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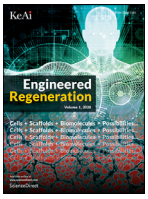

# Wound dressing products: A translational investigation from the bench to the market

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ABSTRACT

Chronic skin wounds affect more than 40 million patients globally and represent a severe growing burden for the healthcare systems, with annual costs expected to exceed $15 billions by 2022. To satisfy the huge demand for effective wound care products, different types of wound dressings have been introduced on the market during the last decades. Based on “the moist wound healing theory” postulated by Prof Winter in 1962, bandages were initially designed to recreate the optimal wound environment to favor the healing process. Then, thanks to the advancements achieved in biomaterial design and processing, biotechnology, imaging and electronic fields, great effort has been devoted to the development of formulations able to actively participate to tissue healing. Indeed, both the literature and the market report the design of medicated wound dressings, i.e., wound care products releasing anti-microbial agents, anti-inflammatory drugs, or bioactive molecules. In this scenario, this review aims at critically describing the currently available wound care products, highlighting their proved effectiveness in wound management. Moreover, an overview of the main strategies exploited to design personalized wound dressings has been reported. Lastly, concerns on regulatory affairs and practical issues limiting the clinical translation of advanced research platforms have also been discussed.

## 1. Introduction

The expression "hard-to-close wounds” refers to damaged tissues characterized by impaired healing processes within approx. 4 weeks of treatment. The physiological wound-healing pathway consists of three sequential and overlapping phases: (i) inflammation, (ii) proliferation and (iii) remodeling. When the healing sequence stalls in one specific phase, leading to unsuccessful tissue closure, wounds are classified as chronic wounds [1]. The main factors responsible for this impeded healing are age, overproduction of matrix metalloproteinases, low vascularization (i.e., ischemia), decreased oxygen and nutrient supply and comorbidities, such as diabetes, peripheral arterial disease and immune deficiency. Moreover, studies revealed that delayed healing could be also attributed to stress-induced aging of cells, which leads to reduced cell viability and impaired healing pathway activation [2]. Among all these factors, bacterial infections represent the most serious issue to be faced in wound management. Indeed, although low levels of bacteria in the wound bed are known to promote tissue regeneration by stimulating the production of proteolytic enzymes [3], bacteria colonization significantly affects wound closure capability by altering the metabolic activity [4]. Furthermore, infected chronic wounds are generally associated with the formation of a biofilm, i.e., a very sturdy polysaccharide coating formed by bacteria, which can limit the diffusion of locally delivered drugs. Hence, to promote tissue regeneration and wound healing, biofilm should be removed. However, it can be barely destroyed by either the immune system or systemic and topical anti-microbial administration and thus, in many cases debridement aimed at disrupting this impermeable film turns out to be essential.

The primary clinical approach to chronic wound treatment consists of glycemic control, revascularization and optimization of the blood flow, removal of exudate, biofilm and necrotic tissue, and control over patients’ co-morbidities. However, this single approach is not strong enough to completely face the problem of chronic skin wounds. Hard-to-close chronic wounds affect more than 40 million patients globally and represent a severe growing burden for the healthcare systems, with annual costs expected to exceed $15 billions by 2022 [5]. Only in Europe, 4 million patients suffer from chronic wounds every year, requiring 25%–50% of acute hospital beds. Furthermore, the costs associated with chronic wound treatments represent at least 4% of the national annual budget, subdivided in 15%–20% for materials, 30%–35% for healthcare providers and more than 50% for hospitalization [6]. As a consequence of this long-term hospitalization, hard-to-close wounds also negatively impact the society wellness, with a significant reduction in the available workforce. Hence, the healthcare systems exert a tremendous pres-

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sure on researchers and pharmaceutical industries towards the development of cost-effective wound treatments. Indeed, since the late 90s, dramatically increasing data on the growth of the global wound care market have been registered, with costs projected to reach $8460 million in 2025 [6]. However, the wide supply of wound treatments developed during these years has not been able to crucially tackle the huge annual incidence of chronic wounds. Such reduced effectiveness could be ascribed to several co-current issues: (i) although wound classifications based on type, origin and regeneration capability do exist, each wound is characterized by a unique healing process and thus, there are no all-purpose ideal dressings; (ii) due to the overwhelming available dressings, there is a lack of full understanding of the advantages offered by commercially available wound care treatments; (iii) there is an extremely wide gap between results coming from the research on advanced wound dressings and the technology level of available products on the market. Hence, much effort should be directed to make the research achievements more easily available for the healthcare providers.

Since ancient time, "the moist wound healing theory" postulated by Prof George D. Winter in 1962 has been the most influencing concept on the design of wound care products [7]. Specifically, Prof Winter proved that significantly faster tissue regeneration kinetics could be achieved in the presence of a moist environment by promoting wound epithelialization. Later, Lawrence highlighted the need to develop wound dressings capable to avoid adherence to damaged tissues [8], while Piskozub investigated the importance to provide dressings with absorbance capability aiming at removing excess exudate [9]. Consequently, the increasing number of available wound dressings and the design of progressively more sophisticated treatments led to the identification of standard test methods to evaluate and compare the wound dressing performances [10]. In general, the ideal wound dressing should fulfill several requirements to effectively manage hard-to-heal wounds [1], as schematically reported in Fig. 1.

Therefore, starting from these principles, a huge number of different types of wound dressings have been designed over the last decades. The following sections aim at providing a critical overview of traditional, medicated and advanced wound dressings currently used in clinical practice with a special focus on their efficacy evaluated through results reported in the literature on randomized clinical trials (RCTs). Furthermore, future directions towards personalized wound dressings are also described. Lastly, an in-depth discussion on the main practical concerns (i.e., regulatory affairs and patch validation processes) limiting the quick market entry of such personalized formulations is reported.

