A View on Polymer-Based Composite Materials for Smart Wound Dressings

S. Baptista-Silva, P. Alves, I. Guimarães, S. Borges, F. Tavaria, P. Granja, M. Pintado, and A. L. Oliveira

Abstract Wound management challenges everyday thousands of health professionals, mainly due to the constant monitoring and difficulties in deciding the correct treatment options. When considering chronic wounds, selecting the ideal dressing defies clinical knowledge, when facing the large amount of different materials, its distinctive properties and the uniqueness of each patient needs. This chapter presents

S. Baptista-Silva (✉) · I. Guimarães · S. Borges · F. Tavaria · M. Pintado · A. L. Oliveira (✉)
Universidade Católica Portuguesa CBQF—Centro de Biotecnologia E Química Fina—Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
e-mail: sara.baptistadasilva@gmail.com

A. L. Oliveira
e-mail: aloliveira@porto.ucp.pt

I. Guimarães
e-mail: ines.gvguimaraes@gmail.com

S. Borges
e-mail: sandraferreiraborges@gmail.com

F. Tavaria
e-mail: ftavaria@porto.ucp.pt

M. Pintado
e-mail: mmpintado@esb.ucp.pt

P. Alves
Universidade Católica Portuguesa, Instituto de Ciências Da Saúde CIIS—Centro de Investigação Interdisciplinar em Saúde, Porto, Portugal
e-mail: Pjalves@porto.ucp.pt

P. Granja
Instituto de Investigação E Inovação Em Saúde (I3S), Universidade Do Porto, Porto, Portugal

Instituto de Engenharia Biomédica (INEB), Universidade Do Porto, Porto, Portugal

Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade Do Porto, Porto, Portugal

Faculdade de Engenharia Da Universidade Do Porto (FEUP), Porto, Portugal

P. Granja
e-mail: pgranja@i3s.up.pt

M. S. Hasnain et al. (eds.), *Polymeric and Natural Composites*,
Advances in Material Research and Technology,
https://doi.org/10.1007/978-3-030-70266-3_14

an overview on the challenges and complexity of a chronic wound, exploring the event of awound infection and discussing the large range of polymer-based composite materials and products in use for each specific wound condition, taking into account the key decision aspects defined by the clinicians. Different tissue engineering strategies are also herein addressed with varied reported clinical success, ranging from non-cellularized to considerably sophisticated cellularized products, reproducing the compositional complexity of both dermis and epidermis. Recent advances in smart dressings and sensors are also brought to discussion as sensing the wound can give us new insights about the series of complex biochemical events related to the healing and regeneration process, while contributing for a better wound assessment.

Keywords Polymers · Composite materials · Wound management · Wound dressings

1 Introduction

Wound treatment is based on a complex approach where it is essential to identify the aetiology, make a correct diagnosis, and ensure that the treatment and therapeutic decisions are the most effective for that case. Wounds can be classified as acute or chronic according to their healing process [1, 2]. Acute wounds are associated with a non-complicated process that is generally organized, sequential and able to restore the anatomic and functional integrity of the tissues involved within a reasonable small amount of time. Meanwhile, chronic wounds are associated with difficult or prolonged healing where the reparative process does not follow in a timely or orderly fashion, and thus fails to produce the previous anatomic and functional integrity of the injured site [3]. According to White et al. [4], chronic wounds may be the result of an association between wounds that have an impaired healing process due to the presence of complex underlying pathologies, such as diabetes mellitus, vascular disease or the presence of malignancy. The epidemiological profile of chronic wounds is not fully known, however, it is estimated that there may be more than 20 million chronic wounds worldwide [5], with 1–2% of the population experiencing a chronic wound during their lifetime [6].

Wound care has high associated costs, as studies from Scandinavian countries have reported, they account for 2–4% of the total health care expenses [7], studies from the UK showed that they account for approximately 4.8 billion pounds [8], and studies from the United States showed that they account for an excess of US$25 billion annually in the treatment of chronic wounds [9]. This burden is expected to grow even more due to aging population and chronic diseases, like diabetes. Aspects such as pain, anxiety and social isolation are aspects that may influence the quality of patient's life besides the wound healing process and cost treatment [10, 11]. Nonetheless these aspects are presently difficult to quantify.

This chapter discusses recent advances in the development of synthetic (including biosynthetic) and biologic (tissue origin) ultimate polymer-based dressing materials

and composites to promote wound care. In specific, this chapter focuses on the current and new solutions that enhance wound healing and tissue regeneration, keeping in mindimportant aspects such as the improvement of wound care system.

2 Complexity of a Chronic Wound

A chronic wound is related to the complexity and senescence of physiological processes and is associated with stagnation. Chronic wound is described as an interruption in the integrity of skin and underlying tissues progresses through a disorganized and complex shape healing process. These injuries hardly progress through a sequence of normal healing, sequential and timely, and there is a precise balance between production and degradation of molecules [12, 13]; to thereby influence the physiology of healing. Chronic wounds can take several years to heal or even have no cure, causing emotional changes, physical and economic to patients, families and health services.

Healing is an intricate, dynamic and continuous systemic process [14], which requires body activation, production and inhibiting of a large number of molecular and cellular components, the interaction of chemical mediators, and extracellular matrix in an orderly and continuous sequence, conduct the entire process of regeneration/ healing. Its understanding is a critical step towards solving chronic wound problem.

2.1 Different Stages of Healing

The healing process is divided into three continuous phases which overlap temporally: inflammatory, proliferative and maturation [15, 16]. Five steps are known during the healing process, including the induction of inflammatory response by injury, reconstitution, migration and proliferation of parenchymal cells and connective tissue; followed by the synthesis of proteins of the extracellular matrix and restructuring of parenchymal elements to restore functionality and finally the connective tissue remodelling [17].

From the moment in which there is tissue loss, platelets come in contact with exposed collagen and other extracellular matrix components, giving the coagulation cascade and the release of vasoactive substances, adhesive proteins, growth factors and proteases [17, 18]. The formation of this provisional matrix is essential for cell migration, in addition to serving as a reservoir for cytokines and growth factors that will be released during the following phases of the healing process [19, 20]. At this stage the Celsius signals are evident as redness, heat, pain, tumor and possible loss of function [21]. The pro-inflammatory cytokines produce proteases that are present in the exudate and breakthe damaged extracellular matrix proteins [22], a process termed proteolysis. Beyond processes described, there is the phagocytosis of bacteria,

cell debris and foreign bodies, as well as the production of growth factors involved in these inflammatory cells to prepare the wound for the proliferative phase when the endothelial fibroblasts and cells also are incorporated [23]. When the inflammatory process does not lapse, a complex response is triggered that can lead to chronic inflammation [24, 25] . The proliferative phase starts with the granulation tissue formation, connective tissue cell proliferation and migration and re-epithelialization of the wound surface. The epithelial cell proliferation starts with a mitogenic and chemotactic stimulation of keratinocytes, with increased microvascular permeability that allows, through the leakage of proteins, cytokines and cell elements, to the formation of a provisional extracellular matrix which is necessary for the migration and proliferation of endothelial cells [26]. The last stage—Maturation is characterized by deposition of extracellular matrix (ECM) remodelling of tissues and wound contraction [27, 28]. In the course of maturation and remodelling process, most fibroblasts and inflammatory cells disappeared from the wound site, giving rise to apoptosis and cell death processes to scar formation.

2.2 Wound Types and Therapeutic Requirements

From the universe of chronic wounds, the most common are pressure ulcers, venousarterial ulcers, and diabetic ulcers (Fig. 1) [29]. Injuries associated with the lower limbs, venous ulcers are the most common type, accounting for about 80–90% of the wounds, and the remaining arterial and neuropathic [30].

The choice of the most adequate dressing is influenced by the aetiology, specific characteristics of the wound bed, type of tissue present, odour, infection signals and amount of exudation [31, 32]. There is a considerable number of different dressings and techniques available for managing wounds according to its characteristics (Table 1).

Thus, the wound bed, and its tissue type are indicative of the required material, the healing phase, progression and treatment effectiveness:

Necrosis: usually black, indicative of tissue death, hard or soft consistency;

Fibrin: yellowish and can be presented adherent to the wound bed (slough);

Granulation: Reddish colored, slightly damp and firm, it is indicative of good evolution of the healing process;

Epithelialization: pinkish tissue, indicative of wound closure and thus usually arises from their edges.

The first principle of wound bed preparation is the removal of this tissue type and should be performed using debridement; which quantitatively reduces bacterial load, toxins and other substances affecting the immune system [33]. It is clear that healing is systemic, but the choice of the most adequate local treatment technique among the available options or the possibility of an infection event are factors that can contribute to accelerate or delay this process.

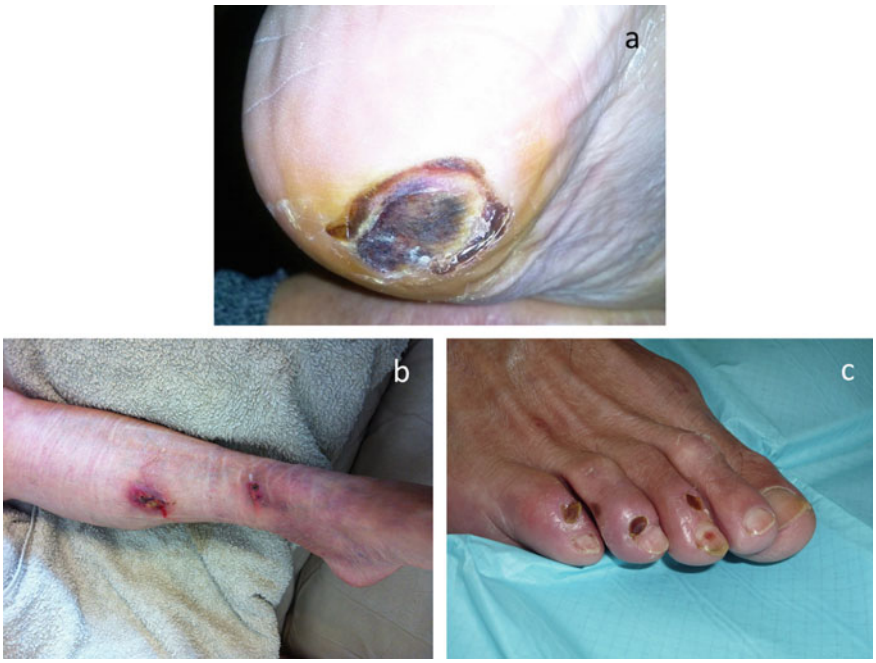

Fig. 1 Most common pressure ulcers: **a** Calcaneous pressure injury, **b** Venous Leg ulcer and **c** Diabetic foot—Charcot foot

The evaluation of the risk factors of the patient with wound should be an element guide to all decisions for the prevention and treatment of wounds. Several factors that interfere with the healing process are identified in the literature, and they are consensual that this continuous process may be hampered by systemic and local factors [27, 28]. According Nazarko [34], in addition to these local and systemic risk factors, extrinsic factors must be evaluated (affecting the condition of the person) and assessing the intrinsic (referring to the wound characteristics) that interfere with the healing process (Table 2).

3 The Event of a Wound Infection

When the skin integrity is disrupted becomes prone to infection. As a multi-functional organ, skin possesses particular biochemical and physical properties that influence its microbiology. Some of these properties include a slightly acidic pH, low moisture content, high lipid content (which confers hydrophobic characteristics) and the presence of antimicrobial peptides [35].

