces and to wound edges. The main advantage of these devices is that they are wire-less with no need for an external power source and can be applied and changed easily without the requirement for someone specially trained in ES.

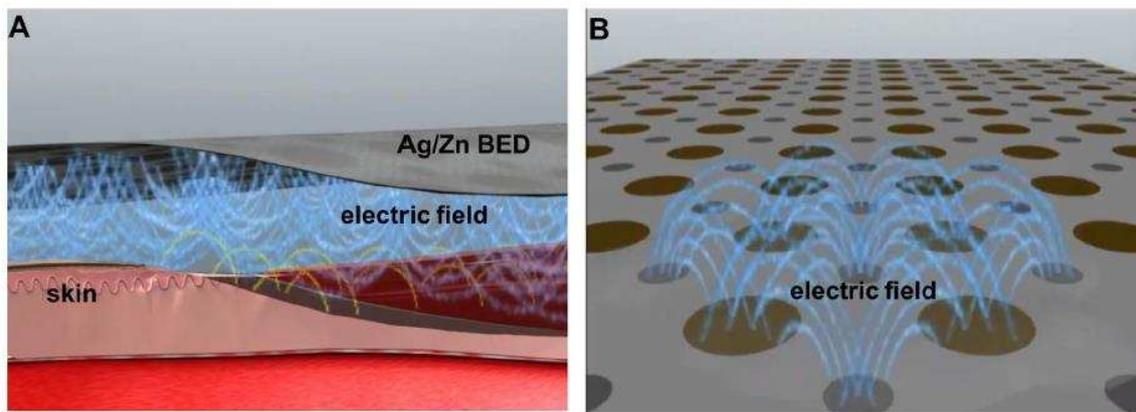


Figure 4. Schematic diagram of the design, application (A), and electric fields (B) generated by Procellera[®] bioelectric dressing.³⁴⁴

8.3.2 Pulsed electromagnetic therapy

The use of pulsed radio-frequency electromagnetic field (PEMF) therapy, has shown notable success in healing of chronic wounds. PEMF is a non-ionizing energy at the shortwave radio frequency band of the electromagnetic spectrum, commonly at a frequency of 27.12MHz and widely used in the field of orthopedics. This therapy is non-invasive and can also be applied to the wound area through wound dressings to aid healing of chronic wounds such as venous leg ulcers.³⁴⁹ Furthermore, it has been reported that PEMF can also provide analgesic benefit to patients following surgery or other soft tissue trauma, with few reports of side effects.³⁵⁰ PEMF devices such as Provant[®] (Regenesis Biomedical, USA) are already used for the treatment of chronic ulcers and postoperative pain, and a new wearable PEMF device was

successfully used for treatment and healing of four patients with non-healing wounds.³⁵¹ However, though the application of electromagnetic fields to the wound area significantly improved both diabetic and normal wound healing in mice,³⁵² there is no clinically relevant evidence to show that electromagnetic therapy increases the rate of healing of venous leg ulcers in patients, and further research is therefore needed.^{349,353}

8.3.3 Low level laser therapy

Low-level laser therapy (LLLT) is a medical procedure that uses red and near-infrared monochromatic light (600 - 1000 nm) to enhance the body's natural healing processes. When the light source is placed in contact with the skin, the light energy (photons) penetrates into the tissue, where it alters the healing process at a cellular level. It is not exactly clear how low-level laser therapy works, but some reports³⁵⁴ suggest that photons are absorbed by the mitochondria and stimulate more ATP production and low levels of reactive oxygen species (ROS). These then activate transcription factors such as NF- κ B, to induce many gene transcript products which provide the beneficial effects.³⁵⁴ The way light interacts with the biological tissues will depend on the characteristics and parameters of light devices but there is evidence that coherent (laser) and non-coherent (LED) light produce similar healing effects on tissues.³⁵⁵ LLLT has been reported to promote osteogenesis,³⁵⁶ wound healing,³³⁴ and the eradication of bacterial biofilms.³³⁵ Currently, a large number of basic studies have reported bio-stimulative effects of LLLT on different types of chronic wounds both in animal models and in humans, but until now there is insufficient evidence to establish the usefulness of LLLT as an effective tool in wound care management.^{357,359,360} Further work is therefore required to confirm its clinical effectiveness in a conclusive way including randomised clinical trials.

9 CONCLUDING REMARKS

Chronic wounds and other difficult to heal wounds have significant health, social and economic burdens on both patients and society in general and therefore of current topical interest worldwide.

In this review, we have covered the current state of the art in chronic wound healing technologies involving the active treatment of these wounds, with emphasis on advanced therapeutically active systems and methods for healing of chronic and other difficult to heal wounds. The driving forces for the development of advanced dressings as improvements over currently used traditional and modern moist dressings, the evolution of the different advanced wound dressings reported in the literature and available commercially, have also been discussed. The major driving forces include the rise in an aging population and therefore increased incidence of pressure and venous leg ulcers, increase in obesity and associated type II diabetes, linked to diabetic chronic ulcers as well as the rise of super antibiotic resistant microorganisms (mainly bacteria) all of which increase the risk of delayed wound healing and potential morbidity (including amputations) and in severe cases, mortality. Other driving forces include the need to reduce cost to National Health Providers, by reducing hospital stays and nursing staff time spent with chronic wound patients.

The review has covered many advanced wound dressings including biological dressings from natural biomaterial polymers (e.g. chitosan, collagen and hyaluronic acid), medicated modern dressings using agents such as antimicrobials (antibiotics, silver, PHMB, antimicrobial peptides) biological based dressings (comprising mainly GFs, stem cells, nucleic acids and other genetic materials), tissue engineered skin substitutes, dressings containing naturally derived wound agents such as Aloe and honey as well as more recent advances in NPWT, oxygen related dressings, electrical stimulation and laser therapy. Several challenges still remain in tackling the problems associated with chronic wounds and it is clear that even single advanced dressings and other advanced physical wound healing procedures, do not always address the problems encountered in chronic wounds for every single patient and therefore a combination of the above mentioned advanced systems will be required.

It is plausible that this will be the way forward in future developments for an ideal advanced dressing that will tackle the problems of chronic wounds including pain and inflammation, odor, infection caused by resistant bacteria, delayed healing and associated costs to health systems and populations worldwide. This is important given the many phases of wound healing and differences in complications observed in different patients. Therefore, a multi-targeted approach appears to be the best way forward and it is hoped that this review has contributed towards identifying the critical factors that need to be tackled to make this a reality in the near to medium term future.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest with respect to the authorship, and/or publication of this article.

REFERENCES

1. Boateng JS, Matthews KH, Stevens HN, Eccleston GM 2008. Wound healing dressings and drug delivery systems: a review. *J Pharm Sci* 97(8):2892-2923.
2. Enoch S, Leaper DJ 2008. Basic science of wound healing. *Surgery (Oxford)* 26(2):31-37.
3. Guo S, Di Pietro LA 2010. Factors Affecting Wound Healing. *J Dental Res* 89(3):219-229.
4. Gurtner GC, Callaghan MJ, Longaker MT 2007. Progress and potential for regenerative medicine. *Annu Rev Med* 58:299-312.
5. Gurtner GC, Werner S, Barrandon Y, Longaker MT 2008. Wound repair and regeneration. *Nature* 453(7193):314-321.
6. Martin P 1997. Wound healing - Aiming for perfect skin regeneration. *Sci* 276(5309):75-81.
7. Nawaz Z, Bentley G 2011. Surgical incisions and principles of wound healing. *Surg* 29(2):59-62.
8. Reinke JM, Sorg H 2012. Wound repair and regeneration. *Eur Surg Res* 49(1):35-43.
9. Velnar T, Bailey T, Smrkolj V 2009. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res* 37(5):1528-1542.
10. Thu HE, Zulfakar MH, Ng SF 2012. Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing. *Int J Pharm* 434(1-2):375-383.
11. Percival J 2002. Classification of wounds and their management. *Surg* 20:114-117.
12. Moore K, McCallion R, Searle RJ, Stacey MC, Harding KG 2006. Prediction and monitoring the therapeutic response of chronic dermal wounds. *Int Wound J* 3(2):89-96.
13. Broderick N 2009. Understanding chronic wound healing. *The Nurse Practitioner* 34(10).
14. Trent JT, Kirsner RS 2003. Wounds and malignancy. *Adv Skin Wound Care* 16(1):31-34.

15. Cutting KF, White RJ 2002. Maceration of the skin and wound bed. 1: Its nature and causes. *J Wound Care* 11(7):275-278.
16. Krasner D, Kennedy KL, Rolstad BS, Roma AW 1993. The ABCs of wound care dressings. *Ostomy Wound Manage* 39(8):66, 68-69.
17. Ferreira MC, Tuma P, Jr., Carvalho VF, Kamamoto F 2006. Complex wounds. *Clinics (Sao Paulo)* 61(6):571-578.
18. Kirketerp-Møller K, Zulkowski K, James G. 2011. Chronic Wound Colonization, Infection, and Biofilms. *Biofilm Infections*, ed.: Springer New York. p 11-24.
19. Skorkowska-Telichowska K, Czemplik M, Kulma A, Szopa J 2013. The local treatment and available dressings designed for chronic wounds. *J Am Acad Dermatol* 68(4):e117-126.
20. Peh K, Khan T, Ch'ng H 2000. Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing. *J Pharm Pharm Sci* 3(3):303-311.
21. Geroult S, Phillips RO, Demangel C 2014. Adhesion of the ulcerative pathogen *Mycobacterium ulcerans* to DACC-coated dressings. *J Wound Care* 23(8):417-418, 422-414.
22. Bessis D, Kempf M, Marsollier L 2015. *Mycobacterium ulcerans* Disease (Buruli Ulcer) in Mali: A New Potential African Endemic Country. *Acta Derm Venereol* 95(4):489-490.
23. Bjarnsholt T, Kirketerp-Møller K, Jensen PO, Madsen KG, Phipps R, Krogh K, Hoiby N, Givskov M 2008. Why chronic wounds will not heal: a novel hypothesis. *Wound Repair Regen* 16(1):2-10.
24. Carter MJ, Tingley-Kelley K, Warriner RA, 3rd 2010. Silver treatments and silver-impregnated dressings for the healing of leg wounds and ulcers: a systematic review and meta-analysis. *J Am Acad Dermatol* 63(4):668-679.
25. Falanga V 2000. Classifications for wound bed preparation and stimulation of chronic wounds. *Wound Repair Regen* 8(5):347-352.
26. Nwomeh BC, Liang HX, Cohen IK, Yager DR 1999. MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. *J Surg Res* 81:189–195.
27. Nwomeh BC, Liang HX, Diegelmann RF, Cohen IK, Yager DR 1998. Dynamics of the matrix metalloproteinases MMP-1 and MMP-8 in acute open human dermal wounds. *Wound Repair Regen* 6:127–134.