## 2. Traditional wound dressings

### 2.1. Gauzes

Gauzes are the oldest and the most inexpensive, available and highly absorbent traditional wound dressing (Fig. 2). Moreover, being easily adaptable to any defect shape, gauzes are widely used to cover both infected and not infected wounds, in which large amounts of exudate are present. However, despite their large use, gauzes are not ideal wound dressings as they can cause trauma, mechanical debridement and thus, patient's pain when removed. Furthermore, they could leave residues (i.e., fibers, particles) activating the immune system towards granuloma formation [1]. A step forward in the field was achieved through the introduction of wet-to-dry bandages, i.e., wound dressings applied in their wet state and allowed to dry in the ulcer cavity entrapping necrotic tissue. Hence, compared to traditional bandages, such systems should be able to more precisely control the mechanical debridement occurring during the dressing take off. However, many other associated drawbacks have been reported. For instance, they turned out to lead to: (i) vasoconstriction, (ii) increased affinity of hemoglobin to oxygen, (iii) hypoxia as a consequence of local tissue cooling during evaporation, and (iv) patient discomfort deriving from their removal in the dry state. Moreover, wet-to-dry dressings have been reported to be responsible for cross-contamination and not-selective debridement [1]. Lastly, irrespective of their nature, gauzes turned out to be highly susceptible to bacteria contamination [11] and thus, characterized by higher infection rates compared to other dressings (e.g., films or hydrocolloids) [12,13]. These mentioned side effects were partially solved through the introduction of impregnated gauzes, i.e., gauzes containing iodine, zinc, and bismuth. Indeed, conversely to traditional bandages, they keep a moist environment and avoid tissue cooling during evaporation [14]. However, all loaded substances have been reported to show cytotoxic effects and low anti-microbial activity.

### 2.2. Transparent films

Transparent film dressings (Fig. 2) can be considered the evolution of gauzes into more sophisticated bandages, being able to simultaneously provide a moist wound environment, ensure gas exchange and prevent contamination from external bacteria. Moreover, film dressings are easy to adapt and to remove without causing patient's pain. However, they do not possess swelling capability and thus, their application is not recommended for the treatment of wounds, which produce large amounts of exudate as well as for infected wounds. Indeed, the simultaneous presence of exudate and infection further enhances bacteria proliferation [1]. In a recently reported study, Wei et al. compared the effectiveness of a newly developed poly (dimethylsiloxane)-based bi-layer film with that of the commercially available Tegaderm™ film (3M, Minnesota, USA) in the treatment of diabetic chronic wounds [15]. Specifically, the authors focused on the need to keep a moist wound environment and a tight contact between the wound dressing and the wound bed, thus highlighting the importance of specific mechanical features wound dressings should comply with. Indeed, experiments conducted on both normal and diabetic rats evidenced faster healing rates when ulcers were treated with the developed bi-layer films compared to Tegaderm™ ones. Such improvement was exclusively attributed to the lower tensile strength and greater elongation at break registered for the bi-layer film compared to Tegaderm™ film, leading at the end to a highly flexible and adaptable wound dressing.

### 2.3. Foams

Conversely to transparent film dressings, foam dressings are able to absorb large amounts of fluids while ensuring thermal insulation and gas exchange (Fig. 2). Hence, foams are indicated for the treatment of exudate-rich wounds. Due to their remarkable high absorbance capability, this type of dressing could be left in place for up to 7 days in not-infected wounds, while daily changes are recommended in the presence of infections [16]. Many research studies have reported the design of foams for wound healing applications, mainly focusing on the identification of the highest-performance material in terms of absorption capability [17–19]. On the other hand, Anderson et al. investigated the absorbance capability of two widely used commercial foam dressings, i.e., Allevyn (Smith and Nephew, London, UK) and Biatain (Coloplast, Humblebaek, Denmark) [20]. Specifically, through an RCT conducted on patients affected by lower leg ulcers, the authors found out that the significantly higher absorbance rate of Biatain foams compared to Allevyn ones (i.e., 76% *vs.* 7%, respectively) resulted in fewer dressing changes and thus, reduced associated costs. Similar clinical investigations on patients affected by diabetic foot ulcers were conducted by Gwak et al. [21]. Specifically, the authors compared the efficacy of two commercial poly (urethane)-based foams, i.e., Betafoam and Medifoam (Genewel, Scongnam, Korea), differing for the presence in the former of 3% povidone-iodine as anti-microbial agent. No differences in bacteria regression and healing rate were observed between the two groups, thus further highlighting that the main mode of action of foam dressings could be ascribed to their absorption capability.