Table 1 Wound characteristics and established therapeutic options

Color	Tissues	Exudation	Objectives	Considerations	Therapeutic options
	Necrosis Necrotic tissue	Null (dry)	Debride Hydrate	Caution: - Arterial occlusion - Heel - Malignant lesions - Coagulation Levels	Surgical Debridment
		Scarce	Debride Hydrate& Maintain Humidity		Autolytic or enzymatic debridement
	Devitalized \ Fibrin Slough Tissue	Scarce	Removal of necrotic tissue Reduce the bacterial load	Perilesional skin protection	Surgical debridement if necessary Autolytic or enzymatic debridement
		Moderate \ abundant	Removal of necrotic tissue Reduce the bacterial load	Perilesional skin protection	Autolytic or enzymatic debridement Absorbing dressings
	Granulation tissue	Scarce	Promote Granulation & Maintain Humidity	Perilesional skin protection Atraumatic treatments	Hydrogels Absorbing dressings
		Moderate \ abundant	Promote Granulation & Management of exudate	Protect new tissues	Absorbing dressings Tissue regeneration material
	Epithelization	Null	• Promote epithelialization • Protection	• Protect new tissues • Decrease Frequency of Treatments	Silicone films Thin Hydrocolloids

Adapted from WUWHS [31]

Table 2 Risk factors for impaired healing

Intrinsic factors	Extrinsic factors
Age	Aetiologies
Skin condition	Wound site
Associated pathologies	Wound size
Life style	Wound bed tissues—Debris
Nutritional status	Exudation—Maceration
Mobility	Wound edges
Psychological well-being	Surrounding skin
Pain	Pain associated with wound
Immune status	Mechanical stress
	Chemical stress
	Dressing materials
	Medication
	Temperature

The outer surface of adult skin is colonized by a handful of stable inhabitants (resident microorganisms) with rare or transient species contributing to interpersonal variation [36]. Skin physiology determines the pattern of colonization by skin microbes. *Staphylococcus* spp. and *Corynebacterium* spp. are the most abundant in moist sites, while lipophilic microorganisms such as *Propionibacterium* spp. and *Malassezia* spp. dominate in sebaceous areas [37]. The role of transient microorganisms in infection remains largely unknown, although it is likely that they influence the infection life cycle.

Microorganisms are present in all wounds. In acute wounds, the short healing time allows for only a small number of skin contaminates to take residence while in chronic wounds, the continued exposure of devitalized tissue is likely to facilitate the colonization of a wide variety of microorganisms and trigger infection [38]. Bacteria, fungi, viruses or protozoa can cause human infection. However, the presence of microorganisms such as bacteria does not necessarily indicate that an infection exists or that will lead to impair wound healing [39].

The role of bacteria in wound healing has been debated over the years. Some have suggested that bacteria may play a beneficial role in normal wound healing and wounds will heal despite the presence of large numbers of microorganisms [40]. Nonetheless, the detrimental effects of specific pathogens, such as *Clostridium perfringens* and *Streptococcus pyogenes*, have been well recognized. These are typically invasive bacteria that are not normal members of the human skin microbiota. In contrast, some resident microorganisms such as *Staphylococcus aureus*, which are part of the microbiota of many humans, also cause wound infections (Table 3).

Polymicrobial wounds are those containing several potential pathogens. This typically delays wound healing, raising the risk for other complications [42] and is the norm in chronic wounds. The concept of wound bioburden involves the bacterial burden, which is the presence of replication microorganisms within a wound, the bacterial load, the virulence of the microorganism and the host reaction [43]. This leads to increased metabolic load imposed by the multiplying microorganisms in the wound bed, and their ability to spread in tissues and produce toxins [44]. The

Table 3 Bacterial species isolated from chronic wounds

	Chronic wounds (specimens from 19 wounds)*	
Bacterial genus	Swab culture	Tissue PCR
Staphylococcus	28	68
Enterococcus	12	18
Pseudomonas	32	28
Proteus	126	–
Citrobacter	8	28
Corynebacteria	0	68
Anaerobes	0	70

* Frank et al. [41].

interactions of multiple microbial populations in chronic infections are still poorly understood, mainly due to controversies in culturing methods [45]. However, over the past several years, molecular-based methods have been increasingly applied in skin and wound microbiology research. Molecular studies of wound microbiology have revealed very diverse bacterial communities. These studies involved polymerase chain reaction (PCR) amplification of the bacterial gene encoding the small ribosomal subunit RNA (16S). More recent studies have used additional molecular methods including metagenomics for the evaluation of chronic wound microbiota [41]. The results of these studies indicate that chronic wounds contain diverse polymicrobial communities and similar community features, such as the presence of strictly anaerobic bacteria, even though the studies were from diverse geographic regions.

Evidence exists that bacteria colonizing human chronic wounds exist as biofilm communities and not in the planktonic form [35, 46]. Bacteria within a biofilm live in microcolonies encapsulated in a matrix composed of an extracellular polymeric substance. This acts as physical barrier to the permeation and action of antimicrobial agents. Besides this, the biofilm confers a habitat where bacteria can communicate with each other (quorum sensing), which may lead to increased virulence and propensity to cause infection [47]. More than 80% of human infections are caused by biofilms [48]. Chronic wounds have a fertile environment for biofilm formation; necrotic tissues and superficial debris facilitate infection whereas the altered vascularization and subsequent ischemia hinder the immune system to develop an efficient defensive response [49]. It is estimated that 60% of all chronic wounds are colonized by biofilms compared to only 6% of acute wounds [50]. This explains how biofilms can impair the healing process, by being often associated with a persistent inflammatory state, tissue disruption and difficulty in healing. The cascade of events leading to a chronic wound is schematized in Fig. 2. Adding to this, the polymicrobial nature of these infections further complicates diagnosis and treatment. When compared chronic wounds (venous leg ulcers, diabetic foot ulcers and pressure ulcers) with acute, generally are colonized by more anaerobic bacteria and fungi. Diabetic foot ulcers have a high incidence of species of *Bacteroides*, *Peptoniphilus*, *Finegoldia*, *Anaerococcus* and *Peptostreptococcus* [51].

It is tempting for the clinician to start antibiotic treatment, but in case of established mature biofilms, this treatment often has only temporary effect on both inflammation and healing. In addition, the clinician has to rely on the results from a swab or biopsy, which rarely reflects all specimens present in the wound. The bacteria in biofilm are up to 1000 times less susceptible to antibiotics [53], and MIC is not reached in the chronic wound fluid. Even silver as an antimicrobial incorporated in several wound dressings, has limited effect in biofilm *in vitro* [54]. With this in mind, the clinician should exercise restraint in administering antibiotics. This favours biofilm persisting bacteria and promotes resistance. Mechanical removal of wound debris (by ultrasound assisted surgery) and even granulation tissue is an effective way of diminishing the bacterial load and is an important part of treatment protocols. “Biofilm managing strategies” have been implemented, but none have yet proved to be more effective than others [55]. The need for new and more efficient treatment regimens (new biofilm penetrating drugs, new substances to disrupt biofilms) and

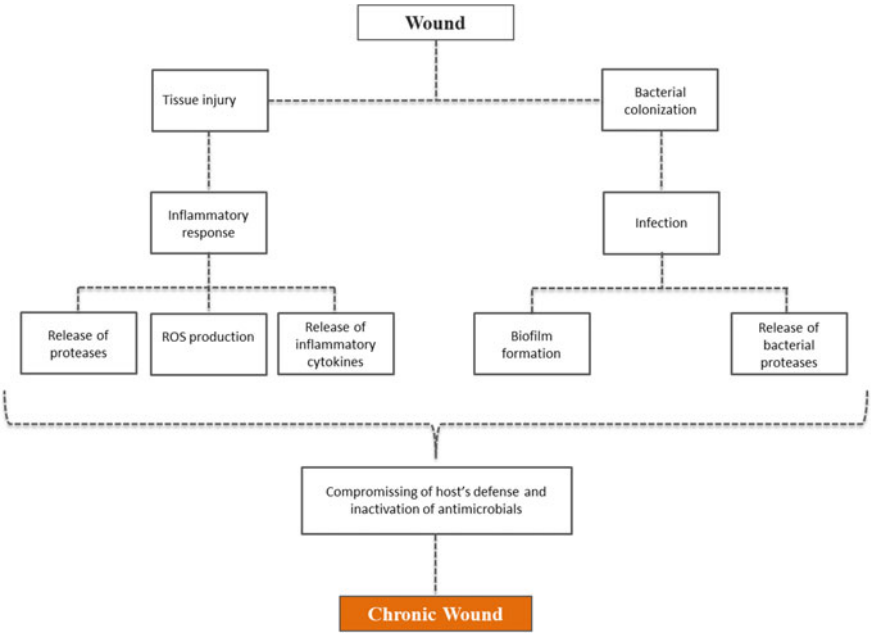

Fig. 2 Cascade of events contribution to a chronic wound. Adapted from McCarty and Percival [52]

research in biofilm (QS manipulation, resistance to antimicrobials) may provide wound care specialists with new, more effective ways to heal wounds.

4 Established Wound Dressing Options

Very few, if any, current wound care products have the capacity to cross the healing process towards tissue restoration [56]. The contexts, realities and needs in wound care around the world are simultaneously equal and different and there are actually a large variety of different dressings for prevention and treatment [57–60] for the most various aetiology's or wound characteristics. These wound care dressings presently can be divided into two broad categories: synthetic (including biosynthetic) and biologic (tissue origin) polymer-based materials and their composites [56].

Biologic-derived polymers for wound dressings are not recent, they are used since the Egyptian's [60], however only after the 60's the first study's started and more specialized biological-derived dressings have been developed since there [56, 61]. Their effectiveness has increased greatly with recent innovative developments, where various skin substitutes were tested over time, such as human skin allograft, xenograft and amnion, are being used at various wound care centres. The skin substitutes provide faster wound coverage solution that may require less vascularized wound

bed, increase in the dermal component of healed wound, reduce or removed inhibitory factors of wound healing, reduced inflammatory response and subsequent scarring [62]. Nevertheless, these skin substitutes need specific expertise/experience and have a higher cost [63].

4.1 Synthetic/Biosynthetic Dressings in Wound Care

Synthetic/biosynthetic dressings have been designed primarily to promote moist wound healing and function as a barrier against infection, while simultaneously collaborate in the growth of granulation tissue and epithelisation. Currently, chronic wounds are treated with a broad variety of dressings tailored to the requirements of the wound (dry or exuding, clean or infected, superficial or deep) (Table 2). These materials are generically categorized as textiles, polyurethane films, foams, hydrogels, hydrocolloids, and collagen/alginate combination of wound dressings as example in Table 1 [60]. Nonetheless the most common wound dressing are alginates and hydrofibers, well studied, applied and documented in literature. Alginate has relevant properties as a gel-forming [64] and film-forming [65]. Alginate dressings are highly absorbent, being this dressing a good choice for highly exudative wounds. These dressings are described to absorb 15–20 times their weight of fluid, which can be a substantial lifestyle improvement for patients with draining ulcers [66]. Hydrofibers are dressings highly absorbent, they have a similar function of alginates but can absorb three times more. They have been demonstrated to be useful in partial-thickness donor sites and partial thickness burns. All together, these polymer-based materials and their compositescan present a wide range of properties resulting in interesting possibilities for the final wound dressing product [67]. The selection of various reinforcements and polymer matrices is very critical in designing a desired product for wound care. Figure 3, shows a schematic illustration of a typical decision tree in wound care.