28. Lobmann R, Zacheja S, Houdek P, Moll I, Lobmann R 2008. Expression of matrix metalloproteinases, cytokines, and connexins in diabetic and nondiabetic human keratinocytes before and after transplantation into an ex vivo wound-healing model. *Diabetes Care* 31:114–120.
29. Bullen EC, Longaker MT, Updike DL, Benton R, Ladin D, Hou Z, Howard EW 1995;. Tissue inhibitor of metalloproteinases-1 is decreased and activated gelatinases are increased in chronic wounds. *J Invest Dermatol* 104:236–240.
30. Weiss SJ 1989. Tissue destruction by neutrophils. *N Engl J Med* 320:365–376.
31. Rao CN, Ladin DA, Liu YY, Chilukuri K, Hou ZZ, Woodley DT 1995. Alpha 1-antitrypsin is degraded and non-functional in chronic wounds but intact and functional in acute wounds: the inhibitor protects fibronectin from degradation by chronic wound fluid enzymes. *J Invest Dermatol* 105:572–578.
32. Grinnell F, Zhu M 1996. Fibronectin degradation in chronic wounds depends on the relative levels of elastase, alpha1-proteinase inhibitor, and alpha2-macroglobulin. *J Invest Dermatol* 106:335–341.
33. Falanga V 1992. Growth factors and chronic wounds: the need to understand the microenvironment. *J. Dermatol* 19:667–672.
34. Bennett NT, Schultz GS 1993. Growth factors and wound healing: Part II. Role in normal and chronic wound healing. *Am J Surg* 166:74–81.
35. Fonder MA, Lazarus GS, Cowan DA, Aronson-Cook B, Kohli AR, Mamelak AJ 2008. Treating the chronic wound: A practical approach to the care of nonhealing wounds and wound care dressings. *J Am Acad Dermatol* 58(2):185-206.
36. <http://www.oxforddictionaries.com/>. (Accessed on 11/05/2015)
37. Langer R 1980. Polymeric delivery systems for controlled drug release. *Chem Eng Commun* 6:1-48.
38. Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA 2009. Hydrogels in regenerative medicine. *Adv Mater* 21(32-33):3307-3329.
39. Martin A, Bustamante P, Chun A. 1993. Diffusion and dissolution in physical pharmacy: Physico-chemical principles. In Mundorff G, editor *The Pharmaceutical Sciences*, 4th edition ed., Philadelphia. p 324-362.
40. Peppas NA 1983. A model of dissolution-controlled solute release from porous drug delivery polymeric systems. *J Biomed Mater Res* 17(6):1079-1087.

41. DuBose JW, Cutshall C, Metters AT 2005. Controlled release of tethered molecules via engineered hydrogel degradation: model development and validation. *J Biomed Mater Res A* 74(1):104-116.
42. Suzuki Y, Tanihara M, Nishimura Y, Suzuki K, Kakimaru Y, Shimizu Y 1998. A new drug delivery system with controlled release of antibiotic only in the presence of infection. *J Biomed Mater Res* 42(1):112-116.
43. Mogosanu GD, Grumezescu AM 2014. Natural and synthetic polymers for wounds and burns dressing. *Int J Pharm* 463(2):127-136.
44. Rajwade JM, Paknikar KM, Kumbhar JV 2015. Applications of bacterial cellulose and its composites in biomedicine. *Appl Microbiol Biotechnol* 99(6):2491-2511.
45. De Olyveira GM, Manzine Costa LM, Basmaji P, L. XF 2011. Bacterial nanocellulose for medicine regenerative. *J Nanotechnol Eng Med* 2.
46. Altman GH, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, Lu H, Richmond J, Kaplan DL 2003. Silk-based biomaterials. *Biomaterials* 24(3):401-416.
47. Grant GT, Morris ER, Rees DA, Smith PJC, Thom D 1973. Biological Interactions between Polysaccharides and Divalent Cations - Egg-Box Model. *Febs Lett* 32(1):195-198.
48. Goh CH, Heng PWS, Chan LW 2012. Cross-linker and non-gelling Na⁺ effects on multi-functional alginate dressings. *Carbohydr Polymers* 87(2):1796-1802.
49. Lee KY, Mooney DJ 2012. Alginate: properties and biomedical applications. *Progress Polymer Sci* 37(1):106-126.
50. Gilchrist T, Martin AM 1983. Wound treatment with Sorbsan--an alginate fibre dressing. *Biomater* 4(4):317-320.
51. Sayag J, Meaume S, Bohbot S 1996. Healing properties of calcium alginate dressings. *J Wound Care* 5(8):357-362.
52. Frenkel JS 2012. The role of hyaluronan in wound healing. *Int Wound J* 11(2):159-163.
53. Humbert P, Mikosinki J, Benchikhi H, Allaert FA 2013. Efficacy and safety of a gauze pad containing hyaluronic acid in treatment of leg ulcers of venous or mixed origin: a double-blind, randomised, controlled trial. *Int Wound J* 10(2):159-166.
54. Catanzano O, D'Esposito V, Acierno S, De Caro C, Avagliano C, Ambrosio M, Russo P, Russo R, Miro A, Ungaro F, Calignano A, Formisano P, Quaglia F 2015. Alginate-Hyaluronan composite hydrogels accelerate wound healing process. *Carbohydr Polym* In press.

55. Ermolov AS, Smirnov SV, Khvatov VB, Istranov LP, Koniushko OI, Kolokolchikova EG, Sychevsky MV, Bocharova VS 2008. The use of bioactive wound dressing, stimulating epithelial regeneration of IIIa-degree burn wounds. *Bull Exp Biol Med* 146(1):153-157.
56. Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, Guenou H, Malissen B, Tajbakhsh S, Galy A 2011. Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. *Development* 138(17):3647-3656.
57. Urciuolo A, Quarta M, Morbidoni V, Gattazzo F, Molon S, Grumati P, Montemurro F, Tedesco FS, Blaauw B, Cossu G, Vozzi G, Rando TA, Bonaldo P 2013. Collagen VI regulates satellite cell self-renewal and muscle regeneration. *Nat Commun* 4:1964.
58. Jayakumar R, Prabakaran M, Sudheesh Kumar PT, Nair SV, Tamura H 2011. Biomaterials based on chitin and chitosan in wound dressing applications. *Biotechnol Adv* 29(3):322-337.
59. Ueno H, Yamada H, Tanaka I, Kaba N, Matsuura M, Okumura M, Kadosawa T, Fujinaga T 1999. Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs. *Biomater* 20(15):1407-1414.
60. Unnithan AR, Barakat NA, Pichiah PB, Gnanasekaran G, Nirmala R, Cha YS, Jung CH, El-Newehy M, Kim HY 2012. Wound-dressing materials with antibacterial activity from electrospun polyurethane-dextran nanofiber mats containing ciprofloxacin HCl. *Carbohydr Polym* 90(4):1786-1793.
61. Sinha M, Banik RM, Haldar C, Maiti P 2013. Development of ciprofloxacin hydrochloride loaded poly(ethylene glycol)/chitosan scaffold as wound dressing. *J Porous Mater* 20:799–807.
62. Calo E, Khutoryanskiy V 2015. Biomedical applications of hydrogels: A review of patents and commercial products. *Eur Polym J* 65 (2015) 252–267.
63. Hoare TR, Kohane DS 2008. Hydrogels in drug delivery: Progress and challenges. *Polym* 49 1993e, 2007
64. Jones A, Vaughan D 2005. Hydrogel dressings in the management of a variety of wound types: A review. *J Orthopaed Med* 9: S1-S11.
65. Watson NFS, Hodgkin W 2005. Wound dressings. *Surg (Oxford)*, 23(2): 52-55.
66. Ahmed EM 2015. Hydrogel: preparation, characterization and applications: a review. *6(2): 105-121.*

67. Rosiak JM, Olejniczak J 1993. Medical application of radiation formed hydrogels. *Radiat Phys Chem* 42: 903.
68. Rosiak JM 1991. Hydrogel dressings. In *Radiation Effects on Polymers*, p. 271. ACS Book Ser. 475, Washington, D.C.
69. Fan LH, Zhou XY, Wu PH, Xie WG, Zheng H, Tan W, Liu SH, Li QY 2014. Preparation of carboxymethyl cellulose sulfates and its application as anticoagulant and wound dressing. *Int J Biol Macromol* 66:245-253.
70. Nayak S, Kundu SC 2014. Sericin-carboxymethyl cellulose porous matrices as cellular wound dressing material. *J Biomed Mater Res A* 102(6):1928-1940.
71. Ng SF, Jumaat N 2014. Carboxymethyl cellulose wafers containing antimicrobials: a modern drug delivery system for wound infections. *Eur J Pharm Sci* 51:173-179.
72. Lin WC, Lien CC, Yeh HJ, Yu CM, Hsu SH 2013. Bacterial cellulose and bacterial cellulose-chitosan membranes for wound dressing applications. *Carbohydr Polym* 94(1):603-611.
73. Wu J, Zheng Y, Song W, Luan J, Wen X, Wu Z, Chen X, Wang Q, Guo S 2014. In situ synthesis of silver-nanoparticles/bacterial cellulose composites for slow-released antimicrobial wound dressing. *Carbohydr Polym* 102:762-771.
74. Wu J, Zheng Y, Wen X, Lin Q, Chen X, Wu Z 2014. Silver nanoparticle/bacterial cellulose gel membranes for antibacterial wound dressing: investigation in vitro and in vivo. *Biomed Mater* 9(3):035005.
75. De Moraes MA, Beppu MM 2103. Biocomposite membranes of sodium algi-nate and silk fibroin fibers for biomedical applications. *J Appl Polym Sci* 130:3451–3457.
76. Gobin AS, Froude VE, Mathur AB 2005. Structural and mechanical characteristics of silk fibroin and chitosan blend scaffolds for tissue regeneration. *J Biomed Mater Res A* 74(3):465-473.
77. Liu TL, Miao JC, Sheng WH, Xie YF, Huang Q, Shan YB, Yang JC 2010. Cyto-compatibility of regenerated silk fibroin film: a medical biomaterial applicable to wound healing. *J Zhejiang Univ Sci* 11:10-16.
78. Mishra RK, Majeed ABA, Banthia AK 2011. Development and characterization of pectin/gelatin hydrogel membranes for wound dressing. *Int J Plast Technol* 15:82–95.
79. Munarin F, Tanzi MC, Petrini P 2012. Advances in biomedical applications of pectin gels. *Int J Biol Macromol* 51(4):681-689.