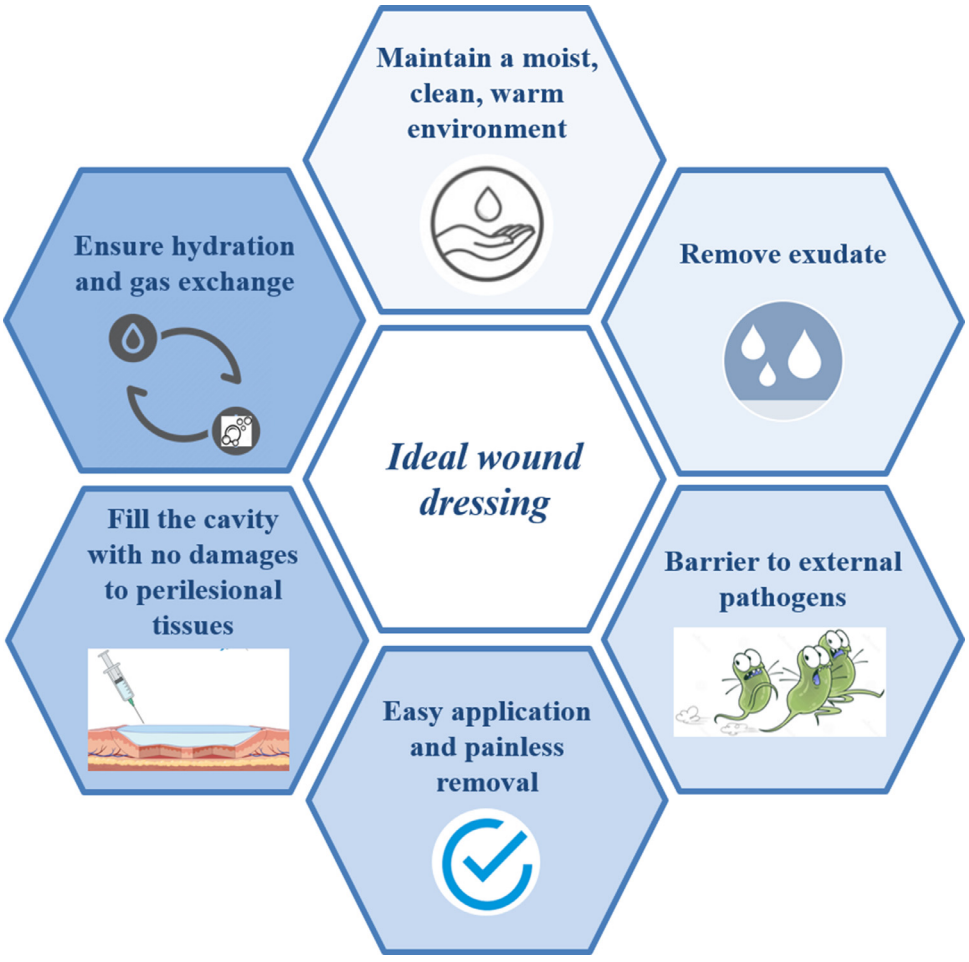


**Fig. 1.** Schematic representation of the main requirements an ideal wound dressing should fulfill to successfully enhance the regeneration of hard-to-close wounds.

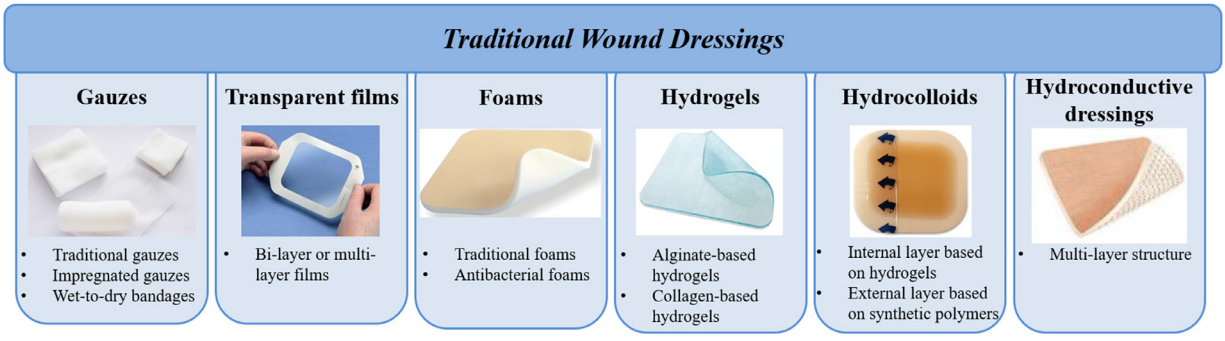


**Fig. 2.** Traditional wound dressings: gauzes, transparent films, foam dressings, hydrogels, hydrocolloids and hydroconductive dressings.

### 2.4. Hydrogels

Among other wound care treatments, hydrogel-based wound dressings (Fig. 2) are widely used as they show discrete sorption capability, allow gas exchange (i.e., oxygen, $CO_2$, $H_2O$), avoid patient's pain during their removal, enhance tissue granulation and are able to lower the wound bed temperature up to 5 °C [22,23]. Moreover, as a consequence of their strong hydrophilic nature (they are composed of 80%–90% of water), hydrogels are able to maintain a moist wound environment, which in turns promotes autolytic debridement. However, hydrogel dressings are less effective in facing bacteria contamination compared to occlusive dressings and thus, they are generally used in conjunction with anti-microbial agents. Moreover, they usually require frequent replacements. Nevertheless, the effectiveness of hydrogels in improving wound healing has been widely investigated. For instance, in 1990 Darkovich et al. carried out an RCT on 90 patients affected by pressure ulcers to compare the effectiveness of a commercial hydrogel dressing, i.e., BioFilm® (Akron, OH), with that offered by a commercial hydrocolloid, i.e., Duoderm® (Convatec, Skillman, NJ) [24]. The authors observed that both dressings were able to improve the healing process compared to traditional medications (with slightly higher percentages registered for patients treated with BioFilm®, i.e., 90% *vs.* 78%), but BioFilm® was able to double the number of healed wounds compared to Duoderm® (i.e., 43% *vs.* 24%). Hence, this study clearly highlighted the superior beneficial effects provided by hydrogel dressings compared to hydrocolloids ones. Furthermore, the significantly higher efficacy of hydrogel-based dressings with respect to gauzes was also assessed [25]. Therefore, during the years, research interest on the design of innovative

**Table 1**
List of alginate-based commercial hydrogel wound dressings.

| Hydrogel wound dressings based on alginate | |
|---|---|
| Product | Company |
| Tegagel® | 3M GmnH, USA |
| Algosteril® | Laboratories BROTHIER, France |
| Nu-Gel® | Systagenix, UK |
| Sorbsan® | Aspen Medical, Europe Ltd |
| Curasorb® Alginate | Medtronic Covidien, Ireland |

**Table 2**
List of collagen-based commercial hydrogel wound dressings.

| Hydrogel wound dressings based on collagen | |
|---|---|
| Product | Company |
| Wun'dres® | Coloplast AG Humbleblack, Denmark |
| Biobrane® | Smith & Nephew, London, UK |
| CellerateRX® | Wound Care Innovations LLC, Addison, USA |
| Regenecare® Wound Gel | MPM Medical Inc., USA |
| CollaSorb® | Paul Hartmann AG, Deutschland |
| Stimulen™ | Southwest Technologies Inc., Kansan City, USA |
| Fibracol® | Acelity, Sant'Antonio, USA |
| Medfil® | Human Bio Science Inc., USA |

hydrogels for wound healing applications has significantly increased, as confirmed by the huge amount of literature published on this topic. In this regard, the Pubmed database reports an exponential increase in the number of publications concerning the use of hydrogels in wound healing over the last five decades, with around 550 works published in 2020. Specifically, the literature reports the design of both naturally-derived and synthetic polymer-based hydrogels for wound healing applications. However, despite the wide availability of materials, commercially available hydrogel wound dressings are mainly based on alginate (Table 1).