The wound stage and its characteristics will implicate the best dressing choice. While films will be the ideal solution for superficial wounds; foams will be more adequate for exudative and granulating; hydrogels will be best applied in eschar, deep or tunneling wounds and in wounds with slough; and hydrocolloids will be used in superficial, eschar and granulating wounds and also wounds with slough [68]. It is highlighted the key steps prior dressing application, like wound bed preparation (cleanse, debridement and measurement), the wound evaluation (considering the different levels of exudate) and the consequent ideal solution for each type of wound. Likewise, some common commercial products are listed below.

In this chapter novel and promising polymer-based composite dressings in the different categories, are reported for wound healing.

Foams

Foams are one of the most variable and versatile dressings for chronic wounds, including thin and thick, adhesive and non-adhesive, coated or uncoated [69]. Typically, the most used foams are constituted of polyurethane or a silicone core with a

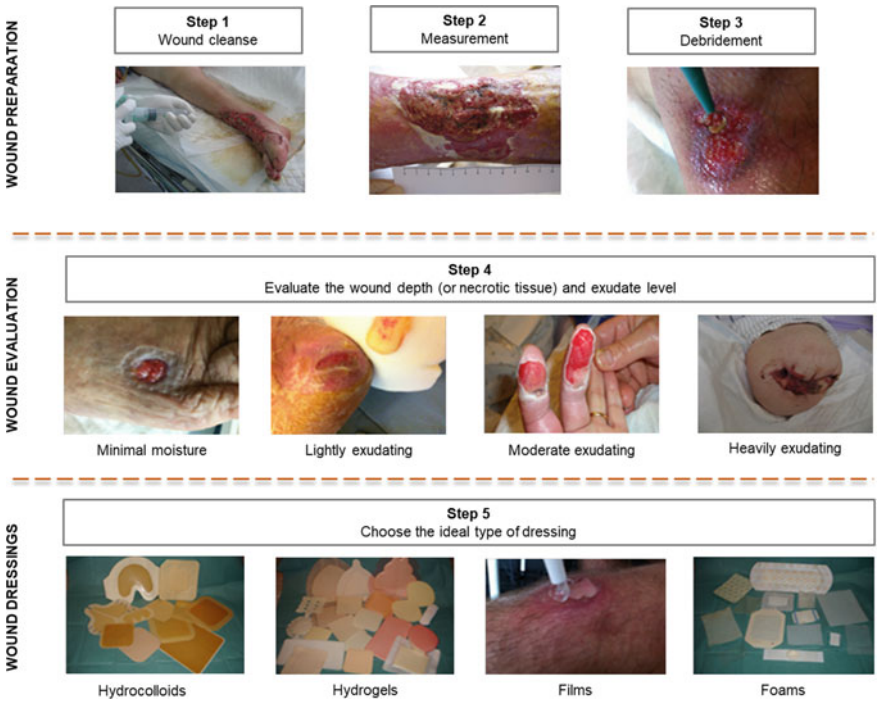

Fig. 3 Schematic illustration of wound care treatment

semi-occlusive out layer. This layer is water vapor permeable and attends to protect against microbial penetration and proliferation, while the polyurethane/silicon serves to give absorptive qualities [66]. Foams are applied in wounds with moderate to high exudate, granulating or necrotic wound, and can be used on infected wounds (Fig. 3). It has been developed foams with antimicrobial activity, as for example silver-containing arabinoxylan foams [70]. There are also available commercial products incorporating silver, such as PolyMem silver® (Ferris Cor.) and Mepilex® Ag (Mölnlycke Health Care).

Foams afford an atmosphere for autolytic debridement and reduce granulation tissue. Thick foams can be used for venous ulcers to offer an improvement of local compression, which allow to control edema and promote healing [69]. Treatment of wounds using negative pressure therapy uses foams that incorporate tubing to a vacuum source. Generally, two types of foams are used: polyurethane foams, for example, VAC GranuFoam® (Kinetic Concepts [KCI], San Antonio, TX); Flexzan® (Dow B. Hickam, Inc.), Hydrasorb® (Tyco Health Care/The Kendall Co.) or polyvinyl alcohol foams, for example, VAC VersaFoam, (Kinetic Concepts [KCI], San Antonio, TX) [71].

Foams can dry wounds with minimal or mild exudate and may require a saline soak during dressing change to avoid pain and trauma [68]. The absorbent capacity

of wound fluids is dependent on the polymeric material used and the thickness of the foam. They are extremely absorbent, protective and adaptable to body surfaces. Additionally, foams are easily manipulated and can be adjusted to the wound size [72].

Foams are appropriate for deep wounds with exposed bony areas such as the ankle or sacrum or exudative cavities, however they should be frequently changed, since daily to once or twice weekly, because the dressing becomes soaked with exudate. Foams can be adherent or non-adherent, in the latter case it is necessary a secondary dressing to avoid shifting [66]. Nevertheless, they possess some drawbacks, for example they can dehydrate dry wound and also they are opaque and the wound visualization can be compromised. In addition, adhesive foams may be responsible for some cases of contact dermatitis [73].

Recently, several innovative composite foams have been developed. A new foam combining the attributes of volume filling and rapid coagulation of shape memory polymers (SMP) with the ability to swell and fill hydrogels has been developed by Landsman et al. [74]. This SMP polyurethane foam is coated with n-vinylpyrrolidone hydrogel (NVP) and polyethylene glycol diacrylate (PEGDA). In a new addition, this composite contains iodine in the form of a complex (PVP-I2 or povidone-iodine), widely used as a surgical antiseptic. The iodine-containing hydrogel gives the composite an antibacterial effect (reducing the viability of common bacteria by 80%) while increasing fluid uptake by 19 times over uncoated SMP foams. In another study by Namviriyachote et al. [75] polyurethane combined (PUC) foam dressings with various biomacromolecules (i.e. carboxymethylcellulose, chitosan, alginate, hydroxypropyl methylcellulose) were fabricated with the adsorption of asiaticoside and silver nanoparticles for traumatic wound treatment. The selected PU-alginate combined foam dressing adsorbed with asiaticoside and silver nanoparticles proved advantages for traumatic dermal wound management.A multilayer dressing consisting of polyvinyl alcohol foam (PVA) and electrospun sodium carboxymethyl cellulose (CMC) surface mesh was developed and characterized by He et al. [76] and co-workers. This composite was further loaded into the PVA foam, with the antimicrobial drug stearyl trimethyl ammonium chloride (STAC) for infection control and the CMC surface mesh offered an effective hemostatic function. Another study shows the potential of alginate-pectin composite foams with different blending ratios using calcium ion cross-linking [77]. In this study, the results suggest that controlling the pectin content in alginate-pectin foams is the key to adjust their mechanical properties, water absorption, and drug-release ability. Alginate-pectin composite foams showed to be promising candidates in different wound-dressing applications. A series of foams composed of PVA)/ alginate (PACFs) were prepared through a crosslinking reaction and lyophilization process [78]. The effect of different alginate content on the physicochemical properties and on the hemostatic function of the PACF was analyzed. The results showed that PACF absorbed plasma, but also stimulated blood cells, further promoting blood clotting, with therefore promising results as wound dressings.

Films

Films are thin, elastic and offer a barrier to microbial colonization [68]. Generally, commercial films are transparent, for example Transeal® (DeRoyal), Bioclusive® (Johnson & Johnson Medical), Mefilm® (Molnlycke Health Care), among others. A suitable wound-dressing film must have crucial properties, including capacity to absorb exudate, to regulate the moisture permeation, to maintain the moisture of the wound and to release the retained bioactive compound [79].

Films can be used in superficial wounds, namely burns, catheter sites and epidermal skin graft harvest sites, being suitable for minimally exudative wounds (Fig. 3) [68]. Films permit an environment for softening dry eschar by autolytic debridement, permit a protection from friction and contribute for pain reduction. A quantity of amorphous hydrogel may be added to the film in order to accelerate the autolytic debridement. These dressings may not be applied on wounds with heavy exudate. Films should be changed when exudate escapes onto intact skin or at a minimum of every 7 days [69].

Films are easy to use in wounds with different shapes, generally allowing for an easy wound visualization and flexibility to use as a primary or as a secondary dressing cover in alginates, foams and hydrogels [66]. Due to their adherence, films are easy to apply and do not need a second dressing [73]. On the other hand, they have non-absorbent characteristics that cause an accumulation of exudate and a maceration of wound edges, being necessary to change them frequently. The adhesive properties of films may potentially injury the skin, mostly in patients with delicate skin, such as elderly people and those with cutaneous atrophy. Therefore, in this case they should reduce the dressing changes to minimum or even avoid the use thereof [68].

Antimicrobial protection has been also addressed over the years and there are already films with this functionality in the market, such as 3 M™ Tegaderm™ CHG Chlorhexidine Gluconate I.V. Securement Dressings (3 M Healthcare) and Acticoat 7 (Smith and Nephew) that contains silver. In this sense Kim and coworkers [80], investigated a nitric oxide-releasing chitosan film. That film demonstrated a stronger antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and, simultaneously, the film accelerated wound healing and epithelization in a rat model. Nevertheless, innovative solutions are presently under development. Novel chitosan and cellulose acetate polymer composites were prepared by solvent-casting method [81]. The formed films were loaded with nanosized cerium oxide, and the results revealed to be promising as potential wound covering material. Alginate films containing pyrogenic silica supported silver nanoparticles were prepared via solid state sintering route without the use of any solvent and reducing agent [82]. Films exhibited antimicrobial and antibiofilm activities against *S. aureus* and *P. aeruginosa* and showed no cytotoxicity towards human skin keratinocytes and human fibroblasts HuDe, with promising evidences as wound dressing toward infected tissues. Novel adhesive composite films were prepared for mupirocin dermal delivery. Natural polymers as chitosan, sodium alginate and carbopol were used for films' development to evaluate possible interactions and the impaired drug release properties [83]. Solvent evaporation method was used for the films' preparation. The formulation was found more effective compared to the market product (Bactroban® cream)

for wound healing at Balb-c mice, which highlights the potential for application as a wound care system.

In a different perspective a series of cross-linked films based on the combination of an elastin-derived biomimetic polypeptide (Human elastin-like polypeptide (HELP)) with alginate (ALG) were studied by Bergonzi et al. to obtain a composite with enhanced antioxidant properties [84]. ALG/HELP composite films loaded with the hydrophobic natural antioxidant curcumin were prepared by solvent casting method followed by the cross-linking with calcium chloride. The antioxidant activity correlated to the increase of HELP content, suggested the applicability of these composites as smart biomaterials for different biomedical applications.

Hydrocolloids

Hydrocolloids are moist wound dressings, that usually comprise a backing material (e.g. semi-permeable films, foams or non-woven polyester fibers) and a layer with hydrophilic/colloidal particles that may contain biocompatible gels made of proteins (e.g. collagen, gelatin) or of polysaccharides (e.g. cellulose and its derivatives) [72, 85]. Hydrocolloids are commonly primary dressings, biodegradable, non-breathable, and adherent to the skin, so that no separate taping is required. They are also waterproof, allowing regular water contact with skin.