80. Boateng JS, Pawar HV, Tetteh J 2013. Polyox and carrageenan based composite film dressing containing anti-microbial and anti-inflammatory drugs for effective wound healing. *Int J Pharm* 441(1-2):181-191.
81. Pawar HV, Boateng JS, Ayensu I, Tetteh J 2014. Multifunctional medicated lyophilised wafer dressing for effective chronic wound healing. *J Pharm Sci* 103(6):1720-1733.
82. Pawar HV, Tetteh J, Boateng JS 2013. Preparation, optimisation and characterisation of novel wound healing film dressings loaded with streptomycin and diclofenac. *Coll Surf B Biointerf* 102:102-110.
83. Zivanovic S, Li J, Davidson PM, Kit K 2007. Physical, mechanical, and antibacterial properties of chitosan/PEO blend films. *Biomacromol* 8(5):1505-1510.
84. Elbadawy A. Kamoun, El-Refaie S. Kenawy, Tamer M. Tamer, Mahmoud A. El-Meligy, Eldin. MSM 2015. Poly (vinyl alcohol)-alginate physically crosslinked hydrogel membranes for wound dressing applications: Characterization and bio-evaluation. *Arabian J Chem* 8(1):38–47.
85. Hwang MR, Kim JO, Lee JH, Kim YI, Kim JH, Chang SW, Jin SG, Kim JA, Lyoo WS, Han SS, Ku SK, Yong CS, Choi HG 2010. Gentamicin-loaded wound dressing with polyvinyl alcohol/dextran hydrogel: gel characterization and in vivo healing evaluation. *AAPS PharmSciTech* 11(3):1092-1103.
86. Kamoun EA, Kenawy ERS, Tamer TM, El-Meligy MA, Eldin MSM 2015. Poly (vinyl alcohol)-alginate physically crosslinked hydrogel membranes for wound dressing applications: Characterization and bio-evaluation. *Arabian J Chem* 8(1):38-47.
87. Sakai S, Tsumura M, Inoue M, Koga Y, Fukano K, Taya M 2013. Polyvinyl alcohol-based hydrogel dressing gellable on-wound via a co-enzymatic reaction triggered by glucose in the wound exudate. *J Mater Chem B* 1(38):5067-5075.
88. Kontogiannopoulos KN, Assimopoulou AN, Tsvintzelis I, Panayiotou C, Papageorgiou VP 2011. Electrospun fiber mats containing shikonin and derivatives with potential biomedical applications. *Int J Pharm* 409(1-2):216-228.
89. Lee JS, Kim JK, Chang YH 2007. Preparation of collagen/poly(l-lactic acid) composite material for wound dressing. *Macromol Res* 15 15:205–210.
90. Luckachan GE, Pillai CKS 2011. Biodegradable polymers – a review on recent trends and emerging perspectives. *J Polym Environ* 19:637–676.
91. Shingel KI, Di Stabile L, Marty JP, Faure MP 2006. Inflammatory inert poly(ethylene glycol)--protein wound dressing improves healing responses in partial- and full-thickness wounds. *Int Wound J* 3(4):332-342.

92. Bader RA, Herzog KT, Kao WJ 2009. A study of diffusion in poly(ethyleneglycol)-gelatin based semi-interpenetrating networks for use in wound healing. *Polym Bull* 62:381–389.
93. Gultekin G, Atalay-Oral C, Erkal S, Sahin F, Karastova D, Tantekin-Ersolmaz SB, Guner FS 2009. Fatty acid-based polyurethane films for wound dressing applications. *J Mater Sci Mater Med* 20(1):421-431.
94. Yari A, Yeganeh H, Bakhshi H 2012. Synthesis and evaluation of novel absorptive and antibacterial polyurethane membranes as wound dressing. *J Mater Sci Mater Med* 23(9):2187-2202.
95. Brett D 2008. A Review of Collagen and Collagen-based Wound Dressings. *Wounds-a Compendium of Clinical Research and Practice* 20(12):347-356.
96. Fleck CA, Simman R 2010. Modern collagen wound dressings: function and purpose. *J Am Col Certif Wound Spec* 2(3):50-54.
97. Saarai A, Kasparkova V, Sedlacek T, Saha P 2013. On the development and characterisation of crosslinked sodium alginate/gelatine hydrogels. *J Mech Behav Biomed Mater* 18:152-166.
98. Boateng J, Burgos-Amador R, Okeke O, Pawar H 2015. Composite alginate and gelatin based bio-polymeric wafers containing silver sulfadiazine for wound healing. *Int J Biol Macromol* 79:63-71.
99. Anisha BS, Sankar D, Mohandas A, Chennazhi KP, Nair SV, Jayakumar R 2013. Chitosan-hyaluronan/nano chondroitin sulfate ternary composite sponges for medical use. *Carbohydr Polym* 92(2):1470-1476.
100. Mohandas A, Anisha BS, Chennazhi KP, Jayakumar R 2015. Chitosan-hyaluronic acid/VEGF loaded fibrin nanoparticles composite sponges for enhancing angiogenesis in wounds. *Coll Surf B Biointerf* 127:105-113.
101. Dai M, Zheng X, Xu X, Kong X, Li X, Guo G, Luo F, Zhao X, Wei YQ, Qian Z 2009. Chitosan-alginate sponge: preparation and application in curcumin delivery for dermal wound healing in rat. *J Biomed Biotechnol* 2009:595126.
102. Naseri N, Algan C, Jacobs V, John M, Oksman K, Mathew AP 2014. Electrospun chitosan-based nanocomposite mats reinforced with chitin nanocrystals for wound dressing. *Carbohydr Polym* 109:7-15.
103. Huang X, Sun Y, Nie J, Lu W, Yang L, Zhang Z, Yin H, Wang Z, Hu Q 2015. Using absorbable chitosan hemostatic sponges as a promising surgical dressing. *Int J Biol Macromol* 75:322-329.

104. Zhang D, Zhou W, Wei B, Wang X, Tang R, Nie J, Wang J 2015. Carboxyl-modified poly(vinyl alcohol)-crosslinked chitosan hydrogel films for potential wound dressing. *Carbohydr Polym* 125:189-199.
105. Dumville JC, O'Meara S, Deshpande S, Speak K 2013. Alginate dressings for healing diabetic foot ulcers. *Cochrane Database Syst Rev* 6:CD009110.
106. Hrynyk M, Martins-Green M, Barron AE, Neufeld RJ 2012. Alginate-PEG sponge architecture and role in the design of insulin release dressings. *Biomacromol* 13(5):1478-1485.
107. Josef E, Zilberman M, Bianco-Peled H 2010. Composite alginate hydrogels: An innovative approach for the controlled release of hydrophobic drugs. *Acta Biomater* 6(12):4642-4649.
108. Miralles G, Baudoin R, Dumas D, Baptiste D, Hubert P, Stoltz JF, Dellacherie E, Mainard D, Netter P, Payan E 2001. Sodium alginate sponges with or without sodium hyaluronate: in vitro engineering of cartilage. *J Biomed Mater Res* 57(2):268-278.
109. Kaiser D, Hafner J, Mayer D, French LE, Lauchli S 2013. Alginate dressing and polyurethane film versus paraffin gauze in the treatment of split-thickness skin graft donor sites: a randomized controlled pilot study. *Adv Skin Wound Care* 26(2):67-73.
110. Powers JG, Morton LM, Phillips TJ 2013. Dressings for chronic wounds. *Dermatol Ther* 26(3):197-206.
111. White RJ, Cooper R, Kingsley A 2001. Wound colonization and infection: the role of topical antimicrobials. *Br J Nurs* 10(9):563-578.
112. White RJ, Cutting K, Kingsley A 2006. Topical antimicrobials in the control of wound bioburden. *Ostomy Wound Manage* 52(8):26-58.
113. Lipsky BA, Hoey C 2009. Topical Antimicrobial Therapy for Treating Chronic Wounds. *Clin Infec Dis* 49(10):1541-1549.
114. Bowler PG, Duerden BI, Armstrong DG 2001. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 14(2):244-269.
115. Phillips D, Davey C 1997. Wound cleaning versus wound disinfection: a challenging dilemma. *Perspectives* 21(4):15-16.
116. Brook I 1996. Aerobic and anaerobic microbiology of necrotizing fasciitis in children. *Pediatr Dermatol* 13(4):281-284.
117. Halbert AR, Stacey MC, Rohr JB, Jopp-McKay A 1992. The effect of bacterial colonization on venous ulcer healing. *Australas J Dermatol* 33(2):75-80.

118. Sehgal SC, Arunkumar BK 1992. Microbial flora and its significance in pathology of sickle cell disease leg ulcers. *Infection* 20(2):86-88.
119. Twum-Danso K, Grant C, al-Suleiman SA, Abdel-Khader S, al-Awami MS, al-Breiki H, Taha S, Ashoor AA, Wosornu L 1992. Microbiology of postoperative wound infection: a prospective study of 1770 wounds. *J Hosp Infect* 21(1):29-37.
120. Ayton M 1985. Wound care: wounds that won't heal. *Nurs Times* 81(46):suppl 16-19.
121. Hickey J, Panicucci R, Duan Y, Dinehart K, Murphy J, Kessler J, Gottardi W 1997. Control of the amount of free molecular iodine in iodine germicides. *J Pharm Pharmacol* 49(12):1195-1199.
122. Levine NS, Lindberg RB, Mason AD, Jr., Pruitt BA, Jr. 1976. The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. *J Trauma* 16(2):89-94.
123. Church D, Elsayed S, Reid O, Winston B, Lindsay R 2006. Burn wound infections. *Clin Microbiol Rev* 19(2):403-434.
124. Revathi G, Puri J, Jain BK 1998. Bacteriology of burns. *Burns* 24(4):347-349.
125. Vindenes H, Bjercknes R 1995. Microbial colonization of large wounds. *Burns* 21(8):575-579.
126. Oncul O, Yildiz S, Gurer US, Yeniiz E, Qyrdedi T, Top C, Gocer P, Akarsu B, Cevikbas A, Cavuslu S 2007. Effect of the function of polymorphonuclear leukocytes and interleukin-1 beta on wound healing in patients with diabetic foot infections. *J Infect* 54(3):250-256.
127. Japoni A, Farshad S, Ziyaeyan M, Ziaian S 2009. Detection of Van-positive and negative vancomycin resistant enterococci and their antibacterial susceptibility patterns to the newly introduced antibiotics. *Pak J Biol Sci* 12(11):844-851.
128. Hunt TK 1981. Surgical wound infections: an overview. *Am J Med* 70(3):712-718.
129. MacMillan BG 1980. Infections following burn injury. *Surg Clin North Am* 60(1):185-196.
130. Eddy JJ, Gideonsen MD, Mack GP 2008. Practical considerations of using topical honey for neuropathic diabetic foot ulcers: a review. *WMJ* 107(4):187-190.
131. Rizzello L, Pompa PP 2014. Nanosilver-based antibacterial drugs and devices: mechanisms, methodological drawbacks, and guidelines. *Chem Soc Rev* 43(5):1501-1518.