Alginate is a polysaccharide able to form ionically crosslinked hydrogels under mild conditions. Moreover, unlike other hydrogel systems, alginate is able to absorb large amounts of fluids and thus, can be successfully exploited for the treatment of wounds characterized by high level of exudate or for bleeding wounds due to their hemostatic effects [26]. Furthermore, it was *in vitro* demonstrated that alginate is also able to promote tissue regeneration by reducing the concentration of pro-inflammatory cytokines in the wound bed and by enhancing angiogenesis and production of collagen type I [27,28]. However, although a very high number of alginate-based dressings are used annually, there are only few works highlighting their superior effectiveness compared to other commercial solutions [1]. For instance, in an RCT involving 36 patients affected by abdominal wound dehiscence, Cannavo et al. observed no statistically improved healing rate for alginate-treated wounds compared to moistened gauzes- or combined dressings-treated ulcers [29]. Conversely, in another RCT involving patients with leg ulcers, the alginate-based Sorbsan® (Aspen Medical Europe Ltd) turned out to significantly improve the healing rate with respect to gauzes (i.e., 31% *vs.* 4%, respectively) [30]. Hence, one crucial issue in the identification of the most promising product closely satisfying the target requirements lies in data derived from previous RCTs, as they often generated divergent conclusions. Furthermore, studies conducted on pigs revealed that secondary dressings used in conjunction with alginate-based bandages exert a determining role in maintaining a moist environment and thus, in promoting tissue regeneration [31].

Beside alginate, chitosan is another polysaccharide widely investigated in the design of wound dressings showing hemostatic, antibacterial and fungistatic properties and enhanced healing capability [32,33]. However, differently from alginate, for which the scientific interest has been translated into many commercial products, chitosan-based research outputs have generated only few commercially available solutions (e.g., Chitoderm® plus - Trusetal, ChitoClear® - Primex, Axiostat® - Axiobio and Opticell® - Medline). Such limited translation to industrial applications is probably mainly due to regulatory affairs correlated with the animal origin of this polymer (chitosan is derived from chitin, present in the crustacean shell). Furthermore, reproducibility issues could also play an important role. Indeed, the high batch-to-batch variability of chitosan strongly affects polymer average molecular weight [34,35], which in turns, influences its bioactive properties and workability [36,37]. Moreover, manufacturing of chitosan products is somewhat more complex than for alginate, requiring dissolution in acidic solutions. Hence, the easy manufacturing of alginate products represents a key advantage, respect to chitosan, from an industrial perspective.

Among naturally-derived polymers, many works report on the use of collagen as hydrogel constituent material for wound healing applications. Indeed, collagen dressings are characterized by high absorbance capability and suitable mechanical properties for wound healing applications. Moreover, being a protein commonly present in tissue extracellular matrix, collagen is also able to promote wound healing by recruiting fibroblasts, endothelial cells and keratinocytes and enhancing the production of native collagen [38,39]. Therefore, many collagen-based wound dressings are commercially available (Table 2). However, despite the diffusion of a huge number of collagen-based dressings, recent studies evidenced two main obstacles, which could potentially limit their further exploitation: (i) the risks associated to pathogen transmission based on the collagen source, and (ii) collagen enzymatic degradation, which inevitably leads to lower dressing stability and thus, the need for more frequent changes [40–42].

On the other hand, hydrogel dressings based on synthetic polymers are not affected by risks and drawbacks associated with animal origin and show the advantage to be potentially *ad hoc* engineered to exert specific functions. However, synthetic polymers are not able to actively participate to the healing process, limiting, therefore, their effectiveness. Hence, the combination of natural and synthetic materials in bioartificial hydrogel-based dressings has attracted wide interest, resulting in the commercialization of partially synthetic wound care products, such as FlexiGel® (Smith & Nephew, London, UK – mixture of poly (acrylamide) and polysaccharides) and Oakin® (Amerigel, blend of poly(ethylene glycol) and oak extracts).

### 2.5. Hydrocolloids

Hydrocolloids are gelling systems typically composed of an elastic matrix containing hydrophilic polymers such as sodium carboxymethyl cellulose, gelatin, pectin and sodium alginate [43]. Due to their nature, hydrocolloids are able to absorb large amounts of fluids and consequently, change into gelly-like masses [44]. Similar to hydrogel-based dressings, hydrocolloid wound care products (Fig. 2) have also been successfully used, being able not only to absorb fluids, but also to keep a moist environment and protect the wound bed from bacteria and foreign pathogens. Furthermore, unlike hydrogels, the outer layer of hydrocolloids can more efficiently seal the wound bed and, thus, act as an efficient barrier without the need of secondary dressings [1]. Moreover, hydrocolloid dressings are able to speed the healing process up by intensifying the autolysis and thus, the endogenous debridement of necrotic tissues [44]. However, this type of wound dressings is not recommended for infected wounds due to their occlusive properties. Moreover, they can cause trauma to perilesional tissues during their removal due to their strong adhesive properties. During the years, many animal studies and RCTs have been carried out to investigate hydrocolloid superior effectiveness compared to other wound dressings. For instance, Chvapil et al. assessed the enhanced healing capability of Duoderm® (Convatec, Skillman, NJ) hydrocolloid compared to gauzes, hypothesizing that this result derived from a promoted inflammatory reaction [45]. In another study, Varghese et al. compared the exudate pH, measured in wounds

treated with Duoderm® hydrocolloids and Opsite® (Smith & Nephew, London, UK) film dressings to evaluate their effect in bacteria management [46]. Specifically, the authors suggested that the acidic exudate characteristic of wounds treated with Duoderm® was effectively able to inhibit the growth of some specific bacteria, thus leading to faster healing. However, if compared with Clearsite® hydrogel dressing (ConMed, Utica, NY), Duoderm® turned out to be less effective in accelerating wound closure and promoting epithelialization [47].