Hydrocolloid dressings have been carefully addressed by Broussard et al. [66] in are view on wound dressings. The most commonly used are composed of a polyurethane external layer and an internal layer of a hydrophilic polymer such as gelatin e, pectin or carboxymethyl cellulose [86]. In their native stage they are impermeable to water, but once in contact with the wound exudate they are able to absorb it and form a gel, progressively more permeable to water vapour and air, which allows the excess of fluid to be removed without wound desiccation [87]. The moist conditions produced under the dressing and the control of the exudate are intended to promote fibrinolysis, angiogenesis and wound healing, to encourage the production of granulation tissue and to increase the quantity of synthesized collagen, leading to an increase on tissue regeneration, without causing softening and breaking down of the tissue [86]. On the other hand, these dressings also contribute for a better management of pain, due to the hydration enhancement, which will help the autolytic debridement, and will also provide a physical barrier to external microorganisms [86, 88]. Nevertheless, because these products are non-breathable theyare not recommended prior to infection control [87].

There are a great variety of commercially available hydrocolloid dressings such as Granuflex®, Aquacel™, Comfeel™, Tegasorb™, Exuderm®, Duoderm®, Ultec™ or Tegaderm™ and these are adequate solutions for both acute and/or chronic wounds, moist or dry, to form a semipermeable thin sheet and to produce a flat, occlusive and adhesive dressing [89]. These dressings are made in sheets that can easily be cut to fit the desired size or shape of ulcers, traumatic injuries, surgical wounds, graft donor sites, superficial wounds, and some burns without the need of separate taping [88]. Due to its diversity and availability at a relatively low cost, to introduce innovation on hydrocolloid dressings, becomes a difficult task. Hydrocolloid drug loading has been attempted by several authors [87, 89, 90] Thu et al. [87] developed a novel bilayer hydrocolloid film based on alginate, which was investigated

as slow-release wound healing vehicle. The bilayer was composed of an upper layer impregnated with model drug (ibuprofen) and a drug-free lower layer, which acted as a rate-controlling membrane [87]. Successful results suggested that they can be exploited as slow-release wound dressings for low exudate wounds. In another study, novel chitosan (Ch) and hyaluronan (HA) wound dressings were developed loaded tiopronin and captopril as antiinflammatory drugs. Composite biomembranes were examined in skin wounds of ischemic rabbits to accelerate the process of healing. Data proved that the biomembranes composed of Ch/HA/tiopronin or Ch/HA/captopril facilitated healing of skin wounds compared to untreated animals and animals treated with Ch/HA membranes [91]. In a different report, a dressing based on PLGA and Aloe vera was developed containing lipid nanoparticles (NLCs). NLCs were added to prevent dressing from adhering to the wound and improve handling. Consequently, the PLGA-AV-NLC membrane promises to be a promising strategy for the treatment of chronic wounds, since it has improved handling compared to formulations without the lipid character of NLCs [92]. To overlap the adhesion loss of hydrocolloid wound dressings which seriously reduces the therapeutic efficiency and patient experience, hydrocolloid dressings were investigated using sodium carboxymethyl cellulose (CMC)-filled hydrocolloid dressings exposing to physiological environment as model. The results promoted the designing of hydrocolloid dressings with both excellent humidity control and sustained self-adhesiveness [93].

Hydrogels

Hydrogels are commonly defined as polymer three-dimensional networks that may be composed of crosslinked natural polymers (e.g. alginate, chitosan, gelatine, silk) or synthetic macromolecules (e.g. polyethylene glycol, polyvinyl alcohol) [94]. Hydrogels have been reviewed by Moura et al. [72] in a wound dressing report about diabetic wound healing and regeneration.Wound dressing hydrogels can be applied either as an amorphous gel or as an elastic film or solid sheet. These dressings usually require a secondary covering such as gauze, that need to be changed frequently, while hydrogel films or sheets may be used as both primary or secondary dressings [95].

Commercially available hydrogels (ActiformCool®, Stimulen™, Regenecare®, Intrasite Gel, Solosite Gel, Kendall™, 2nd skin®, Tegagel™) are flexible, rubbery and soft, nonreactive or irritant, biocompatible, and permeable to metabolites [96]. Typically, hydrogels are non-adherent and cool to the surface of the wound, which may lead to a better management of pain and therefore a better patient acceptability. Commonly they are suitable for cleansing of dry, sloughy or necrotic wounds by rehydrating dead tissues and enhancing autolytic debridement (Fig. 5). Nonetheless hydrogel dressings due to their high content of water (70–90%) are not suitable to be applied in heavily exuding wounds, once fluid accumulation can lead to skin maceration and bacterial proliferation.

The highly-hydrated network of a hydrogel can be held together via physical or chemical crosslinks, can be made biodegradable, and responsive to specific stimuli such as pH and temperature, and can be engineered to deliver therapeutic cells, drugs and soluble factors in a sustained and controlled way [97]. The success of application of hydrogels as a delivery system in wound healing will largely depend

on biomimetic design and engineering, harnessing cell–material interactions in the cell fate and functions [97].

In Table 3 are summarized a few studies on advances in hydrogel formulation for wound healing and regeneration. Shukla et al. [98] studied an apigenin loaded hydrogel using gellan gum—chitosan with polyethylene glycol as a cross linker. Results proven that the prepared hydrogel seems to be highly suitable for wound healing due to adequate properties of biocompatibility, biodegradability, moist nature and antioxidant effectiveness. Agubata et al. [96] developed and evaluated wound healing hydrogels containing hydroxypropyl methylcellulose, ofloxacin and biodegradable microfibres from surgical sutures. These formulations promoted high collagen deposition after twenty-one days of wounding, with minimal scar formation. Evidences support the promising use of these hydrogels containing for effective wound healing.

Zeng et al. [99], developed injectable gelatin microcryogels which could load cells for enhanced cell delivery and cell therapy for wound healing. In this study, human adipose-derived stem cells (hASCs) laden in gelatine microcryogels, were instigated as primed injectable 3D micro-niches for a new cell delivery methodology for skin wound healing. Results showed wound bed recovery and a direct effect on wound basal layer for healing enhancement. Gong et al. [100] studied a biodegradable *in situ* thermosensitive hydrogel as a controlled drug delivery system composed of curcumin loaded polymeric micelles for successful cutaneous wound repair. Despite advances in the design and development of hydrogels it is still a challenge to develop a hydrogel with good stability and strong mechanical attributes for hemostasis and wound healing. In this sense, a recent study has developed a new polysaccharide hydrogel based of fenugreek gum-cellulose composite. A fenugreek gum was combined with cellulose through hydrogen bonding to form a hydrogel to improve the mechanical properties of the compound hydrogel [101]. Notably, hemostasis and wound healing have been confirmed, which highlights the promising medical potential of the compound hydrogel to promote wound healing [101]. The preparation of hydrogel-based materials with high antibacterial activities and good biosafety at the same time can also be challenging. In order to answer this crucial problem, Yang et al. [102] has developed a physical hydrogel composed of multifunctional chitosan/ carboxymethyl chitosan/ silver polyelectrolyte (CTS/ CMCTS/ AgNPs). A physical hydrogel composed and built by in situ photoreduction of silver ions with CMCTS α-hydroxy and acidification sol by semi-dissolving gel transition methods (SD-A-SGT) using natural polymers with no chemical reducer involved. This composite showed desired biosafety and antibacterial activities simultaneously, with great application potentials as wound dressing.

In a different point of view Lin et al,. studied the importance of anti-inflammation and angiogenesis in wound healing [103]. Therefore, the team developed a composite hydrogel dressing with stepwise delivery of diclofenac sodium (DS) and basic fibroblast growth factor (bFGF) to be applied in the inflammation stage and new tissue formation stage respectively for wound repair. The *in vivo* wound healing of rats revealed that this composite hydrogel showed a better healing effect with a wound contraction of 96% at 14 d, less inflammation and higher angiogenesis, than all

control groups. This is promising data for hydrogel wound dressings [103]. Hamdi et al developed chitosan and protein isolate composite hydrogels, for carotenoids-controlled delivery and wound healing. The concentration of the protein isolate was increased to turn chitosan hydrogels more elastic, not exceeding 15% (w/w) of protein isolate concentration in thechitosan-protein isolate composite hydrogels revealed low cytotoxicity towards MG-63 osteosarcoma cells. The topical application of adhesives based on this hydrogel compound and enriched with carotenoids, allowed the acceleration of wound healing and complete regeneration being a promising new biomaterial [104].

4.2 Tissue Engineered Skin Substitutes and Advanced Wound Healing

Regenerative medicine is a recent but already widely accepted and expanding field involving the development and/or manipulation of molecules cells, tissues or organs to repair, replace or support injured body parts in order to recover their function [105]. Tissue engineering can be perceived now as among the available regenerative medicine strategies and can be defined as the science of persuading the body to heal itself through the supply of molecular signals, cells and scaffolds, to the adequate anatomic sites [106]. For skin regeneration, it essentially consists in expanding skin or stem cells, cultivating in a biomaterial support structure or scaffold, eventually combining biomolecules of interest such as growth factors, and then implanting the cell-scaffold construct for restoring the barrier function (initial step in burns) or for promoting wound healing (e.g., chronic wounds) [107].

This field has been assuming increasing clinical relevance due to the successful clinical tissue engineering-based products already available, namely for skin regeneration. Its clinically proven potential, associated to the limitation of the previously described technologies, allow tissue engineering, and regenerative medicine in general, to bring hope as solution for several clinical problems presently unsolved.

Tissue engineered skin substitutes, given their potential higher similarity to the natural skin tissue, are capable of overcoming several of the limitations previously described for skin grafts, namely donor shortage, and conventional wound dressings, namely undesirable adhesion to the lesion, impossibility associated to the difficulty in reproducing skin appendages and incapacity to replace the lost tissue, particularly the dermis [85, 108–110].

Tissue engineering has been particularly successful in the field of wound healing, and in particular for the treatment of burns and chronic wounds. This is actually the more mature and only area of application where several different products are already available, recurring to distinct strategies and with varied clinical success, ranging from non-cellularized products, composed of a biodegradable and porous polymeric matrix ready for implantation, to considerably sophisticated cellularized products, reproducing the compositional complexity of both dermis and epidermis

[111–113]. The biomaterial scaffold used can be produced using natural, synthetic or hybrid polymers and serves as a template for cell adhesion, proliferation and differentiation, playing a crucial role in guiding neo tissue morphogenesis [109, 114–116]. Additionally, natural skin healing can be stimulated through the incorporation, into these products, of a myriad of biomolecules such as genes, drugs, cytokines or growth factors [109, 111, 112].

Clinically available skin substitutes can be broadly divided into epidermal, dermal and dermo-epidermal products [109, 111, 117], although other categorization modalities exist. For regenerating superficial wounds, several commercial epidermal substitutes exist, using either autologous or allogeneic keratinocytes, namely MySkin® (CellTran, UK), consisting of a silicone layer seeded with autologous keratinocytes, Epicel® (Genzyme Biosurgery, USA), made of petrolatum gauze covered by autologous keratinocytes sheets, Epidex® (Eurodern, Switzerland), consisting of a silicone membrane cultured with autologous keratinocytes from the outer root sheath, and ReCell® (Avita Medical, Australia), where autologous keratinocytes are directly sprayed into the lesion [118–120]. Although generally providing an efficient epidermal coverage epidermal constructs present several limitations, namely long fabrication time due to the obtention and expansion of keratinocytes, difficult handling due to their fragile nature, variable engraftment rates and high cost [109, 120].