132. Luu YK, Kim K, Hsiao BS, Chu B, Hadjiargyrou M 2003. Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA-PEG block copolymers. *J Control Rel* 89(2):341-353.
133. Dutta NK, Annadurai S, Mazumdar K, Dastidar SG, Kristiansen JE, Molnar J, Martins M, Amaral L 2007. Potential management of resistant microbial infections with a novel non-antibiotic: the anti-inflammatory drug diclofenac sodium. *Int J Antimicrob Agents* 30(3):242-249.
134. Dutta NK, Mazumdar K, Baek MW, Kim DJ, Na YR, Park SH, Lee HK, Lee BH, Park JH 2008. In vitro efficacy of diclofenac against *Listeria monocytogenes*. *Eur J Clin Microbiol Infect Dis* 27(4):315-319.
135. Dutta NK, Mazumdar K, Seok SH, Park JH 2008. The anti-inflammatory drug Diclofenac retains anti-listerial activity in vivo. *Lett Appl Microbiol* 47(2):106-111.
136. Burt S 2004. Essential oils: their antibacterial properties and potential applications in foods – a review. *Int J Food Microbiol* 94:223–253.
137. Llorens E, Calderon S, Del Valle LJ, Puiggali J 2015. Polybiguanide (PHMB) loaded in PLA scaffolds displaying high hydrophobic, biocompatibility and antibacterial properties. *Mater Sci Eng C Mater Biol Appl* 50:74-84.
138. Aoyagi S, Onishi H, Machida Y 2007. Novel chitosan wound dressing loaded with minocycline for the treatment of severe burn wounds. *Int J Pharm* 330(1-2):138-145.
139. Stinner DJ, Noel SP, Haggard WO, Watson JT, Wenke JC 2010. Local antibiotic delivery using tailorable chitosan sponges: the future of infection control? *J Orthop Trauma* 24(9):592-597.
140. Labovitiadi O, Lamb AJ, Matthews KH 2012. In vitro efficacy of antimicrobial wafers against methicillin-resistant *Staphylococcus aureus*. *Ther Deliv* 3(4):443-455.
141. Labovitiadi O, Lamb AJ, Matthews KH 2012. Lyophilised wafers as vehicles for the topical release of chlorhexidine digluconate--release kinetics and efficacy against *Pseudomonas aeruginosa*. *Int J Pharm* 439(1-2):157-164.
142. Labovitiadi O, O'Driscoll NH, Lamb AJ, Matthews KH 2013. Rheological properties of gamma-irradiated antimicrobial wafers and in vitro efficacy against *Pseudomonas aeruginosa*. *Int J Pharm* 453(2):462-472.
143. Klueh U, Wagner V, Kelly S, Johnson A, Bryers JD 2000. Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. *J Biomed Mater Res* 53(6):621-631.

144. Davies RL, Etris SF 1997. The development and functions of silver in water purification and disease control. *Catalysis Today* 36(1):107-114.
145. Yamanaka M, Hara K, Kudo J 2005. Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl Environ Microbiol* 71(11):7589-7593.
146. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PK, Chiu JF, Che CM 2007. Silver nanoparticles: partial oxidation and antibacterial activities. *J Biol Inorg Chem* 12(4):527-534.
147. Rai M, Yadav A, Gade A 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv* 27(1):76-83.
148. Ong SY, Wu J, Moochhala SM, Tan MH, Lu J 2008. Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. *Biomaterials* 29(32):4323-4332.
149. Madhumathi K, Sudheesh Kumar PT, Abhilash S, Sreeja V, Tamura H, Manzoor K, Nair SV, Jayakumar R 2010. Development of novel chitin/nanosilver composite scaffolds for wound dressing applications. *J Mater Sci Mater Med* 21(2):807-813.
150. Pant B, Pant HR, Pandeya DR, Panthi G, Nam KT, Hong ST, Kim CS, Kim HY 2012. Characterization and antibacterial properties of Ag NPs loaded nylon-6 nanocomposite prepared by one-step electrospinning process. *Coll Surf A* 395:94-99.
151. Ciloglu NS, Mert AI, Dogan Z, Demir A, Cevan S, Aksaray S, Tercan M 2014. Efficacy of silver-loaded nanofiber dressings in *Candida albicans*-contaminated full-skin thickness rat burn wounds. *J Burn Care Res* 35(5):e317-320.
152. Lin YH, Hsu WS, Chung WY, Ko TH, Lin JH 2014. Evaluation of various silver-containing dressing on infected excision wound healing study. *J Mater Sci-Mater Med* 25(5):1375-1386.
153. Archana D, Singh BK, Dutta J, Dutta PK 2015. Chitosan-PVP-nano silver oxide wound dressing: in vitro and in vivo evaluation. *Int J Biol Macromol* 73:49-57.
154. Gaisford S, Beezer AE, Bishop AH, Walker M, Parsons D 2009. An in vitro method for the quantitative determination of the antimicrobial efficacy of silver-containing wound dressings. *Int J Pharm* 366(1-2):111-116.

155. Said J, Dodoo CC, Walker M, Parsons D, Stapleton P, Beezer AE, Gaisford S 2014. An in vitro test of the efficacy of silver-containing wound dressings against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in simulated wound fluid. *Int J Pharm* 462(1-2):123-128.
156. Fries CA, Ayalew Y, Penn-Barwell JG, Porter K, Jeffery SL, Midwinter MJ 2014. Prospective randomised controlled trial of nanocrystalline silver dressing versus plain gauze as the initial post-debridement management of military wounds on wound microbiology and healing. *Injury* 45(7):1111-1116.
157. Gee Kee EL, Kimble RM, Cuttle L, Khan A, Stockton KA 2015. Randomized controlled trial of three burns dressings for partial thickness burns in children. *Burns*.
158. Lindsay S. Silver White Paper, Everything you ever wanted to know about the use of silver in wound therapy, 2011. http://www.systagenix.co.uk/cms/uploads/1458_Silver_WhitePaperA4_LP3_060.pdf (Accessed on 17 February 2015)
159. Altman H, Steinberg D, Porat Y, Mor A, Fridman D, Friedman M, Bachrach G 2006. In vitro assessment of antimicrobial peptides as potential agents against several oral bacteria. *J Antimicrob Chemother* 58(1):198-201.
160. Reddy KV, Yedery RD, Aranha C 2004. Antimicrobial peptides: premises and promises. *Int J Antimicrob Agents* 24(6):536-547.
161. Alves D, Olivia Pereira M 2014. Mini-review: Antimicrobial peptides and enzymes as promising candidates to functionalize biomaterial surfaces. *Biofouling* 30(4):483-499.
162. Ng VW, Chan JM, Sardon H, Ono RJ, Garcia JM, Yang YY, Hedrick JL 2014. Antimicrobial hydrogels: a new weapon in the arsenal against multidrug-resistant infections. *Adv Drug Deliv Rev* 78:46-62.
163. O'Driscoll NH, Labovitiadi O, Cushnie TP, Matthews KH, Mercer DK, Lamb AJ 2013. Production and evaluation of an antimicrobial peptide-containing wafer formulation for topical application. *Curr Microbiol* 66(3):271-278.
164. Gomes AP, Mano JF, Queiroz JA, Gouveia IC 2015. Incorporation of antimicrobial peptides on functionalized cotton gauzes for medical applications. *Carbohydr Polym* 127:451-461.
165. Miao J, Pangule RC, Paskaleva EE, Hwang EE, Kane RS, Linhardt RJ, Dordick JS 2011. Lysostaphin-functionalized cellulose fibers with antistaphylococcal activity for wound healing applications. *Biomater* 32(36):9557-9567.

166. Fischetti VA 2008. Bacteriophage lysins as effective antibacterials. *Curr Opin Microbiol* 11(5):393-400.
167. Hadaway L 2010. Polyhexamethylene Biguanide Dressing – Another Promising Tool to Reduce Catheter-related Bloodstream Infection. *J Assoc Vascul Access* 15(4):203–205.
168. Piatkowski A, Drummer N, Andriessen A, Ulrich D, Pallua N 2011. Randomized controlled single center study comparing a polyhexanide containing bio-cellulose dressing with silver sulfadiazine cream in partial-thickness dermal burns. *Burns* 37(5):800-804.
169. Dilamian M, Montazer M, Masoumi J 2013. Antimicrobial electrospun membranes of chitosan/poly(ethylene oxide) incorporating poly(hexamethylene biguanide) hydrochloride. *Carbohydr Polym* 94(1):364-371.
170. White RJ 2009. Wound infection-associated pain. *J Wound Care* 18(6):245-249.
171. Glaser R, Kiecolt-Glaser J, PT. M 1999. Stress-related changes in pro inflammatory cytokine production in wounds. *Arch Gen Psychiatry* 56:450–456.
172. World Union of Wound Healing Societies (WUWHS). 2004. Principles of best practice: Minimising pain at wound dressing-related procedures. A consensus document., ed., London.
173. Glynn C. 2002. The control of pain associated with chronic leg ulcers. *The Oxford European wound healing course handbook*, ed., Oxford: Positif Press. p 99–109.
174. Jorgensen B, Friis GJ, Gottrup F 2006. Pain and quality of life for patients with venous leg ulcers: proof of concept of the efficacy of Biatain-Ibu, a new pain reducing wound dressing. *Wound Repair Regen* 14(3):233-239.
175. Arapoglou V, Katsenis K, Syrigos KN, Dimakakos EP, Zakopoulou N, Gjodsbol K, Glynn C, Schafer E, Petersen B, Tsoutos D 2011. Analgesic efficacy of an ibuprofen-releasing foam dressing compared with local best practice for painful exuding wounds. *J Wound Care* 20(7):319-320, 322-315.
176. Canton I, McKean R, Charnley M, Blackwood KA, Fiorica C, Ryan AJ, MacNeil S 2010. Development of an Ibuprofen-releasing biodegradable PLA/PGA electrospun scaffold for tissue regeneration. *Biotechnol Bioeng* 105(2):396-408.