### 2.6. Hydroconductive dressings

More recently an innovative class of wound dressings has been introduced, namely hydroconductive dressings (Fig. 2). These dressings show a specific multilayer structure able to absorb exudate, remove debris from the wound bed and then move these by-products away in their core [1]. Drawtex® (SteadMed Medical, USA) was the first commercially available hydroconductive dressing characterized by high absorbance capability (up to 30-50 fold its own weight), not-traumatic removal and capability to preserve its structure up to 7 days. Due to the potentially higher effectiveness of Drawtex® dressing, much attention has been paid in the investigation of its properties. For instance, Ortiz et al. first *in vitro* evaluated Drawtex® capability to reduce bacteria colonies in the medium put in contact with it [48]. Specifically, the authors observed the simultaneous bacteria decrease in the medium and increase in the dressing itself, thus proving its capability to entrap pathogens within its structure. A similar approach was also exploited by Ochs et al. to evaluate both bacteria and matrix metalloproteinase reduction in a chronic wound model [49]. These *in vitro* results were further confirmed by RCTs involving 10 patients affected by non-healing venous leg ulcers [50]. Indeed, 9 of 10 patients underwent faster healing processes within the 4 weeks of observation with respect to standard required times. However, this study revealed that Drawtex® capability to improve tissue regeneration cannot be ascribed to bacteria destruction rather than to rapid exudate removal.

As a matter of fact, the described wound dressings show peculiar features which make them more suitable to treat specific chronic wound types, but none of them can be universally considered an ideal wound dressing. Table 3 summarizes the main *pros* and *cons* of commercially available wound care products belonging to the traditional wound-dressing category.

## 3. Medicated wound dressings

With the final aim of improving wound dressing effectiveness, a step forward was achieved by combining the previously described products with functional components, which can actively take part to the wound healing process. Specifically, these potentially more effective wound care products are generically referred to as medicated wound dressings. Based on the nature of the loaded functional compounds, they are classified as bioactive wound dressings and drug-loaded wound dressings releasing anti-microbial, anti-inflammatory and analgesic therapeutic agents or biological factors.

### 3.1. Bioactive wound dressings

Bioactive dressings (Fig. 3) are wound dressings constituted by precursors showing endogenous activity, which can be exploited to actively enhance tissue regeneration [51]. Generally, they are based on naturally-derived polymers, such as alginate, chitosan, chitin, collagen, which have been reported in the literature to play a key role in tissue regeneration. Beside these renowned polymers, synthetic materials can also be properly engineered to exert specific functions. For instance, Laurano et al. recently explored the potentialities of polyurethane (PU) chemistry to synthesize polymers able to exert anti-microbial activities without the need of additional anti-microbial agents [52]. Specifically, PU activity against both Gram-negative and Gram-positive bacteria and fungi resulted from the successful synthesis of polymers with a proper hydrophilic/hydrophobic balance and from the exposure of positively-charged functional groups (i.e., secondary amino groups). Another widely exploited strategy to further improve wound dressing bioactivity lies in the addition of selected metal ions as dressing constituents during their preparation. For instance, zinc ions could be introduced as ionic crosslinker in alginate-based systems, resulting in improved anti-bacterial properties against *Escherichia Coli* as reported by Straccia et al. [53]. Alternatively, Poor et al. were able to potentiate the anti-microbial activity of alginate-based dressings through their non-thermal plasma-treatment [54], while Murakami et al. combined alginate with chitin, chitosan and fucoidan to prepare wound dressings with enhanced healing capability [55]. More recently, chitosan has also been blended with synthetic polymers to prepare wound dressings with improved healing rate, enhanced epithelialization and reduced inflammation phase. For example, Chen et al. reported the preparation of chitosan/poly(vinyl alcohol) sponges [56], meanwhile Nacer Khodja et al. evaluated the healing activity of poly(vinyl alcohol)/chitosan hydrogels [57]. Among other naturally-derived materials, hyaluronic acid was also exploited as bioactive macromer in the preparation of wound bandages, as it enhances collagen deposition, epithelialization and wound vascularization [58,59]. Due to its promising advantages, many hyaluronic acid-based wound dressings are already available on the market, such as Hyalofill® and Hyalosafe® (Anika Therapeutics, Bedford, MA) and Hyalo Regen (Fidia Pharma USA, NJ).