For the regeneration of full thickness wounds dermal tissue is required and mechanical stability is important to prevent wound contraction [109, 121]. For wound coverage dermal substitutes are usually associated to a permanent epidermal substitute using autologous split-thickness skin grafts or cultured epithelial autografts [19]. Available products in the market providing effective dermal regeneration include several cell-free products, such as Integra® (Integra LifeSciences, USA), a nanofibrous composite bilayer mesh composed of crosslinked collagen and glycosaminoglycan layer and a semi-permeable polysiloxane layer, Hyalomatrix® (Anika Therapeutics, USA), a bilayered hyaluronic acid-based scaffold covered with a silicone sheath, Matriderm® (MedskinSolutions, Germany), a composite collagen and elastin scaffold, and AlloDerm® (Lifecell, USA), a donated human dermis processed to remove cells, as well as cellularized scaffolds containing fibroblasts, such as Dermagraft® (Organogenesis, USA), a cryopreserved human fibroblast-derived dermal substitute, generated by the culture of neonatal dermal fibroblasts onto a bioresorbable poly(lactic-co-glycolic acid) (PLGA) mesh scaffold [109, 120, 122, 123].

Dermal-epidermal substitutes are the most advanced tissue engineered currently available in the clinics since they mimic both skin layers (dermis and epidermis) for full skin regeneration. Dermal-epidermal substitutes available in the market include PermaDerm® (Regenicin, USA), constituted by a biodegradable collagen scaffold cultured with autologous fibroblasts and keratinocytes, and Apligraf® (Organogenesis, USA), a composite bi-layered product composed of two distinct nanofibrous layers, being the lower dermal layer constituted by bovine type I collagen cultured with human allogeneic fibroblasts and the upper epidermal layer constituted by

cultured human allogeneic keratinocytes [109, 111]. Although providing more effective regeneration of full-thickness skin defects than conventional treatments, these products still present several limitations, namely inefficient wound closure due to rejection of allogeneic cells, high production costs [109].

Overall, tissue engineered skin substitutes present several advantages when compared with conventional treatments, including faster regeneration, increased dermal component in the healed wound, lower vascularization requirements, and reduced presence of inhibitory factors [124]. However, they still also present several limitations as previously pointed out, including lack of skin appendages, such as hair follicles, sebaceous glands and sweat glands, poor cell, inefficient vascularization, wound contraction, fibrosis, scarring at graft margins, use of animal-derived serum in cell culture, and high manufacturing costs [109, 112, 120, 124–126].

To overcome these limitations, several advanced skin regeneration strategies are under development in order to address both the fundamental issues underlying the limited understanding of the phenomena involved, as well as the technological barriers inhibiting their implementation. For instance, in order to promote the formation of skin appendages recent studies explore the culture of specific cells, including stem cells, such as Schwann cells, hair follicle cells, or melanocytes into scaffolds [113, 127]. Other strategies explore the used of advanced fabrication technologies, such as electrospinning or 3D printing, to fabricate scaffolds combined with cells and adequate biomolecules with improved complexity in terms of compositional and architectural biomimicry and providing better control over cell seeding [113, 128–132].

5 Sensing the Wound

The management of chronic wounds can greatly benefit from sensing tools able to predict in real time the need for a specific therapeutic intervention and whether the therapy is working or not. Adding diagnostic and theranostic sensors to wound management is an exciting possibility. The immediate benefits for the clinicians and patients are obvious: an increase of the treatment efficiency, the reduction of treatment time, and in extreme cases, lowering the risk of amputation. On the other hand, sensing the wound can give us new insights about the series of complex biochemical events related to the healing and regeneration process, contributing for a better wound assessment.

5.1 Detectable Biomarkers

Research on biomarkers for the assessment of wound status is of extreme relevance. However, this is a slowly progressing field due to the difficulty on isolating specific biochemical and physiological events that could be used to represent each wound

Table 4 Progress on biomarkers for wound healing Adapted from WUWHS [134]

Main classes of biomarkers (as identified in 2007 consensus meeting)
• Bacterial load/specific microbial species/biofilms • Cytokine release in response to specific microbial antigens • DNA—e.g. gene polymorphisms • Enzymes and their substrates—e.g. matrix metalloproteinases and extracellular matrix • Exposed bone • Growth factors and hormones—e.g. platelet-derived growth factor • (PDGF), sex steroids (androgens/oestrogens), thyroid hormones • Immunohistochemical markers—e.g. integrins, chemokine receptors and transforming growth factor beta II receptors • Inflammatory mediators—e.g. cytokines and interleukins • Nitric oxide • Nutritional factors—e.g. zinc, glutamine, vitamins • pH of wound fluid • Reactive oxygen species • Temperature • Transepidermal water loss from periwound skin
Newly identified biomarkers
• Uric acid • Glucose • H_2O_2

event [133]. Almost a decade ago Harding et al. [134] have gathered in a consensus meeting for discussing the progress of wound monitorization and have generated a list of potential wound markers, which served as basis to the studies in the following years. More recently other markers have been identified and added to this list, as summarized in Table 4.

Some of these markers have been recently gaining importance while others have led to contradictory results and difficulties upon their detection. Recently, Dragaville et al. [135] reviewed the state-of-the-art on some of the most effective markers, either embedded in dressings or as point-of-care (POC) techniques for wound assessment and monitoring. These include temperature, oxygen, bacteria, pH and biochemical signals.

Among the different wound types, chronic non-healing wounds have been particularly studied for biochemical markers, through several clinical investigations [136, 137]. Proteases, protease inhibitors, and pro-inflammatory cytokines are presently under study either locally at the wound site and/or systemically using high-throughput screening (metabolomic, proteomic, genomic and lipidomic analysis) [138]. Of these, proteases (serine, metalloproteinases, cysteine, aspartic) and specifically matrix metalloproteinases have received the most attention in studies of chronic wounds, showing great potential as targets for wound assessment [137, 139]. In a recent review

by Lindley et al. [138], some steps are proposed for the validation and implementation of these clinically applicable biomarkers, including those measured in tissue (ex. β-catenin), wound fluid (matrix metalloproteinases and interleukins), swabs, wound microbiota, and serum (ex. procalcitonin and matrix metalloproteinases).

The ongoing research on the above mentioned biomarkers or on others certainly discovered in the future gains more relevance when sustained in the idea of developing appropriated sensor tools. Since a wound is a dynamic environment, there is a strong need to develop systems that can diagnose the wound parameters in a minimally invasive way and report continuously on the type of environment inside the wound. Ideally it would consist of individual or combined sensors for pH, temperature, humidity, oxygen, bacteria sensors, etc [135, 140]. To monitor these parameters is not a difficult task, however the generated information needs to be precisely correlated to the events taking place in the wound bed. For instances a pH variation can occur following an inflammatory process or due to an infection [141, 142]. On the other hand, variations in wound pH will influence proteolytic activity and oxygen content and so measurements of enzyme activity may not be relevant unless correlated with pH [142].

5.2 Multicomponent Biosensor Dressings

The combination of sensors and dressings with active properties is nowadays considered as the gold standard, although numerous challenges still need to be overcome. A biosensor integrated within the dressing should be able to detect low levels of a certain biomarker (ex: resulting from a bacterial contamination) and consequently emit a recognizable output indicative of infection risk. Ideally this smart sensor should then trigger a material response towards a therapeutic effect, e.g., the controlled release of a pre-loaded drug. This could be achieved by integrating switchable surfaces or stimuli-responsive materials into the dressingto generate smart composites [135, 140, 143, 144]. At the same time the sensor should provide information about different parameters indicative of the status of the wound, in particular pH, temperature, moisture, and exudate production, etc. In doing so, these smart dressings will help shifting the paradigm of chronic wound care from routine management and time-based dressing changes toward cost effective personalized care and knowledge-based treatment [140, 144].

There is a significant effort in the research community to develop near-patient or wearable devices to enable wound care professionals to objectively measure the wound status. There have been advances in monitoring moisture [145–147], pH [145, 146], oxygen, protease [140, 144], and bacterial load [142, 146, 148, 149]; however, only a few of these systems are available for commercial use. At the present time, it is striking how few wound care devices have made it into clinical use. Recent advances on sensor research in wound monitoring have been made mostly on generic physiological status indicators such as, moisture [144, 145], pH [142, 146], oxygen [142, 150], and bacterial load [148, 149] and temperature.

In the case of moisture sensors, only slight advances have been made. A sensor to measure moisture content has been commercialized by Ohmedics Ltd (Glasgow, UK) following research on the moisture status of advanced wound dressings [146]. The WoundSense™ device is a disposable moisture sensor, suitable for use in any dressing and allows moisture monetarization without the need to disturb the dressing. Recently, Milne et al. [140] have reported the first large-scale observational study using this system. The results suggest that a large number of unnecessary dressing changes are being made, with disturbance of the wound bed and impact on healing and costs associated.

Presently, a considerable attention is being paid to wound pH monitoring, as it affects fibroblasts/keratinocytes activity, microbial proliferation and oxygen release to the tissues, altering the immune response of the wound [142]. As reported in the literature [151], the pH of healthy human skin is in the range of 4.0–7.0. In chronic venous leg ulcers and in pressure ulcers, an increase in pH (i.e. alkaline or neutral pH) is a sign of infection, if compared with the normal surrounding skin. Although the ideal pH sensor is yet to be discovered, there have been considerable developments in recent years, mostly in response to the limitations of the traditional glass potentiometric system related to its fragility and inability to measure multiple wound regions simultaneously and continuously [143]. These only provide localized measurements and are not feasible for complex measurements across the surface of a heterogeneous wound surface. Other strategies have been explored over the last years for continuous monitoring of the pH, either through electrochemical [148, 149, 151] or colorimetric methods [152, 153]. Electrochemical sensors measure the concentration of H^+ ions based on the rate of electrochemical reactions. Recently, Sharp et al. [150]. has proposed a version of printed electrodes on flexible acetate sheets that incorporate uric acid for monitoring wound pH. This sensor can detect pH in a broad range, from 4.0 to 10. Guinovart et al. [148]have modified a commercial adhesive bandage to create a pH sensor by screen printing Ag/AgCl and carbon electrodes onto it. This wearable pH sensor has shown to detect variations between 5.5 and 8 of pH values. More recently Rahimi et al. [149] have developed an inexpensive flexible array of pH sensors fabricated on a polymer-coated commercial paper, to be integrated in low-cost dressings as a way to map the pH at various wound sites. Another approach that has attracted noticeable attention is the use of pH sensitive materials and dyes for detection of skin pH [138, 140, 152, 153]. These colorimetric sensors are usually easy-to-read and can be utilized without integrated electronics. However, a key challenge for fabrication and use of these systems is to prevent the dye from leaching out of the dressing onto the skin. In addition, the sensitivity of the dye should cover the entire range of pH variation observed in skin disorders and wounds (pH = 4–9). Recently Tamayol et al. [153], developed a composite hydrogel alginate-based microfibers containing mesoporous particles loaded with a pH-responsive dye. T fabricated pH-responsive microfibers were flexible and able to maintain contact with skin, minimizing the leakage of the dye from the fibers. Non-invasive luminescence imaging is also of great interest for studying biological parameters in wound healing. Schreml et al. [154] have developed the first method for 2D luminescence imaging

of pH *in vivo* on humans. More recently they have described a sprayable luminescent pH sensor able to be applied to very uneven wound tissues [155].