177. Fogh K, Andersen MB, Bischoff-Mikkelsen M, Bause R, Zutt M, Schilling S, Schmutz JL, Borbujo J, Jimenez JA, Cartier H, Jorgensen B 2012. Clinically relevant pain relief with an ibuprofen-releasing foam dressing: results from a randomized, controlled, double-blind clinical trial in exuding, painful venous leg ulcers. *Wound Repair Regen* 20(6):815-821.
178. Romanelli M, Dini V, Polignano R, Bonadeo P, Maggio G 2009. Ibuprofen slow-release foam dressing reduces wound pain in painful exuding wounds: preliminary findings from an international real-life study. *J Dermatolog Treat* 20(1):19-26.
179. Bigliardi PL, Neumann C, Teo YL, Pant A, Bigliardi-Qi M 2015. Activation of the delta-opioid receptor promotes cutaneous wound healing by affecting keratinocyte intercellular adhesion and migration. *Br J Pharmacol* 172(2):501-514.
180. Stein C, Kuchler S 2013. Targeting inflammation and wound healing by opioids. *Trends Pharmacol Sci* 34(6):303-312.
181. Heilmann S, Kuchler S, Wischke C, Lendlein A, Stein C, Schafer-Korting M 2013. A thermosensitive morphine-containing hydrogel for the treatment of large-scale skin wounds. *Int J Pharm* 444(1-2):96-102.
182. Chen FM, Zhang M, Wu ZF 2010. Toward delivery of multiple growth factors in tissue engineering. *Biomater* 31(24):6279-6308.
183. Taipale J, Keski-Oja J 1997. Growth factors in the extracellular matrix. *FASEB J* 11(1):51-59.
184. Schultz GS, Wysocki A 2009. Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen* 17(2):153-162.
185. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M 2008. Growth factors and cytokines in wound healing. *Wound Repair Regen* 16(5):585-601.
186. Barrientos S, Brem H, Stojadinovic O, Tomic-Canic M 2014. Clinical application of growth factors and cytokines in wound healing. *Wound Repair Regen* 22(5):569-578.
187. Smith & Nephew website. REGRANEX (Becaplermin) Gel 0.01%, <http://www.smith-nephew.com/key-products/advanced-wound-management/regranex-becaplermin-gel/>. (Accessed on 31/01/2015)
188. Food and Drug Administration. Regranex, 2008. <http://www.fda.gov/downloads/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/UCM142821.pdf>. (Accessed on 31/01/2015)

189. Fang RC, Galiano RD 2008. A review of becaplermin gel in the treatment of diabetic neuropathic foot ulcers. *Biologics* 2(1):1-12.
190. Steed DL 1995. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. Diabetic Ulcer Study Group. *J Vasc Surg* 21(1):71-78; discussion 79-81.
191. Wieman TJ, Smiell JM, Su Y 1998. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. *Diabetes Care* 21(5):822-827.
192. Margolis DJ, Bartus C, Hoffstad O, Malay S, Berlin JA 2005. Effectiveness of recombinant human platelet-derived growth factor for the treatment of diabetic neuropathic foot ulcers. *Wound Repair Regen* 13(6):531-536.
193. Tanaka A, Nagate T, Matsuda H 2005. Acceleration of wound healing by gelatin film dressings with epidermal growth factor. *J Vet Med Sci* 67(9):909-913.
194. Ulubayram K, Nur Cakar A, Korkusuz P, Ertan C, Hasirci N 2001. EGF containing gelatin-based wound dressings. *Biomater* 22(11):1345-1356.
195. Ribeiro MP, Morgado PI, Miguel SP, Coutinho P, Correia IJ 2013. Dextran-based hydrogel containing chitosan microparticles loaded with growth factors to be used in wound healing. *Mater Sci Eng C Mater Biol Appl* 33(5):2958-2966.
196. Lai HJ, Kuan CH, Wu HC, Tsai JC, Chen TM, Hsieh DJ, Wang TW 2014. Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing. *Acta Biomater* 10(10):4156-4166.
197. Wang W, Lin S, Xiao Y, Huang Y, Tan Y, Cai L, Li X 2008. Acceleration of diabetic wound healing with chitosan-crosslinked collagen sponge containing recombinant human acidic fibroblast growth factor in healing-impaired STZ diabetic rats. *Life Sci* 82(3-4):190-204.
198. Zamani M, Prabhakaran MP, Ramakrishna S 2013. Advances in drug delivery via electrospun and electrosprayed nanomaterials. *Int J Nanomedicine* 8:2997-3017.
199. Yang Y, Xia T, Zhi W, Wei L, Weng J, Zhang C, Li X 2011. Promotion of skin regeneration in diabetic rats by electrospun core-sheath fibers loaded with basic fibroblast growth factor. *Biomater* 32(18):4243-4254.

200. Choi JS, Leong KW, Yoo HS 2008. In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF). *Biomater* 29(5):587-596.
201. Kulkarni A, Diehl-Jones W, Ghanbar S, Liu S 2014. Layer-by-layer assembly of epidermal growth factors on polyurethane films for wound closure. *J Biomater Appl* 29(2):278-290.
202. Jazwa A, Kucharzewska P, Leja J, Zagorska A, Sierpniowska A, Stepniowski J, Kozakowska M, Taha H, Ochiya T, Derlacz R, Vahakangas E, Yla-Herttuala S, Jozkowicz A, Dulak J 2010. Combined vascular endothelial growth factor-A and fibroblast growth factor 4 gene transfer improves wound healing in diabetic mice. *Genet Vaccines Ther* 8:6.
203. Mazzucco L, Borzini P, Gope R 2010. Platelet-derived factors involved in tissue repair-from signal to function. *Transfus Med Rev* 24(3):218-234.
204. Barsotti MC, Losi P, Briganti E, Sanguinetti E, Magera A, Al Kayal T, Feriani R, Di Stefano R, Soldani G 2013. Effect of platelet lysate on human cells involved in different phases of wound healing. *PLoS One* 8(12):e84753.
205. Rossi S, Faccendini A, Bonferoni MC, Ferrari F, Sandri G, Del Fante C, Perotti C, Caramella CM 2013. "Sponge-like" dressings based on biopolymers for the delivery of platelet lysate to skin chronic wounds. *Int J Pharm* 440(2):207-215.
206. Sandri G, Bonferoni MC, Rossi S, Ferrari F, Mori M, Del Fante C, Perotti C, Scudeller L, Caramella C 2011. Platelet lysate formulations based on mucoadhesive polymers for the treatment of corneal lesions. *J Pharm Pharmacol* 63(2):189-198.
207. Sandri G, Bonferoni MC, Rossi S, Ferrari F, Mori M, Del Fante C, Perotti C, Caramella C 2012. Thermosensitive eyedrops containing platelet lysate for the treatment of corneal ulcers. *Int J Pharm* 426(1-2):1-6.
208. Mori M, Rossi S, Bonferoni MC, Ferrari F, Sandri G, Riva F, Del Fante C, Perotti C, Caramella C 2014. Calcium alginate particles for the combined delivery of platelet lysate and vancomycin hydrochloride in chronic skin ulcers. *Int J Pharm* 461(1-2):505-513.
209. Branski LK, Pereira CT, Herndon DN, Jeschke MG 2007. Gene therapy in wound healing: present status and future directions. *Gene Ther* 14(1):1-10.
210. Hengge UR, Chan EF, Foster RA, Walker PS, Vogel JC 1995. Cytokine gene expression in epidermis with biological effects following injection of naked DNA. *Nat Genet* 10(2):161-166.

211. Cam C, Segura T 2013. Matrix-based gene delivery for tissue repair. *Curr Opin Biotechnol* 24(5):855-863.
212. Breen AM, Dockery P, O'Brien T, Pandit AS 2008. The use of therapeutic gene eNOS delivered via a fibrin scaffold enhances wound healing in a compromised wound model. *Biomater* 29(21):3143-3151.
213. Chandler LA, Gu DL, Ma C, Gonzalez AM, Doukas J, Nguyen T, Pierce GF, Phillips ML 2000. Matrix-enabled gene transfer for cutaneous wound repair. *Wound Repair Regen* 8(6):473-479.
214. Gu DL, Nguyen T, Gonzalez AM, Printz MA, Pierce GF, Sosnowski BA, Phillips ML, Chandler LA 2004. Adenovirus encoding human platelet-derived growth factor-B delivered in collagen exhibits safety, biodistribution, and immunogenicity profiles favorable for clinical use. *Mol Ther* 9(5):699-711.
215. Felgner PL, Rhodes G 1991. Gene therapeutics. *Nat* 349(6307):351-352.
216. Lee PY, Li Z, Huang L 2003. Thermosensitive hydrogel as a Tgf-beta1 gene delivery vehicle enhances diabetic wound healing. *Pharm Res* 20(12):1995-2000.
217. Kong HJ, Kim ES, Huang YC, Mooney DJ 2008. Design of biodegradable hydrogel for the local and sustained delivery of angiogenic plasmid DNA. *Pharm Res* 25(5):1230-1238.
218. Saraf A, Baggett LS, Raphael RM, Kasper FK, Mikos AG 2010. Regulated non-viral gene delivery from coaxial electrospun fiber mesh scaffolds. *J Control Rel* 143(1):95-103.
219. Layliev J, Wilson S, Warren SM, Saadeh PB 2012. Improving Wound Healing with Topical Gene Therapy. *Adv Wound Care (New Rochelle)* 1(5):218-223.
220. Kozielski KL, Tzeng SY, Green JJ 2013. Bioengineered nanoparticles for siRNA delivery. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 5(5):449-468.
221. Nelson CE, Kim AJ, Adolph EJ, Gupta MK, Yu F, Hocking KM, Davidson JM, Guelcher SA, Duvall CL 2014. Tunable delivery of siRNA from a biodegradable scaffold to promote angiogenesis in vivo. *Adv Mater* 26(4):607-614, 506.
222. Kim HS, Yoo HS 2013. Matrix metalloproteinase-inspired suicidal treatments of diabetic ulcers with siRNA-decorated nanofibrous meshes. *Gene Ther* 20(4):378-385.
223. Castleberry S, Wang M, Hammond PT 2013. Nanolayered siRNA dressing for sustained localized knockdown. *ACS Nano* 7(6):5251-5261.