More recently, an innovative and promising strategy to develop wound dressings with intrinsically anti-microbial properties lies in the exploitation of anti-microbial peptides (AMPs). AMPs are low molecular weight peptides (10–50 amino acids) exposing at least two positive charges [60] able to effectively treat a wide spectrum of pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), extended spectrum beta-lactamase (ESBL), vancomycin-resistant *Enterococcus* (VRE) and multidrug-resistant *Acinetobacter baumannii* (MRAB) [61]. The strong activity of AMPs against bacteria is attributed to their unique mode of action, i.e., the capability to selectively identify microbial cells by means of their charged superficial membrane. Furthermore, respect to antibiotics, AMPs do not induce bacteria resistance, due to their multiple mechanisms of actions. Finally, AMPs may also promote tissue regeneration and stimulate angiogenesis [62]. In this regard, many *in vivo* research studies have been performed to investigate the role exerted in wound closure by different AMPs, i.e., Human Cathelicidin LL-37 [63], Innate Defense Regulator 1018 peptide (IDR-1018) [64], Human $\beta$-defensis (hBD-2 and hBD-3) [65], Pexiganan [66] and Tiger17 peptide [67]. However, AMPs are very susceptible to external conditions (e.g., oxygen, alkaline pH) resulting in their fast de-activation. Moreover, AMPs can also easily lose their functions as a consequence of their self-assembly, the contact with saline fluids [68] and their susceptibility to enzymatic degradation [69]. Thus, such low stability in a physiological environment strongly affects the successful AMP application in clinical practice. For instance, despite promising *in vivo* results, the Pexiganan-based Locilex® cream failed during phase III clinical trials as no differences were observed in the regeneration pathway of diabetic foot ulcers treated with Locilex®, placebo or Pexiganan-free cream [62]. Specifically, such failure was ascribed to AMP loss of function over time. Hence, to protect AMP chemical integrity and functionality, they should be embedded into specific micro- and nano-carriers. An in-depth discussion on the state-of-the-art of AMP delivery vehicles can be found in the recently published comprehensive review by Martin-Serrano and coworkers [70]. Although an increasing number of AMP discoveries has been translated into patents, only few formulations have successfully completed the clinical trial assessment. Nevertheless, many efforts have been addressed towards the design and characterization of AMP-loaded wound dressings, aimed at tackling practical concerns in favor of their great potentialities as natural antibiotics [71].

Beside the use of the aforementioned naturally-derived polymers as intrinsically anti-microbial agents, the literature also reports investiga-

**Table 3**
Main *pros* and *cons* of traditional wound dressings currently on the market.

| Wound dressing | Advantages | Drawbacks | Refs. |
|---|---|---|---|
| Gauzes | Very cheap<br>Highly absorbent<br>Easily adaptable to any defect shape | Can cause trauma and mechanical debridement<br>Could leave residues<br>Susceptible to bacteria contamination | [1,11] |
| Wet-to-dry bandages | Highly absorbent<br>Easily adaptable<br>Controlled necrotic tissue removal | Vasoconstriction<br>Increased affinity of haemoglobin to oxygen<br>Hypoxia<br>Patient discomfort<br>Cross-contamination<br>Not-selective debridement<br>Susceptible to bacteria contamination | [1,11] |
| Transparent film dressings | Moist wound environment<br>Ensure gas exchange<br>Prevent external bacteria contamination<br>Easy to apply<br>Removal with no patient's pain | No fluid absorption<br>Unsuitable for infected wounds | [1,15] |
| Foam dressings | High absorption capability<br>Thermal insulation<br>Gas exchange<br>Long residence time for not-infected wounds | Daily change required for infected wounds<br>May provoke skin maceration | [16,20,21] |
| Hydrogel-based dressings | Discrete absorption capability<br>Gas exchange<br>No pain during removal<br>Enhance tissue granulation<br>Able to lower the wound bed temperature<br>Moist wound environment | Low protection against bacteria<br>Frequent wound dressing replacement<br>Need of a secondary dressing | [22–25,31] |
| Hydrocolloid wound dressings | High absorption capability<br>Moist environment<br>Wound bed protection against bacteria<br>No need of a secondary barrier | Unsuitable for infected wounds<br>Traumatic wound dressing removal<br>Too strong adhesive properties | [1,45–47] |
| Hydroconductive wound dressings | Rapid exudate removal<br>Debris and by-product removal<br>Not traumatic removal | Daily-change required | [1,48,50] |