Oxygen is one of the critical factors regulating the wound healing process [156], Acute hypoxia can cause tissue loss in a chronic wound and negatively impact the wound healing process [157]. Thus measuring oxygen concentration in real-time can be an effective tool for monitoring wound status. However, this parameter has only recently gained attention. Mostafalu et al. [158] have created a localized 3D-printed smart wound dressing platform that enables real-time data acquisition of oxygen concentration. The bandage contained a flexible oxygen sensor in a compact package, incorporating a series of off-the-shelf electronic components including a programmable-gain analog front-end, a microcontroller and wireless radio, an integrated electronic system with data readout and wireless transmission capabilities. This flexible platform can allow for a self-operating remote therapy for chronic wounds.

Uric acid (UA) concentration in wound exudate is another key marker which is recently being explored as specific indicator of wound status and infection since it is highly correlated with wound severity [159] and significantly decreases during bacterial infection [160]. Kassal et al. [161] described a new type of smart bandage for determination of uric acid (UA) status, by screen printing an amperometric biosensor directly on a wound dressing. The smart bandage biosensor interfaces with a wearable potentiostat for on-demand wireless data transfer to a computer, tablet, or Smartphone.

As described in this chapter, chronic wounds are extremely susceptible to infection, as the first defense barrier, the skin, is disrupted allowing for bacteria to invade the underlying tissue. Due to the clinical relevance of this problem to diagnose infection on a dressing at early stages and preferably, without its removal is a critical landmark. As herein referred, many of the above described sensors are used in dressings for detecting bacterial infection. But other strategies have been recently proposed, exploring different biochemical markers. For example, in a study by Krismastuti et al. [162] a porous anodized aluminum oxide (pAAO) based biosensor was developed as a biosensing platform to detect proteinase K, an enzyme which is a readily available model system for the proteinase produced by *P. aeruginosa.* As a proof-of-concept, this platform was successfully tested with human wound fluid, highlighting the potential for detection of bacterial infections in chronic wounds. Hajnsek and coworkers [163] developed an electrochemical sensor for fast detection of wound infection based on the quantification of myeloperoxidase activity as a marker for bacterial infection.

The use of carbon fibre tow as an electrochemical sensing matrix for assessing pyocyanin production, a substance produced by *P. aeruginosa* as a result of quorum sensing during wound colonization, was proposed by Sharp et al. [164]. The proposed small and inexpensive sensor assembly is suggested for use in monitoring *P. aeruginosa* growth. Ciani et al. [165] have reported the design and characterization of an electrochemical biosensor system and impedance detection method capable of the multiparameter detection of TREM-1 (Triggering Receptor-1 Expressed on

Myeloid cells), MMP-9 (Matrix MetalloPeptidase 9) and HSL (N-3-oxo-dodecanoyl-l-HomoSerineLacton), relevant in bacterial quorum sensing. These antigens are used without amplification and with minimal pre-analytical requirements on screen printed electrodes (SPEs), which are cheap, commercially available.

Temperature is an important parameter to assess chronicity, is frequently used for monitoring at-risk patients and anticipate ulceration [166]. However, there is little research using temperature sensors embedded in wound dressings. This can be achieved by incorporating miniaturized wireless sensors within wound dressings, as proposed by Matzeu et al. [167]. They fabricated a sensor using multiwall carbon nanotubes and electrodes produced by electroplating nickel and gold over the copper tracks prepared through a lithography process. Due to the wireless communication ability, this system can be used under a bandage or a wound dressing for minimally invasive, remote monitoring of temperature.

Most of the current methods for incorporating biosensors in dressings to monitoring chronic wounds have been independently developed and therefore are predominantly single-parameter. However due to the complex, multi-stage progression of wound healing the development of wearable integrated systems with sensors and readout telectronics is a major goal in the continuous monitoring of a chronic wound. Some steps are being done in this integrative approach. For instances, Mehmood et al. [167] presented a low-power portable telemetric system for measuring and transmitting real-time information of wound-site temperature, sub-bandage pressure and moisture level from within the wound dressing. Wang et al. [168] reported the use of a sprayable and thermogelating biomaterial (Poloxamer TM; a.k.a. Pluronic) in optical imaging of pH values, local oxygen and temperature. The polymer sensor particles containing molecular probes (such as for sensing O, pH or temperature) are incorporated in the host material, and the resulted sensor cocktail was sprayed onto surface of interest at low temperature. On increasing temperature, the sprayed thin film forms a gel and tightly adheres to form a stable sensor film. Two of the most relevant fluctuating wound parameters during the healing process which are pH and glucose concentration. Jankowska et al. [169] presented a fluorescent sensing system to monitor the wound status and to distinguish between an autonomously healing and a chronic wound at an early stage. The system allows monitoring simultaneously pH and Glucose using a fluorescent pH indicator dye, carboxynaphthofluorescein, and a metabolite-sensing enzymatic system, based on glucose oxidase and horseradish peroxidase, immobilized on a biocompatible polysaccharide matrix.

6 Future Perspectives

Chronic wounds have a deep impact in patient's health, social life, and it means an economical general burden, being therefore a current topical interest worldwide. In this chapter, we have focused on the current state of the art in chronic wound healing medicines involving the active treatment of these wounds. The evolution of the different advanced wound dressings, skin substitutes and available commercially

products, have been discussed, highlighting the advantages of combining materials, bioactive compounds and sensors to better face the different stages of the wound. The exact moment of deciding which dressing should be used remains controversial and the existing medical literature is not helpful. Only a few, ifany, prospective randomized control trials conclusively prove the superiority of one type ofwound dressing over another [170]. Therefore, more wound care research providing level A evidence is needed. Nevertheless, in daily clinics, the decisions need to be taken and all strategies attempt to achieve thesame goal: the successful healing.

The chapter covered many advanced wound dressings, including several types of polymeric systems and its composites in the form of foams, films, hydrogels and hydrocolloids for wound healing and tissue-engineered skin substitutes, dressings containing (antibiotics, silver, stem cells, etc). Although there is an enormous diversity of solutions, challenges still remain in tackling the problems associated with chronic wounds, and it is clear that one single advanced dressing does not always address the problems encountered in chronic wounds. Therefore, a combination of the above-mentioned advanced systems will be required, which impliesthat there is no single perfect dressing for allwounds. Nevertheless, the increasingly advanced biomaterials and their composite systems, creates favourable conditions for: better management and retention of the exudate; better adjustment to certain anatomical sites, not limiting the mobility of its users; better ease of removal of material for visual inspection, exchange of material for suspected infection; possibility to monitor parameters such as the pH or temperature.

The future of dressings points to the "interaction" between the material and user, as well as between the material and the clinician. It is not always easy to achieve or control all the variables involved in the process, because the dressing is optimised to control one or two needs, being optimal for the purpose of its design hence the professional's assessment of the best material for the priority variable to be controlled at that time. The future will bring possibilities of monitoring, at the wound bed, important events such as:an infection or exudate increase.

As a concluding remark there is no single ideal dressing code for wound healing. In the future it seems crucial to address advanced multi-component dressings that will tackle the problems of chronic wounds such as: pain, inflammation, odour, infection, delayed healing, and associated costs to health systems and populations worldwide. Therefore, a multi-targeted approach seems to be the best way toward wound care, which also should include a more detailed comprehension by the health professionalson the use of these advanced dressings and their recommendations.

Acknowledgements This work was supported by National Funds from FCT—Fundação para a Ciência e a Tecnologia through project UIDB/50016/2020. Sara Baptista-Silva gratefully acknowledges FCT for the grants (ref. SFRH/BPD/116024/2016).

References

1. Schultz GS et al (2004) Wound bed preparation and a brief history of TIME. Int Wound J **1**:19–32
2. Fletcher J (2008) Differences between acute and chronic wounds and the role of wound bed preparation. Nurs Stand 22:62–68
3. Lazarus GS et al (1994) Definitions and Guidelines for Assessment of Wounds and Evaluation of Healing. Arch Dermatol 130:489–493
4. White RJ, Cutting K, Kingsley A (2006) Topical antimicrobials in the control of wound bioburden. Ostomy Wound Manag 52:26–58
5. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J (2005) The global burden of diabetic foot disease. Lancet 366:1719–1724
6. Gottrup F (2004) A specialized wound-healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. Am J Surgery 187:38–43
7. Gottrup F, Holstein P, Jorgensen B, Lohmann M, Karlsmar T (2001) A new concept of a multidisciplinary wound healing center and a national expert function of wound healing. Arch Surg 136:765–772
8. Guest JF et al (2017) Health economic burden that different wound types impose on the UK's national health service. Int Wound J 14:322–330
9. Department of Health and Human Services (DHHS). CMS Manual System (2004) Pub. 100–07 State Operations Provider Certification SUBJECT: Revisions to State Operations Manual (SOM), Appendix PP-"Guidance to Surveyors for Long Term Care Facilities. 10:317
10. Bosanquet N, Brown D, Straub J, Harper DR, Ruckley CV (1999) Perceived health in a randomised trial of treatment for chronic venous ulceration. Eur J Vasc Endovasc Surg 17:155–159
11. Alves P, Costeira A, Vales L (2009) Reduzir a dor e o trauma no tratamento de feridas. Rev Nursing 20–25
12. Edwards J, Howley P, Cohen I (2004) In vitro inhibition of human neutrophil elastase by oleic acid albumin formulations from derivatized cotton wound dressings. Int J Pharm 284:1–12
13. Schönfelder U et al (2005) Influence of selected wound dressings on PMN elastase in chronic wound fluid and their antioxidative potential in vitro. Biomaterials 26:6664–6673
14. Baranoski S, Ayelo E (2006) O essencial sobre o tratamento de feridas—Principios práticos
15. Clark RAF, Richard AF (1996). The molecular and cellular biology of wound repair
16. De Mendonça RJ, Coutinho-Netto J (2009) Aspectos celulares da cicatrização. An Bras Dermatol 84:257–262
17. Diegelmann RF, Evans MC (2004) Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 9:283–289
18. Mandelbaum S, Santis É, Mandelbaum M (2003) Cicatrização: conceitos atuais e recursos auxiliares—Parte I. An Bras Dermatol 78:393–410
19. Werner S, Grose R (2003) Regulation of wound healing by growth factors and cytokines. Physiol Rev 83:835–870
20. Eming SA, Krieg T, Davidson JM (2007) Gene therapy and wound healing. Clin Dermatol 25:79–92
21. William M (2015) *Feridas: Conceitos e Atualidades*
22. Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM (2011) Dynamic reciprocity in the wound microenvironment. Wound repair Regen. Off. Publ. Wound Heal. Soc. [and] Eur. Tissue Repair Soc 19:134–148
23. Singer AJ, Clark RAF (1999) Cutaneous wound healing. N Engl J Med 341:738–746
24. Robbins S et al (2005) Robbins & Cotran: Patologia—Bases patológicas das doenças
25. Balbino CA, Pereira LM, Curi R (2005) Mechanisms involved in wound healing: a revision. Rev Bras Ciencias Farm J Pharm Sci 41:27–51