224. Cha J, Falanga V 2007. Stem cells in cutaneous wound healing. *Clin Dermatol* 25(1):73-78.
225. Chen JS, Wong VW, Gurtner GC 2012. Therapeutic potential of bone marrow-derived mesenchymal stem cells for cutaneous wound healing. *Front Immunol* 3:192.
226. Cherubino M, Rubin JP, Miljkovic N, Kelmendi-Doko A, Marra KG 2011. Adipose-derived stem cells for wound healing applications. *Ann Plast Surg* 66(2):210-215.
227. Branski LK, Gauglitz GG, Herndon DN, Jeschke MG 2009. A review of gene and stem cell therapy in cutaneous wound healing. *Burns* 35(2):171-180.
228. Yolanda MM, Maria AV, Amaia FG, Marcos PB, Silvia PL, Escudero D, Jesús OH 2014. Adult Stem Cell Therapy in Chronic Wound Healing. *J Stem Cell Res Ther* 4(162).
229. Yoshikawa T, Mitsuno H, Nonaka I, Sen Y, Kawanishi K, Inada Y, Takakura Y, Okuchi K, Nonomura A 2008. Wound therapy by marrow mesenchymal cell transplantation. *Plast Reconstr Surg* 121(3):860-877.
230. Hunt NC, Grover LM 2010. Cell encapsulation using biopolymer gels for regenerative medicine. *Biotechnol Lett* 32(6):733-742.
231. Seol D, Magnetta MJ, Ramakrishnan PS, Kurriger GL, Choe H, Jang K, Martin JA, Lim TH 2013. Biocompatibility and preclinical feasibility tests of a temperature-sensitive hydrogel for the purpose of surgical wound pain control and cartilage repair. *J Biomed Mater Res B Appl Biomater*.
232. Dong YX, Hassan WU, Kennedy R, Greiser U, Pandit A, Garcia Y, Wang WX 2014. Performance of an in situ formed bioactive hydrogel dressing from a PEG-based hyperbranched multifunctional copolymer. *Acta Biomater* 10(5):2076-2085.
233. Davis SC, Perez R 2009. Cosmeceuticals and natural products: wound healing. *Clin Dermatol* 27(5):502-506.
234. Ni Y, Yates KM, Tizard IR. 2004. Aloes: The genus Aloe. In Reynolds T, editor, ed., New York: CRC Press LLC.
235. Djeraba A, Quere P 2000. In vivo macrophage activation in chickens with Acemannan, a complex carbohydrate extracted from Aloe vera. *Int J Immunopharmacol* 22(5):365-372.
236. Boudreau MD, Beland FA 2006. An evaluation of the biological and toxicological properties of Aloe barbadensis (miller), Aloe vera. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 24(1):103-154.

237. Inpanya P, Faikrua A, Ounaron A, Sittichokechaiwut A, Viyoch J 2012. Effects of the blended fibroin/aloë gel film on wound healing in streptozotocin-induced diabetic rats. *Biomed Mater* 7(3):035008.
238. Pereira R, Carvalho A, Vaz DC, Gil MH, Mendes A, Bartolo P 2013. Development of novel alginate based hydrogel films for wound healing applications. *Int J Biol Macromol* 52:221-230.
239. Silva SS, Caridade SG, Mano JF, Reis RL 2013. Effect of crosslinking in chitosan/aloë vera-based membranes for biomedical applications. *Carbohydr Polym* 98(1):581-588.
240. Motealleh B, Zahedi P, Rezaeian I, Moghimi M, Abdolghaffari AH, Zarandi MA 2014. Morphology, drug release, antibacterial, cell proliferation, and histology studies of chamomile-loaded wound dressing mats based on electrospun nanofibrous poly(varepsilon-caprolactone)/polystyrene blends. *J Biomed Mater Res B Appl Biomater* 102(5):977-987.
241. Antonio F, Guillem R, Sonia T, Clara M, Piergiorgio G, Valeria C, Gianluca C, Tzanov T 2011. Cross-linked collagen sponges loaded with plant polyphenols with inhibitory activity towards chronic wound enzymes. *Biotechnol J* 6(10):1208-1218.
242. Tan SP, McLoughlin P, O'Sullivan L, Prieto ML, Gardiner GE, Lawlor PG, Hughes H 2013. Development of a novel antimicrobial seaweed extract-based hydrogel wound dressing. *Int J Pharm* 456(1):10-20.
243. Bhatnagar M, Parwani L, Sharma V, Ganguli J, Bhatnagar A 2013. Hemostatic, antibacterial biopolymers from *Acacia arabica* (Lam.) Willd. and *Moringa oleifera* (Lam.) as potential wound dressing materials. *Indian J Exp Biol* 51(10):804-810.
244. Muthukumar T, Prabu P, Ghosh K, Sastry TP 2014. Fish scale collagen sponge incorporated with *Macrotyloma uniflorum* plant extract as a possible wound/burn dressing material. *Coll Surf B Biointerf* 113:207-212.
245. Dorai AA 2012. Wound care with traditional, complementary and alternative medicine. *Indian J Plast Surg* 45(2):418-424.
246. Hammer KA, Carson CF, Riley TV 1999. Antimicrobial activity of essential oils and other plant extracts. *J Appl Microbiol* 86(6):985-990.
247. Liakos I, Rizzello L, Scurr DJ, Pompa PP, Bayer IS, Athanassiou A 2014. All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. *Int J Pharm* 463(2):137-145.

248. Catanzano O, Straccia MC, Miro A, Ungaro F, Romano I, Mazzarella G, Santagata G, Quaglia F, Laurienzo P, Malinconico M 2014. Spray-by-spray in situ cross-linking alginate hydrogels delivering a tea tree oil microemulsion. *Eur J Pharm Sci* 66C:20-28.
249. Altıok D, Altıok E, Tihminlioglu F 2010. Physical, antibacterial and antioxidant properties of chitosan films incorporated with thyme oil for potential wound healing applications. *J Mater Sci Mater Med* 21(7):2227-2236.
250. Kavooosi G, Dadfar SMM, Purfard AM 2013. Mechanical, Physical, Antioxidant, and Antimicrobial Properties of Gelatin Films Incorporated with Thymol for Potential Use as Nano Wound Dressing. *J Food Scie* 78(2):E244-E250.
251. Dias AM, Braga ME, Seabra IJ, Ferreira P, Gil MH, de Sousa HC 2011. Development of natural-based wound dressings impregnated with bioactive compounds and using supercritical carbon dioxide. *Int J Pharm* 408(1-2):9-19.
252. Anghel I, Holban AM, Grumezescu AM, Andronescu E, Ficiu A, Anghel AG, Maganu M, Laz RV, Chifiriuc MC 2012. Modified wound dressing with phytonanostructured coating to prevent staphylococcal and pseudomonal biofilm development. *Nanoscale Res Lett* 7(1):690.
253. Charernsriwilaiwat N, Rojanarata T, Ngawhirunpat T, Sukma M, Opanasopit P 2013. Electrospun chitosan-based nanofiber mats loaded with *Garcinia mangostana* extracts. *Int J Pharm* 452(1-2):333-343.
254. Molan P 2001. Honey as a Topical Antibacterial Agent for Treatment of Infected Wounds. *Am J Of Clin Derm* 2(1):13-19.
255. Mandal S, Mandal M 2011. Honey: Its Medicinal Properties and Antibacteria Activity. *Asian Pacif J Trop Biomed*:15-160.
256. Bansal V, Medhi B, Pandhi 2005. Honey - A remedy Rediscovered And Its Therapeutic Utility. *Kathmandu Univ Med J* 3:305-309.
257. Eteraf-Oskouei T, Najafi M 2013. Traditional and Modern Uses of Natural Honey in Human Diseases: A Review. *Iranian J Basic Med Sci* 16(6):731-742.
258. Molan P 2009. Debridement of Wounds with Honey. *J Woun Tech* 5:12-18.
259. Molan P 2011. The Evidence and The Rationale For The Use Of Honey as a Wound Dressing. *Wound Pract Res* 19(4):201-221.
260. Seckam A, Cooper R 2013. Understanding How Honey Impacts on Wounds: An Update on Recent Research Findings. *Wounds Int* 4(1):20-24.

261. Schneider M, Coyle S, Warnock M, Gow I, Fyfe L 2013. Anti-microbial activity and composition of manuka and portobello honey. *Phytother Res* 27(8):1162-1168.
262. Cantarelli M, Pellerano R, Marchevsky E, Camina JM 2008. Quality of honey from Argentina: Study of chemical Composition and Trace Elements. *J Arg Chem Soc* 96:33-41.
263. White JW, Doner LW. 1980. Honey Composition and Properties. In agriculture USDo, editor *Beekiing In The United States*, ed. p 82-92.
264. Khalil MI, Sulaiman SA 2010. The Potential Role of Honey and Its Polyphenols in Preventing Heart Diseases: A Review. *Afric J Trad Complement Alt Med* 7(4):315-321.
265. Hill KE, Malic S, McKee R, Rennison T, Harding KG, Williams DW, Thomas DW 2010. An in vitro model of chronic wound biofilms to test wound dressings and assess antimicrobial susceptibilities. *J Antimicrob Chemother* 65(6):1195-1206.
266. Molan P 2012. The Antibacterial Activity of Honey and Its Role in Treating Diseases. *Academiaedu*:1-18.
267. White JW, Subers MH, Schepartz AI 1963. The identification of Inhibine, the Antibacterial Factor in Honey as Hydrogen Peroxide and Its Origin in a Honey Glucose-Oxidase System. *Biochim Biophys Acta* 73:57-70.
268. Brudzynski K 2006. Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. *Canadian J Microbiol* 52(12):1228-1237.
269. Molan P 1992. The Antibacterial Activity of Honey.1.The nature of the antibacterial activity. *Bee World* 73(1):5-28.
270. Bogdanov S, Martin P, Lullmann C 1997. Harmonised methods of the European Honey Commission. *Apidologie*:3-59.
271. Molan P 2001. Why Honey Is Effective As A Medicine. *Bee World* 82(1):22-40.
272. Mohrig W, Messner B 1968. Lysozyme as an Antibacterial Agent in Honey and Bee Venom. *Acta Biologica et Medica Germanica* 21:85-95.
273. Estevinho L, Pereira AP, Moreira L, Dias LG, Pereira E 2008. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food Chem Toxicol* 46(12):3774-3779.
274. Jull AB, Cullum N, Dumville JC, Westby MJ, Deshpande S, Walker N 2015. Honey as a topical treatment for wounds. *Cochrane Database Syst Rev* 3:CD005083.