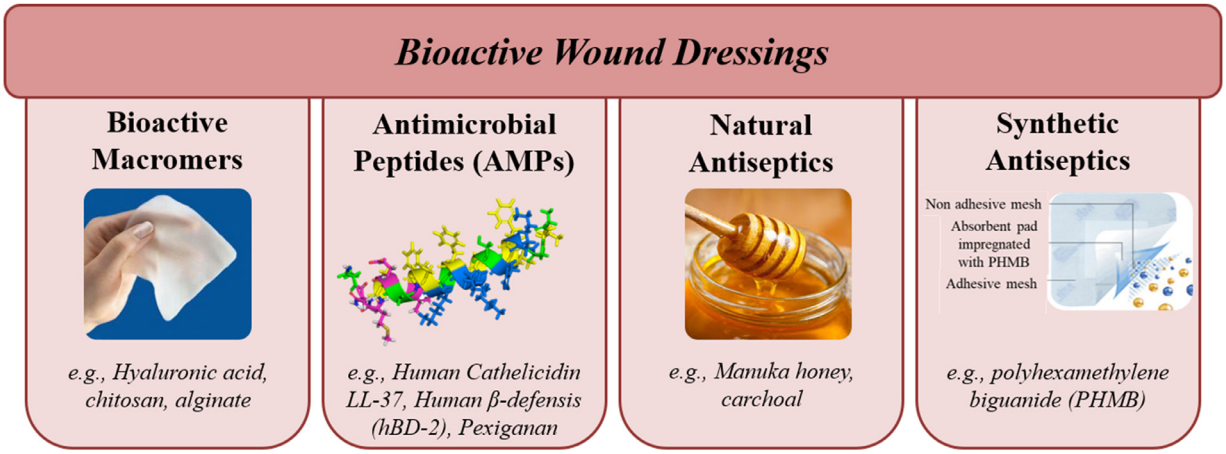


**Fig. 3.** Schematic representation of the main strategies exploited to prepare bioactive wound dressings.

tions on wound dressings containing natural or synthetic antiseptics. For instance, Manuka honey has been widely applied to treat infected wounds and there are many works evidencing its effectiveness in providing anti-bacterial action against a wide spectrum of bacteria and fungi [72], enhancing autolytic debridement and anti-inflammatory effects. Moreover, honey is also able to reduce patient's pain, remove odors and provide the nutrients required for new tissue formation as it contains several amino acids and vitamins (e.g., vitamin C) [73]. An RCT study performed on patients affected by venous ulcers revealed that wounds treated with honey were characterized by increased healing rate and decreased incidence of infections compared to those treated with IntraSite® Gel (Smith & Nephew, UK) [74]. Similar studies were also carried out by Gupta et al., evidencing the higher effectiveness of honey-containing dressings in tissue healing and sterility maintenance compared to silver-loaded bandages [75]. In another RCT involving patients suffering from chronic wounds for more than six weeks, honey-based dressings showed extremely superior performances compared to povidone iodine-based bandages [73]. Specifically, the presence of honey allowed the complete wound healing for 32% patients, while no one treated with povidone iodine showed complete wound closure within the observational period. Moreover, honey was also able to accelerate the healing pathway: after 4 weeks of dressing application, honey-treated patients showed a significantly higher surface area reduction compared to povidone iodine treated ones (i.e., 56% *vs.* 25%).

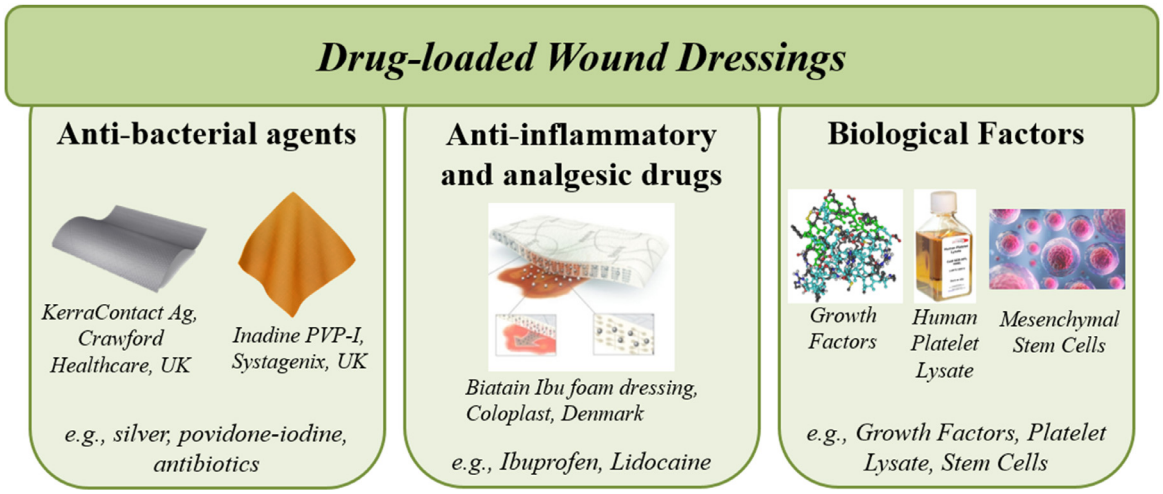


**Fig. 4.** Drug-loaded wound dressings releasing anti-microbial agents, anti-inflammatory and analgesic drugs and biological factors.

**Table 4**
List of commercially available honey-loaded wound dressings.

| Honey-based wound dressings | | |
|---|---|---|
| Product | Company | Wound dressing type |
| SurgihoneyTM | H&R Healthcare | Pure honey |
| MGO$^{TM}$ Manuka Honey | Manuka Health New Zeland Ltd | Pure honey |
| Manuka Fill® | Links Medical | Manuka honey |
| Manuka IG® | Links Medical | Impregnated gauze |
| Activon® | Advancis Medical | Mesh dressing |
| TheraHoney® | Medline | Film dressing |
| Algivon® | Advancis Medical | Alginate dressing |
| Actilite® | Advancis Medical | Foam |
| Medihoney® | Derma Science | Hydrocolloid |

Table 4 reports some commercially available honey-loaded wound dressings.

Among other natural additives, charcoal-based dressings have also been widely used. However, although many studies focused their attention on charcoal activity in wound healing [76–78], supportive research data are still missing. The only assessed charcoal activity lies in its capability to act as deodorizing agent.

On the other hand, poly (hexamethylene biguanide) (PHMB) is a synthetic antiseptic agent, which has been exploited for the preparation of various products, such as contact lenses, cleaning solutions and wet wipes [1]. Specifically, the effectiveness of PHMB as anti-microbial agent was assessed against a wide spectrum of bacteria and fungi at very low concentration (i.e., 0.3%) to simultaneously ensure no-cytotoxic and irritant effects [79]. Moreover, the anti-bacterial activity of PHMB was also evaluated by testing its bacteria removal capability from 38 critically colonized or infected wounds [80]. Results evidenced that PHMB-treated lesions were characterized by a significantly increased reduction of bacterial bio-burden compared to silver-treated wounds. XCell® anti-microbial dressing (0.3% PHMB, Xylos) and Kerlix® AMD sponges (0.2% PHMB, Covidien) are examples of commercially available PHMB-containing products.

### 3.2. Drug-loaded wound dressings

During the years, wound dressings have been engineered to show the additional capability to load and *in situ* release drugs and/or anti-microbial agents, with the aim to more effectively treat chronic lesions, prolonged inflammation phases and delayed wound closure (Fig. 4). Indeed, by exploiting wound dressings as drug carriers it is possible to directly deliver high amounts of drugs in the wound bed, while reducing the administered dosages. Moreover, the possibility to *in situ* release the therapeutic agents could also avoid potential side effects on non-target tissues.