26. Dvorak H (2002) Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol 20:4368–4380
27. Broughton G, Janis J, Attinger C (2006) The basic science of wound healing. Plast Reconstr Surgery 117:12–34
28. Gurtner GC, Werner S, Barrandon Y, Longaker MT (2008) Wound repair and regeneration. Nature 453:314–321
29. Macdonald JM, Geyer MJ, Organization WH (2010) In: John MM, Mary JG (eds) Wound and lymphoedema. vol 7. pp 122
30. Flanagan M (2013) In: Wound healing and skin integrity: principles and practice
31. Swanson T, Angel D (2017) Wound infection in clinical practice update. Aust Nurs midwifery J 24:33
32. Jones V, Grey JE, Harding KG (2006) Wound dressings. BMJ 332:777–780
33. Leaper DJ et al (2012) Extending the TIME concept: what have we learned in the past 10 years?(*). Int Wound J 9 Suppl 2:1–19
34. Nazarko L (2005) Part two: carrying out a thorough assessment. Nurs Resid Care 7:304–306
35. Percival SL, Emanuel C, Cutting KF, Williams DW (2012) Microbiology of the skin and the role of biofilms in infection. Int Wound J 9:14–32
36. Fyhrquist N, Salava A, Auvinen P, Lauerma A (2016) Skin Biomes Curr Allergy Asthma Rep 16:40
37. Grice EA, Segre JA (2011) The skin microbiome. Nat Rev Microbiol 9:244–253
38. Gethin G (2009) Role of topical antimicrobials in wound management. J Wound Care 1–8
39. Dow G, Browne A, Sibbald RG (1999) Infection in chronic wounds: controversies in diagnosis and treatment. Ostomy Wound Manage 45(22–29):23–27
40. Edwards R, Harding KG (2004) Bacteria and wound healing. Curr Opin Infect Dis 17:91–96
41. Frank DN et al (2009) Microbial diversity in chronic open wounds. Wound Repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 17:163–172
42. Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14:244–269
43. Schultz GS et al (2003) Wound bed preparation: a systematic approach to wound management. Wound Repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 11 Suppl 1:S1–28
44. Warriner R, Burrell R (2015) Infection and the chronic wound: a focus on silver. Adv Skin Wound Care 18 Suppl 1:2–12
45. Bjarnsholt T, Moser C, Jensen PØ, Høiby N (2011) Chronic wound colonization, infection, and biofilms. Biofilm Infect 1–314. https://doi.org/10.1007/978-1-4419-6084-9
46. Siddiqui AR, Bernstein JM (2010) Chronic wound infection: facts and controversies. Clin Dermatol 28:519–526
47. Scalise A et al (2015) Microenvironment and microbiology of skin wounds: the role of bacterial biofilms and related factors. Semin Vasc Surg 28:151–159
48. Davies D (2003) Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov 2:114–122
49. Zhao G et al (2013) Biofilms and inflammation in chronic wounds. Adv Wound Care 2:389–399
50. James GA et al (2008) Biofilms in chronic wounds. Wound Repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 16:37–44
51. Percival SL et al (2012) A review of the scientific evidence for biofilms in wounds. Wound Repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 20:647–657
52. McCarty SM, Percival SL (2013) Proteases and delayed wound healing. Adv Wound Care 2:438–447
53. Bjarnsholt T et al (2007) Silver against Pseudomonas aeruginosa biofilms. APMIS 115:921–928
54. Falanga V (2000) Classifications for wound bed preparation and stimulation of chronic wounds. Wound Repair and Regeneration : Official Publication of the Wound Healing Soc [and] the Europ Tissue Repair Soc 8:347–352

55. Kirketerp-Møller K, Zulkowski K, James G (2010) Chronic wound colonization, infection, and biofilms. Biofilm Infections 11–24. https://doi.org/10.1007/978-1-4419-6084-9_2
56. Brown-Etris M, Custshall W, Hiles M (2002) A new biomaterial derived from small intestine submucosa and developed into a wound matrix device. Wounds 14:150–166
57. Clark M et al (2014) Systematic review of the use of prophylactic dressings in the prevention of pressure ulcers. Int Wound J 11:460–471
58. Black J et al (2015) Use of wound dressings to enhance prevention of pressure ulcers caused by medical devices. Int. Wound J 12:322–327
59. Call E et al (2015) Enhancing pressure ulcer prevention using wound dressings: what are the modes of action? Int. Wound J 12:408–413
60. Das S, Baker AB (2016) Biomaterials and nanotherapeutics for enhancing skin wound healing. Front Bioeng Biotechnol 4:82
61. Eaglstein WH, Falanga V (1998) Tissue engineering and the development of Apligraf, a human skin equivalent. Cutis 62:1–8
62. Shores JT, Gabriel A, Gupta S (2007) Skin substitutes and alternatives: a review. Adv Skin Wound Care 20:410–493
63. Halim AS, Khoo TL, Mohd Yussof SJ (2010) Biologic and synthetic skin substitutes: an overview. Indian J. Plast. Surg. Off Publ Assoc Plast Surg India 43:S23–8
64. Spasojević D et al (2016) Lignin model compound in alginate hydrogel: a strong antimicrobial agent with high potential in wound treatment. Int J Antimicrob Agents 48:732–735
65. Rezvanian M, Amin MCIM, Ng S-F (2016) Development and physicochemical characterization of alginate composite film loaded with simvastatin as a potential wound dressing. Carbohydr Polym 137:295–304
66. Broussard KC, Powers JG (2013) Wound dressings: selecting the most appropriate type. Am J Clin Dermatol 14:449–459
67. Cherusseri J et al (2017) Polymer-based composite materials: characterizations BT—composite materials: processing, applications, characterizations. In: Kar KK (ed) Springer, Berlin, Heidelberg, pp 37–77. https://doi.org/10.1007/978-3-662-49514-8_2
68. Dabiri G, Damstetter E, Phillips T (2016) Choosing a wound dressing based on common wound characteristics. Adv Wound Care 5:32–41
69. Seaman S (2002) Dressing selection in chronic wound management. J Am Podiatr Med Assoc 92:24–33
70. Aduba DCJ et al (2016) Fabrication, characterization, and in vitro evaluation of silver-containing arabinoxylan foams as antimicrobial wound dressing. J Biomed Mater Res A 104:2456–2465
71. Liu J, Morykwas MJ, Argenta LC, Wagner WD (2011) Development of a biodegradable foam for use in negative pressure wound therapy. J Biomed Mater Res B Appl Biomater 98:316–322
72. Moura LIF, Dias AMA, Carvalho E, Sousa, De HC (2013) Recent advances on the development of wound dressings for diabetic foot ulcer treatment —a review. Acta Biomater 9:7093–7114
73. Powers JG, Morton LM, Phillips TJ (2013) Dressings for chronic wounds. Dermatol Ther 26:197–206
74. Landsman TL et al (2017) A shape memory foam composite with enhanced fluid uptake and bactericidal properties as a hemostatic agent. Acta Biomater 47:91–99
75. Namviriyachote N, Muangman P, Chinaroonchai K, Chuntrasakul C, Ritthidej GC (2020) Polyurethane-biomacromolecule combined foam dressing containing asiaticoside: fabrication, characterization and clinical efficacy for traumatic dermal wound treatment. Int J Biol Macromol 143:510–520
76. He M et al (2020) Smart multi-layer PVA foam/ CMC mesh dressing with integrated multifunctions for wound management and infection monitoring. Mater Des 194:108913
77. Oh G-W, Nam SY, Heo S-J, Kang D-H, Jung W-K (2020) Characterization of ionic cross-linked composite foams with different blend ratios of alginate/pectin on the synergistic effects for wound dressing application. Int J Biol Macromol 156:1565–1573

78. Wang Y et al (2018) Shape-adaptive composite foams with high expansion and absorption used for massive hemorrhage control and irregular wound treatment. Appl Mater Today 13:228–241
79. Bajpai SK, Jyotishi P, Bajpai M (2016) Synthesis of nanosilver loaded chitosan/poly(acrylamide-co-itaconic acid) based inter-polyelectrolyte complex films for antimicrobial applications. Carbohydr Polym 154:223–230
80. Kim JO et al (2015) Nitric oxide-releasing chitosan film for enhanced antibacterial and in vivo wound-healing efficacy. Int J Biol Macromol 79:217–225
81. Kalaycıoğlu Z et al (2020) Antibacterial nano cerium oxide/chitosan/cellulose acetate composite films as potential wound dressing. Eur Polym J 133:109777
82. Ambrogi V et al (2020) Biocompatible alginate silica supported silver nanoparticles composite films for wound dressing with antibiofilm activity. Mater Sci Eng, C 112:110863
83. Üstündağ Okur N et al (2019) An alternative approach to wound healing field; new composite films from natural polymers for mupirocin dermal delivery. Saudi Pharm J 27:738–752
84. Bergonzi C et al (2020) Alginate/human elastin-like polypeptide composite films with antioxidant properties for potential wound healing application. Int J Biol Macromol 164:586–596
85. Boateng JS, Matthews KH, Stevens HNE, Eccleston GM (2008) Wound healing dressings and drug delivery systems: a review. J Pharm Sci 97:2892–2923
86. Pott FS, Meier MJ, Stocco JGD, Crozeta K, Ribas JD (2014) The effectiveness of hydrocolloid dressings versus other dressings in the healing of pressure ulcers in adults and older adults: a systematic review and meta-analysis. Rev Lat Am Enfermagem 22:511–520
87. Thu H-E, Zulfakar MH, Ng S-F (2012) Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing. Int J Pharm 434:375–383
88. Mazzurco JD, Krach KJ (2012) Use of a hydrocolloid dressing to aid in the closure of surgical wounds in patients with fragile skin. J Amer Acad Dermatol 66:335–336
89. Jin SG et al (2015) Mechanical properties and in vivo healing evaluation of a novel Centella asiatica-loaded hydrocolloid wound dressing. Int J Pharm 490:240–247
90. Jin SG et al (2016) Influence of hydrophilic polymers on functional properties and wound healing efficacy of hydrocolloid based wound dressings. Int J Pharm 501:160–166
91. Valachova K, Svik K, Biro C, Soltes L (2020) Skin wound healing with composite biomembranes loaded by tiopronin or captopril. J Biotechnol 310:49–53
92. Garcia-Orue I et al (2019) Composite nanofibrous membranes of PLGA/Aloe vera containing lipid nanoparticles for wound dressing applications. Int J Pharm 556:320–329
93. Kong D et al (2020) Adhesion loss mechanism based on carboxymethyl cellulose-filled hydrocolloid dressings in physiological wounds environment. Carbohydr Polym 235:115953
94. Marchesan S et al (2013) Self-assembly of ciprofloxacin and a tripeptide into an antimicrobial nanostructured hydrogel. Biomaterials 34:3678–3687
95. Dumville JC, O'Meara S, Deshpande S, Speak K (2011) Hydrogel dressings for healing diabetic foot ulcers. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD009101.pub2
96. Agubata CO, Okereke C, Nzekwe IT, Onoja RI, Obitte NC (2016) Development and evaluation of wound healing hydrogels based on a quinolone, hydroxypropyl methylcellulose and biodegradable microfibres. Eur J Pharm Sci Off J Eur Fed Pharm Sci 89:1–10
97. Toh WS, Loh XJ (2014) Advances in hydrogel delivery systems for tissue regeneration. Mater Sci Eng C Mater Biol Appl 45:690–697
98. Shukla R, Kashaw SK, Jain AP, Lodhi S (2016) Fabrication of apigenin loaded gellan gum-chitosan hydrogels (GGCH-HGs) for effective diabetic wound healing. Int J Biol Macromol 91:1110–1119
99. Zeng Y et al (2015) Preformed gelatin microcryogels as injectable cell carriers for enhanced skin wound healing. Acta Biomater 25:291–303
100. Gong C et al (2013) A biodegradable hydrogel system containing curcumin encapsulated in micelles for cutaneous wound healing. Biomaterials 34:6377–6387
101. Deng Y et al (2020) Novel fenugreek gum-cellulose composite hydrogel with wound healing synergism: facile preparation, characterization and wound healing activity evaluation. Int J Biol Macromol 160:1242–1251