275. Lay-flurrie K 2008. Honey in wound care: effects, clinical application and patient benefit. *Br J Nurs* 17(11):S30, S32-36.
276. Visavadia BG, Honeysett J, Danford M 2008. Manuka honey dressing: an effective treatment for chronic wound infections. *Br J Oral Maxillofac Surg* 46(8):696-697.
277. Kamaratos AV, Tzirogiannis KN, Iraklianos SA, Panoutsopoulos GI, Kanellos IE, Melidonis AI 2014. Manuka honey-impregnated dressings in the treatment of neuropathic diabetic foot ulcers. *Int Wound J* 11(3):259-263.
278. Mavric E, Wittmann S, Barth G, Henle T 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Mol Nutr Food Res* 52(4):483-489.
279. Cooke J, Dryden M, Patton T, Brennan J, Barrett J 2015. The antimicrobial activity of prototype modified honeys that generate reactive oxygen species (ROS) hydrogen peroxide. *BMC Research Notes* 8(20).
280. Dryden M, Tawse C, Adams J, Howard A, Saeed K, Cooke J 2014. The use of Surgihoney to prevent or eradicate bacterial colonisation in dressing oncology long vascular lines. *J Wound Care* 23(6):338-341.
281. Iyyam Pillai S, Palsamy P, Subramanian S, Kandaswamy M 2010. Wound healing properties of Indian propolis studied on excision wound-induced rats. *Pharm Biol* 48(11):1198-1206.
282. McLennan SV, Bonner J, Milne S, Lo L, Charlton A, Kurup S, Jia J, Yue DK, Twigg SM 2008. The anti-inflammatory agent Propolis improves wound healing in a rodent model of experimental diabetes. *Wound Repair Regen* 16(5):706-713.
283. de Almeida EB, Cordeiro Cardoso J, Karla de Lima A, de Oliveira NL, de Pontes-Filho NT, Oliveira Lima S, Leal Souza IC, de Albuquerque-Junior RL 2013. The incorporation of Brazilian propolis into collagen-based dressing films improves dermal burn healing. *J Ethnopharmacol* 147(2):419-425.
284. Pillai CK, Sharma CP 2010. Review paper: absorbable polymeric surgical sutures: chemistry, production, properties, biodegradability, and performance. *J Biomater Appl* 25(4):291-366.
285. Hranjec T, Swenson BR, Sawyer RG 2010. Surgical site infection prevention: how we do it. *Surg Infect (Larchmt)* 11(3):289-294.

286. Mingmalairak C. 2011. Antimicrobial sutures: New strategy in surgical site infections. In Mendez-Vilas A, editor *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances*, ed.: Formatex Research Center. p 313-323.
287. Gomez-Alonso A, Garcia-Criado FJ, Parreno-Manchado FC, Garcia-Sanchez JE, Garcia-Sanchez E, Parreno-Manchado A, Zambrano-Cuadrado Y 2007. Study of the efficacy of Coated VICRYL Plus Antibacterial suture (coated Polyglactin 910 suture with Triclosan) in two animal models of general surgery. *J Infect* 54(1):82-88.
288. Obermeier A, Schneider J, Wehner S, Matl FD, Schieker M, von Eisenhart-Rothe R, Stemberger A, Burgkart R 2014. Novel high efficient coatings for antimicrobial surgical sutures using chlorhexidine in fatty acid slow-release carrier systems. *PLoS One* 9(7):e101426.
289. Blaker JJ, Nazhat SN, Boccaccini AR 2004. Development and characterisation of silver-doped bioactive glass-coated sutures for tissue engineering and wound healing applications. *Biomater* 25(7-8):1319-1329.
290. Zhang SW, Liu XL, Wang HL, Peng J, Wong KKY 2014. Silver nanoparticle-coated suture effectively reduces inflammation and improves mechanical strength at intestinal anastomosis in mice. *J Pediatr Surg* 49(4):606-613.
291. Bigalke C, Luderer F, Wulf K, Storm T, Lobler M, Arbeiter D, Rau BM, Nizze H, Vollmar B, Schmitz KP, Klar E, Sternberg K 2014. VEGF-releasing suture material for enhancement of vascularization: development, in vitro and in vivo study. *Acta Biomater* 10(12):5081-5089.
292. Fuchs TF, Surke C, Stange R, Quandt S, Wildemann B, Raschke MJ, Schmidmaier G 2012. Local delivery of growth factors using coated suture material. *ScientificWorldJournal* 2012:109216.
293. Dines JS, Weber L, Razzano P, Prajapati R, Timmer M, Bowman S, Bonasser L, Dines DM, Grande DP 2007. The effect of growth differentiation factor-5-coated sutures on tendon repair in a rat model. *J Shoulder Elbow Surg* 16(5 Suppl):S215-221.
294. Reckhenrich AK, Kirsch BM, Wahl EA, Schenck TL, Rezaeian F, Harder Y, Foehr P, Machens HG, Egana JT 2014. Surgical sutures filled with adipose-derived stem cells promote wound healing. *PLoS One* 9(3):e91169.
295. Pasternak B, Missios A, Askendal A, Tengvall P, Aspenberg P 2007. Doxycycline-coated sutures improve the suture-holding capacity of the rat Achilles tendon. *Acta Orthop* 78(5):680-686.

296. Guyette JP, Fakharzadeh M, Burford EJ, Tao ZW, Pins GD, Rolle MW, Gaudette GR 2013. A novel suture-based method for efficient transplantation of stem cells. *J Biomed Mater Res A* 101(3):809-818.
297. Lee JE, Park S, Park M, Kim MH, Park CG, Lee SH, Choi SY, Kim BH, Park HJ, Park JH, Heo CY, Choy YB 2013. Surgical suture assembled with polymeric drug-delivery sheet for sustained, local pain relief. *Acta Biomater* 9(9):8318-8327.
298. He CL, Huang ZM, Han XJ 2009. Fabrication of drug-loaded electrospun aligned fibrous threads for suture applications. *J Biomed Mater Res A* 89(1):80-95.
299. Weldon CB, Tsui JH, Shankarappa SA, Nguyen VT, Ma M, Anderson DG, Kohane DS 2012. Electrospun drug-eluting sutures for local anesthesia. *J Control Rel* 161(3):903-909.
300. Costantino U, Ambroggi V, Nocchetti M, Perioli L 2008. Hydrotalcite-like compounds: versatile layered hosts of molecular anions with biological activity. *Microporous Mesoporous Mater* 107:149–160.
301. Catanzano O, Acierno S, Russo P, Cervasio M, Del Basso De Caro M, Bolognese A, Sammartino G, Califano L, Marenzi G, Calignano A, Acierno D, Quaglia F 2014. Melt-spun bioactive sutures containing nanohybrids for local delivery of anti-inflammatory drugs. *Mater Sci Eng C Mater Biol Appl* 43:300-309.
302. Mansbridge JN. 2013. Tissue-Engineered Skin Substitutes. *Biomaterials Science (Third Edition), An Introduction to Materials in Medicine*, ed. p 1276–1288.
303. Limova M 2010. Active Wound Coverings: Bioengineered Skin and Dermal Substitutes. *Surgical Clinics of North America* 90(6):1237-+.
304. Auger FA, Lacroix D, Germain L. 2009. Skin substitutes and wound healing. *Skin Pharmacol Physiol* 22(2): 94-102.
305. Hartmann-Fritsch F, Biedermann T, Braziulis E, Luginbühl J, Pontiggia L, Böttcher-Haberzeth S, van Kuppevelt TH, Faraj KA, Schiestl C, Meuli M, Reichmann E 2012. Collagen hydrogels strengthened by biodegradable meshes are a basis for dermo-epidermal skin grafts intended to reconstitute human skin in a one-step surgical intervention. *J Tissue Eng Regen Med* doi: 10.1002/term.1665.
306. 306. Afeesh R, Unnithan, Nasser A.M, Barakat, P.B, Tirupathi Pichiah, Gopalsamy Gnanasekaran^e, R. Nirmala, Youn-Soo Cha, Che-Hun Jung, Mohamed El-Newehy, Hak Yong Kim 2012. Wound-dressing materials with antibacterial activity from electrospun polyurethane–dextran nanofiber mats containing ciprofloxacin HCl. *Carbohydr Polym* 90(4): 1786-1793.

307. Guorui Jin, Molamma P. Prabhakaran, Dan Kai b,c, Sathesh Kumar Annamalai Kantha D. Arunachalam d, Seeram Ramakrishna 2013. Tissue engineered plant extracts as nanofibrous wound dressing. *Biomater* 34: 724 – 734.
308. Michael S, Sorg H, Peck CT, Reimers K, Vogt PM 2013. The mouse dorsal skin fold chamber as a means for the analysis of tissue engineered skin. *Burns* 39(1):82-88.
309. Morissette Martin P, Maux A, Laterreur V, Mayrand D, V LG, Moulin VJ, Fradette J 2015. Enhancing repair of full-thickness excisional wounds in a murine model: Impact of tissue-engineered biological dressings featuring human differentiated adipocytes. *Acta Biomater* (In press).
310. Netchiporouk E, Armour A, De Oliveira A 2010. Development of epidermal-dermal tissue engineered skin substitutes. *J Am Acad Dermatol* 62(3):Ab144-Ab144.
311. Keck M, Haluza D, Lumenta DB, Burjak S, Eisenbock B, Kamolz LP, Frey M 2011. Construction of a multi-layer skin substitute: Simultaneous cultivation of keratinocytes and preadipocytes on a dermal template. *Burns* 37(4):626-630.
312. Sharma K, Bullock A, Ralston D, MacNeil S 2014. Development of a one-step approach for the reconstruction of full thickness skin defects using minced split thickness skin grafts and biodegradable synthetic scaffolds as a dermal substitute. *Burns* 40(5):957-965.
313. Wahab N, Roman M, Chakravarthy D, Luttrell T 2015. The Use of a Pure Native Collagen Dressing for Wound Bed Preparation Prior to Use of a Living Bi-layered Skin Substitute. *J Am Coll Clin Wound Spec* (In Press).
314. Huang S, Lu G, Wu Y, Jirigala E, Xu Y, Ma K, Fu X 2012. Mesenchymal stem cells delivered in a microsphere-based engineered skin contribute to cutaneous wound healing and sweat gland repair. *J Dermatol Sci* 66(1):29-36.
315. Klar AS, Guven S, Biedermann T, Luginbuhl J, Bottcher-Haberzeth S, Meuli-Simmen C, Meuli M, Martin I, Scherberich A, Reichmann E 2014. Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells. *Biomater* 35(19):5065-5078.
316. Brimson CH, Nigam Y 2013. The role of oxygen-associated therapies for the healing of chronic wounds, particularly in patients with diabetes. *J Eur Acad Dermatol Venereol* 27(4):411-418.
317. Chambers AC, Leaper DJ 2011. Role of oxygen in wound healing: a review of evidence. *J Wound Care* 20(4):160-164.