102. Yang J et al (2020) Preparation of a chitosan/carboxymethyl chitosan/AgNPs polyelectrolyte composite physical hydrogel with self-healing ability, antibacterial properties, and good biosafety simultaneously, and its application as a wound dressing. Compos Part B Eng 197:
103. Lin X et al (2020) An alginate/poly(N-isopropylacrylamide)-based composite hydrogel dressing with stepwise delivery of drug and growth factor for wound repair. Mater Sci Eng, C 115:111123
104. Hamdi M et al (2020) A novel blue crab chitosan/protein composite hydrogel enriched with carotenoids endowed with distinguished wound healing capability: In vitro characterization and in vivo assessment. Mater Sci Eng, C 113:110978
105. Atala A (2009) Foundations of regenerative medicine: clinical and therapeutic applications
106. van Blitterswijk C et al (2008) In: Tissue engineering
107. MacNeil S (2007) Progress and opportunities for tissue-engineered skin. Nature 445:874–880
108. Jurczak F et al (2007) Randomised clinical trial of Hydrofiber dressing with silver versus povidone-iodine gauze in the management of open surgical and traumatic wounds. Int Wound J 4:66–76
109. Groeber F, Holeiter M, Hampel M, Hinderer S, Schenke-Layland K (2011) Skin tissue engineering–in vivo and in vitro applications. Adv Drug Deliv Rev 63:352–366
110. Mayet N et al (2014) A comprehensive review of advanced biopolymeric wound healing systems. J Pharm Sci 103:2211–2230
111. Shevchenko RV, James SL, James SE (2010) A review of tissue-engineered skin bioconstructs available for skin reconstruction. J R Soc Interface 7:229–258
112. Greaves NS, Iqbal SA, Baguneid M, Bayat A (2013) The role of skin substitutes in the management of chronic cutaneous wounds. Wound repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 21:194–210
113. Pereira RF, Barrias CC, Granja PL, Bartolo PJ (2013) Advanced biofabrication strategies for skin regeneration and repair. Nanomedicine (Lond) 8:603–621
114. Kalyanaraman B, Boyce ST (2009) Wound healing on athymic mice with engineered skin substitutes fabricated with keratinocytes harvested from an automated bioreactor. J Surg Res 152:296–302
115. Shevchenko RV et al (2014) The in vitro characterization of a gelatin scaffold, prepared by cryogelation and assessed in vivo as a dermal replacement in wound repair. Acta Biomater 10:3156–3166
116. 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 40:957–965
117. Yildirimer L, Thanh NTK, Seifalian AM (2012) Skin regeneration scaffolds: a multimodal bottom-up approach. Trends Biotechnol 30:638–648
118. Gravante G et al (2007) A randomized trial comparing ReCell system of epidermal cells delivery versus classic skin grafts for the treatment of deep partial thickness burns. Burns 33:966–972
119. Zweifel CJ et al (2008) Initial experiences using non-cultured autologous keratinocyte suspension for burn wound closure. J Plast Reconstr Aesthet Surg 61:1–4
120. Böttcher-Haberzeth S, Biedermann T, Reichmann E (2010) Tissue engineering of skin. Burns 36:450–460
121. Philandrianos C et al (2012) Comparison of five dermal substitutes in full-thickness skin wound healing in a porcine model. Burns 38:820–829
122. van der Veen VC, Boekema BKHL, Ulrich MMW, Middelkoop E (2011) New dermal substitutes. Wound Repair Regen 19:59–65
123. Harding K, Sumner M, Cardinal M (2013) A prospective, multicentre, randomised controlled study of human fibroblast-derived dermal substitute (Dermagraft) in patients with venous leg ulcers. Int Wound J 10:132–137
124. Jayarama Reddy V et al (2013) Nanofibrous structured biomimetic strategies for skin tissue regeneration. Wound Repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 21:1–16

125. Poinern GEJ et al (2010) Nanoengineering a biocompatible inorganic scaffold for skin wound healing. J Biomed Nanotechnol 6:497–510
126. Liu X et al (2012) Antimicrobial electrospun nanofibers of cellulose acetate and polyester urethane composite for wound dressing. J Biomed Mater Res B Appl Biomater 100:1556–1565
127. Sriwiriyanont P et al (2012) Morphogenesis of chimeric hair follicles in engineered skin substitutes with human keratinocytes and murine dermal papilla cells. Experim Dermatol 21:783–785
128. Nichol JW, Khademhosseini A (2009) Modular tissue engineering: engineering biological tissues from the bottom up. Soft Matter 5:1312–1319
129. Zamanian B, Kachouie NN, Nichol JW, Khademhosseini A (2016) Self-assembly of cell-laden hydrogels on the liquid-air interface. 121–131
130. Rustad KC, Sorkin M, Levi B, Longaker MT, Gurtner GC (2010) Strategies for organ level tissue engineering. Organogenesis 6:151–157
131. Jakab K et al (2010) Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication 2:22001
132. Dias JR, Granja PL, Bártolo PJ (2016) Advances in electrospun skin substitutes progress in materials science advances in electrospun skin substitutes. Prog Mater Sci 84:314–334
133. Eming SA, Martin P, Tomic-Canic M (2014) Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med 6:265sr6
134. Becker K et al (2009) Diagnostics and wounds: a consensus document. World Counc Enteros Ther J 29:18–20 22
135. Dargaville TR et al (2013) Sensors and imaging for wound healing: a review. Biosens Bioelectron 41:30–42
136. Eming SA et al (2010) Differential proteomic analysis distinguishes tissue repair biomarker signatures in wound exudates obtained from normal healing and chronic wounds. J Proteome Res 9:4758–4766
137. Broadbent J, Walsh T, Upton Z (2010) Proteomics in chronic wound research: potentials in healing and health. Proteomics Clin Appl 4:204–214
138. Lindley LE, Stojadinovic O, Pastar I, Tomic-Canic M (2016) Biology and biomarkers for wound healing. Plast Reconstr Surg 138:18–28
139. Amato B et al (2015) Role of matrix metalloproteinases in non-healing venous ulcers. Int Wound J 12:641–645
140. Mehmood N, Hariz A, Fitridge R, Voelcker NH (2014) Applications of modern sensors and wireless technology in effective wound management. J Biomed Mater Res B Appl Biomater 102:885–895
141. Schreml S et al (2010) The impact of the pH value on skin integrity and cutaneous wound healing. J Eur Acad Dermatol Venereol 24:373–378
142. Schneider LA, Korber A, Grabbe S, Dissemond J (2007) Influence of pH on wound-healing: a new perspective for wound-therapy? Arch Dermatol Res 298:413–420
143. Salvo P, Dini V, Di Francesco F, Romanelli M (2015) The role of biomedical sensors in wound healing. Wound Med 8:15–18
144. McLister A, McHugh J, Cundell J, Davis J (2016) New developments in smart bandage technologies for wound diagnostics. Adv Mater 28:5732–5737
145. Milne SD et al (2016) A wearable wound moisture sensor as an indicator for wound dressing change: an observational study of wound moisture and status. Int Wound J 13:1309–1314
146. McColl D, Cartlidge B, Connolly P (2007) Real-time monitoring of moisture levels in wound dressings in vitro: an experimental study. Int J Surg 5:316–322
147. McColl D, MacDougall M, Watret L, Connolly P (2009) Monitoring moisture without disturbing the wound dressing. Wounds UK 5:94–99
148. Guinovart T, Valdés-Ramírez G, Windmiller JR, Andrade FJ, Wang J (2014) Bandage-based wearable potentiometric sensor for monitoring wound pH. Electroanalysis 26:1345–1353
149. Rahimi R et al (2016) A low-cost flexible pH sensor array for wound assessment
150. Sharp D (2013) Printed composite electrodes for in-situ wound pH monitoring. Biosens Bioelectron 50:399–405

151. Lambers H, Piessens S, Bloem A, Pronk H, Finkel P (2006) Natural skin surface pH is on average below 5, which is beneficial for its resident flora. Int J Cosmet Sci 28:359–370
152. Mohr GJ, Müller H (2015) Tailoring colour changes of optical sensor materials by combining indicator and inert dyes and their use in sensor layers, textiles and non-wovens. Sens Actuators B Chem 206:788–793
153. Tamayol A et al (2016) Flexible pH-sensing hydrogel fibers for epidermal applications. Adv Healthc Mater 5:711–719
154. Schreml S et al (2011) 2D luminescence imaging of pH in vivo. Proc Natl Acad Sci USA 108:2432–2437
155. Schreml S et al (2012) A sprayable luminescent pH sensor and its use for wound imaging in vivo. Experim Dermatol 21:951–953
156. Schreml S et al (2010) Oxygen in acute and chronic wound healing. Br J Dermatol 163:257–268
157. Sen CK (2009) Wound healing essentials: let there be oxygen. Wound Repair Regen Off Publ Wound Heal Soc [and] Eur Tissue Repair Soc 17:1–18
158. Mostafalu P et al (2016) A toolkit of thread-based microfluidics, sensors, and electronics for 3D tissue embedding for medical diagnostics. Microsyst Nanoeng 2:16039
159. Fernandez ML, Upton Z, Edwards H, Finlayson K, Shooter GK (2012) Elevated uric acid correlates with wound severity. Int Wound J 9:139–149
160. Sharp D, Davis J (2008) Integrated urate sensors for detecting wound infection. Electrochem Commun Electrochem Commun 10:709–713
161. Kassal P et al (2015) Smart bandage with wireless connectivity for uric acid biosensing as an indicator of wound status. Electrochem Commun 56:6–10
162. Krismastuti F, Bayat H, Voelcker N, Schönherr H (2015) Real time monitoring of layer-by-layer polyelectrolyte deposition and bacterial enzyme detection in nanoporous anodized aluminum oxide. Anal Chem 87:3856–3863
163. Hajnsek M et al (2015) Sens Actuators B: Chem An Electrochem Sens Fast Detection of Wound Infection Based on Myeloperoxidase Activity 209:2014–2016
164. Sharp D, Gladstone P, Smith RB, Forsythe S, Davis J (2010) Approaching intelligent infection diagnostics: carbon fibre sensor for electrochemical pyocyanin detection. Bioelectrochemistry 77:114–119
165. Ciani I et al (2012) Development of immunosensors for direct detection of three wound infection biomarkers at point of care using electrochemical impedance spectroscopy. Biosens Bioelectron 31:413–418
166. Dini V et al (2015) Correlation between wound temperature obtained with an infrared camera and clinical wound bed score in venous leg ulcers. Wounds a Compend Clin Res Pract 27:274–278
167. Mehmood N, Hariz A, Templeton S, Voelcker NH (2015) A flexible and low power telemetric sensing and monitoring system for chronic wound diagnostics. Biomed Eng Online 14:17
168. Wang X et al (2015) A water-sprayable, thermogelating and biocompatible polymer host for use in fluorescent chemical sensing and imaging of oxygen, pH values and temperature. Sens Actuators B Chem 221:37–44
169. Jankowska DA et al (2017) Simultaneous detection of pH value and glucose concentrations for wound monitoring applications. Biosens Bioelectron 87:312–319
170. Buck D, Galiano R (2007) In: wound care. grabb and smith's plastic surgery