318. Rodriguez PG, Felix FN, Woodley DT, Shim EK 2008. The role of oxygen in wound healing: a review of the literature. *Dermatol Surg* 34(9):1159-1169.
319. Hunter S, Langemo DK, Anderson J, Hanson D, Thompson P 2010. Hyperbaric oxygen therapy for chronic wounds. *Adv Skin Wound Care* 23(3):116-119.
320. Duzgun AP, Satir HZ, Ozozan O, Saylam B, Kulah B, Coskun F 2008. Effect of hyperbaric oxygen therapy on healing of diabetic foot ulcers. *J Foot Ankle Surg* 47(6):515-519.
321. Kranke P, Bennett MH, Martyn-St James M, Schnabel A, Debus SE 2012. Hyperbaric oxygen therapy for chronic wounds. *Cochrane Database Syst Rev* 4:CD004123.
322. Abidia A, Laden G, Kuhan G, Johnson BF, Wilkinson AR, Renwick PM, Masson EA, McCollum PT 2003. The role of hyperbaric oxygen therapy in ischaemic diabetic lower extremity ulcers: a double-blind randomised-controlled trial. *Eur J Vasc Endovasc Surg* 25(6):513-518.
323. Blackman E, Moore C, Hyatt J, Railton R, Frye C 2010. Topical wound oxygen therapy in the treatment of severe diabetic foot ulcers: a prospective controlled study. *Ostomy Wound Manage* 56(6):24-31.
324. Heng MC, Harker J, Bardakjian VB, Ayvazian H 2000. Enhanced healing and cost-effectiveness of low-pressure oxygen therapy in healing necrotic wounds: a feasibility study of technology transfer. *Ostomy Wound Manage* 46(3):52-60, 62.
325. Kellar RS, Audet RG, Roe DF, Rheins LA, Draelos ZD 2013. Topically delivered dissolved oxygen reduces inflammation and positively influences structural proteins in healthy intact human skin. *J Cosmet Dermatol* 12(2):86-95.
326. Laird KF, Baer D, Leas ML, Renz EM, Cancio LC 2014. Evaluation of an oxygen-diffusion dressing for accelerated healing of donor-site wounds. *J Burn Care Res* 35(3):214-218.
327. Zellner S, Manabat R, Roe DF 2015. A dissolved oxygen dressing: a pilot study in an ischemic skin flap model. *J Int Med Res* 43(1):93-103.
328. Moffatt CJ, Stanton J, Murray S, Doody V, Davis PJ, Franks PJ 2014. A randomised trial to compare the performance of Oxyzyme® and Iodozyme® with standard care in the treatment of patients with venous and mixed venous/arterial ulceration. *Wound Med* 6: 1–10.

329. Vig S, Dowsett C, Berg L, Caravaggi C, Rome P, Birke-Sorensen H, Bruhin A, Chariker M, Depoorter M, Dunn R, Duteille F, Ferreira F, Martinez JM, Grudzien G, Hudson D, Ichioka S, Ingemansson R, Jeffery S, Krug E, Lee C, Malmsjo M, Runkel N, International Expert Panel on Negative Pressure Wound T, Martin R, Smith J 2011. Evidence-based recommendations for the use of negative pressure wound therapy in chronic wounds: steps towards an international consensus. *J Tissue Viability* 20 Suppl 1:S1-18.
330. Thompson JT, Marks MW 2007. Negative pressure wound therapy. *Clin Plast Surg* 34(4):673-684.
331. Shweiki E, Gallagher KE 2013. Negative pressure wound therapy in acute, contaminated wounds: documenting its safety and efficacy to support current global practice. *Int Wound J* 10(1):13-43.
332. Morykwas MJ, Argenta LC, Shelton-Brown EI, McGuirt W 1997. Vacuum-assisted closure: a new method for wound control and treatment: animal studies and basic foundation. *Ann Plast Surg* 38(6):553-562.
333. Argenta LC, Morykwas MJ 1997. Vacuum-assisted closure: a new method for wound control and treatment: clinical experience. *Ann Plast Surg* 38(6):563-576; discussion 577.
334. Borgquist O, Ingemansson R, Malmsjo M 2011. Individualizing the use of negative pressure wound therapy for optimal wound healing: a focused review of the literature. *Ostomy Wound Manage* 57(4):44-54.
335. Gupta S, Baharestani M, Baranoski S, de Leon J, Engel SJ, Mendez-Eastman S, Niezgodna JA, Pompeo MQ 2004. Guidelines for managing pressure ulcers with negative pressure wound therapy. *Adv Skin Wound Care* 17 Suppl 2:1-16.
336. Morykwas MJ, Argenta LC 1993. Use of Negative-Pressure to Increase the Rate of Granulation-Tissue Formation in Chronic Open Wounds. *Faseb Journal* 7(3):A138-A138.
337. Payne JL, Ambrosio AM 2009. Evaluation of an antimicrobial silver foam dressing for use with V.A.C. therapy: morphological, mechanical, and antimicrobial properties. *J Biomed Mater Res B Appl Biomater* 89(1):217-222.
338. Nuccitelli R 2003. A role for endogenous electric fields in wound healing. *Curr Top Dev Biol* 58:1-26.

339. Nishimura KY, Isseroff RR, Nuccitelli R 1996. Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds. *J Cell Sci* 109 (Pt 1):199-207.
340. Thakral G, Lafontaine J, Najafi B, Talal TK, Kim P, Lavery LA 2013. Electrical stimulation to accelerate wound healing. *Diabet Foot Ankle* 4.
341. Ud-Din S, Bayat A 2014. Electrical Stimulation and Cutaneous Wound Healing: A Review of Clinical Evidence. *Healthcare* 2:445-467.
342. Szuminsky NJ, Albers AC, Unger P, Eddy JG 1994. Effect of narrow, pulsed high voltages on bacterial viability. *Phys Ther* 74(7):660-667.
343. Cutting K 2006. Electrical stimulation in the treatment of chronic wounds. *Wounds UK* 2(1): 62–71.
344. Banerjee J, Das Ghatak P, Roy S, Khanna S, Sequin EK, Bellman K, Dickinson BC, Suri P, Subramaniam VV, Chang CJ, Sen CK 2014. Improvement of human keratinocyte migration by a redox active bioelectric dressing. *PLoS One* 9(3):e89239.
345. Blount AL, Foster S, Rapp DA, Wilcox R 2012. The use of bioelectric dressings in skin graft harvest sites: a prospective case series. *J Burn Care Res* 33(3):354-357.
346. Harding AC, Gil J, Valdes J, Solis M, Davis SC 2012. Efficacy of a bio-electric dressing in healing deep, partial-thickness wounds using a porcine model. *Ostomy Wound Manage* 58(9):50-55.
347. Kim H, Makin I, Skiba J, Ho A, Housler G, Stojadinovic A, Izadjoo M 2014. Antibacterial efficacy testing of a bioelectric wound dressing against clinical wound pathogens. *Open Microbiol J* 8:15-21.
348. Kim H, Izadjoo MJ 2015. Antibiofilm efficacy evaluation of a bioelectric dressing in mono- and multi-species biofilms. *J Wound Care* 24 Suppl 2:S10-14.
349. Aziz Z, Cullum N, Flemming K 2013. Electromagnetic therapy for treating venous leg ulcers. *Cochrane Database Syst Rev* 2:CD002933.
350. Guo L, Kubat NJ, Isenberg RA 2011. Pulsed radio frequency energy (PRFE) use in human medical applications. *Electromagn Biol Med* 30(1):21-45.
351. Rawe IM, Vlahovic TC 2012. The use of a portable, wearable form of pulsed radio frequency electromagnetic energy device for the healing of recalcitrant ulcers: a case report. *Int Wound J* 9(3):253-258.
352. Callaghan MJ, Chang EI, Seiser N, Aarabi S, Ghali S, Kinnucan ER, Simon BJ, Gurtner GC 2008. Pulsed electromagnetic fields accelerate normal and diabetic wound healing by increasing endogenous FGF-2 release. *Plast Reconstr Surg* 121(1):130-141.

353. Aziz Z, Cullum NA, Flemming K 2011. Electromagnetic therapy for treating venous leg ulcers. *Cochrane Database Syst Rev* (3):CD002933.
354. Farivar S, Malekshahabi T, Shiari R 2014. Biological effects of low level laser therapy. *J Lasers Med Sci* 5(2):58-62.
355. Chaves ME, Araujo AR, Piancastelli AC, Pinotti M 2014. Effects of low-power light therapy on wound healing: LASER x LED. *An Bras Dermatol* 89(4):616-623.
356. Aoki A, Mizutani K, Schwarz F, Sculean A, Yukna RA, Takasaki AA, Romanos GE, Taniguchi Y, Sasaki KM, Zeredo JL, Koshy G, Coluzzi DJ, White JM, Abiko Y, Ishikawa I, Izumi Y 2015. Periodontal and peri-implant wound healing following laser therapy. *Periodontology 2000* 68(1):217-269.
357. Loreti EH, Pascoal VLW, Nogueira BV, Silva IV, Pedrosa DF 2015. Use of Laser Therapy in the Healing Process: A Literature Review. *Photomed Laser Surg* 33(2):104-116.
358. Percival SL, Francolini I, Donelli G 2015. Low-level laser therapy as an antimicrobial and antibiofilm technology and its relevance to wound healing. *Future Microbiol* 10(2):255-272.
359. da Silva JP, da Silva MA, Almeida AP, Lombardi Junior I, Matos AP 2010. Laser therapy in the tissue repair process: a literature review. *Photomed Laser Surg* 28(1):17-21.
360. Beckmann KH, Meyer-Hamme G, Schroder S 2014. Low level laser therapy for the treatment of diabetic foot ulcers: a critical survey. *Evid Based Complement Alternat Med* 2014:626127.