#### 3.2.1. Wound dressings releasing anti-bacterial agents

The most widely and simplest adopted strategy to tackle infected wounds consists of the addition of anti-microbial agents during the preparation of wound dressings. Among them, silver ions have been used for their anti-microbial properties since ancient time, due to their renowned effectiveness against bacteria, viruses and fungi and their capability to reduce inflammation [1]. Silver is characterized by several modes of action, comprising disruptions of bacteria cell walls, inactivation of bacterial enzymes and interference with the synthesis of bacteria DNA [1]. However, silver ions are not able to penetrate through thick wounds [81].

A huge number of different forms of commercially available wound dressings has been engineered to release silver ions at different concentrations (minimum clinically relevant concentration = 5 – 50 ppm). The first commercialized silver-loaded wound dressing, named Actisorb® (Systagenix, UK), was launched in 1980 [51], followed by Hydrogel Ag (1% silver sulfadiazine, Gentell, USA), SilvaSorb® sheets (0.13% silver chloride, Medline, USA), Acticoat® (Smith & Nephew, UK), Program Prisma$^{TM}$ (Systagenix, UK), Allevyn (Smith & Nephew, UK) and many others (Table 5).

Due to the great interest coming from the market of silver-loaded wound dressings, many researchers are still focusing their efforts towards the design of more sophisticated systems aimed at prolonging the release kinetics of encapsulated silver ions. For instance, Neibert et al. described the preparation of alginate fibers embedding silver nanoparticles [82], meanwhile Nguyen et al. investigated the influence of silver nanoparticle concentration loaded into chitosan/poly(vinyl alcohol) hydrogels on anti-bacterial activity [83]. Results evidenced that, although all tested concentrations (i.e., 15, 30 and 60 ppm) effectively reduced bacteria contamination *in vitro*, only the formulation loaded with silver nanoparticles at 30 ppm was able to improve wound healing in *in vivo* tests. Additionally, the study reported by Masood et al. further confirmed the beneficial effects in rat wound healing deriving from the impregnation of chitosan/poly(ethylene glycol) sponges with silver nanoparticles [84]. However, the simultaneous release of high silver ion concentrations could result in cytotoxic effects. Thus, many researchers are actively working on the identification of alternative strategies to more safely deliver silver ions in the wound site. For instance, to increase the amount of administered silver, while avoiding

**Table 5**
List of commercially available silver-loaded wound dressings.

| Silver-containing wound dressings | | |
|---|---|---|
| Product | Company | Wound dressing type |
| Silverseal | DermaScience | Fibrous gauze |
| Tegaderm Ag Mesh | 3M | Fibrous gauze |
| Vliwaktiv Ag | Lohmann and Rauscher | Fibrous gauze |
| Urgotul SSD | Laboratories Urgo | Fibrous gauze |
| Acticoat | Smith & Nephew | Film |
| Restore Contact Layer with Silver | Hollister Wound Care LLC | Film |
| Arglaes film | Medline | Film |
| Silvercel™ | Systagenix | Film |
| Silvercel™ non-adherent dressing | Systagenix | Film |
| Acticoat Moisture Control | Smith & Nephew | Foam |
| Allevyn Ag | Smith & Nephew | Foam |
| Biatain Ag | Coloplast | Foam |
| Mepilex Ag | Molnlycke | Foam |
| Optifoam Ag Adhesive | Medline | Foam |
| Optifoam Ag Non-Adhesive | Medline | Foam |
| PolyMem Silver Island | Ferris Mfg. Corp. | Foam |
| PolyWic Silver | Ferris Mfg. Corp. | Foam |
| Restore non-adherent foam with silver | Hollister Wound Care LLC | Foam |
| Silverlon Negative Pressure | Argentum Medical, LLC | Foam |
| SilverSite | Centurion | Foam |
| UrgoCell Silver/Cellosorb Ag | Urgo Medical | Foam |
| V.A.C. GranuFoam Silver | KCI | Foam |
| Contreet Hydrocolloid | Coloplast | Hydrocolloid |
| Silverseal Hydrocolloid | DermaScience | Hydrocolloid |
| SureSkin | EuroMed | Hydrocolloid |
| Aquacel Ag | ConvaTec | Hydrogel |
| Elta Silvergel | Elta | Hydrogel |
| ExcelGinate Ag | MPM | Hydrogel |
| Gentel Ag Hydrogel Wound dressing | Gentell | Hydrogel |
| Silvasorb Gel | Medline | Hydrogel |
| SilverMed Antimicrobial Silver | MPM | Hydrogel |
| Silverseal | DermaScience | Hydrogel |
| Silver-Sept Antimicrobial Gel | Anacapa Tech Inc | Hydrogel |
| Durafiber Ag | Smith & Nephew | Hydrogel |

cytotoxic effects, and to prolong its release kinetics over time, Torre et al. incorporated silver ions into ordered mesoporous carbons [85]. Such strategy successfully combined the biological effects of silver ions and the high biocompatibility of carbon-based particles. The characterization of silver-decorated mesoporous carbons evidenced high cell viability, strong anti-bacterial activity and promoted re-epithelialization and angiogenesis. A comprehensive review on the state-of-the-art of silver-based delivery systems has been recently published by Haidari et al. [86]. However, although *in vitro* investigations produced promising results, similar achievements were not always observed in *in vivo* studies. Additionally, RCTs revealed that evidences were insufficient to clearly assess the superior effectiveness of silver-containing dressings in reducing infections [87–89]. Conversely, the use of silver-loaded wound dressings improved the ulcer healing rate compared to traditional dressings. For instance, through an RCT conducted on 31 patients affected by diabetic foot ulcers, Tsang et al. demonstrated the superior capability of nanocrystalline silver dressings in reducing wound size compared to Manuka honey and conventional bandages [90]. However, in accordance with the above-mentioned conclusions on the efficacy of silver as anti-bacterial agent, no significant differences were registered in terms of bio-burden reduction among the three tested groups.

Beside silver, iodine has been also widely employed as antiseptic agent since the first demonstrations of its anti-microbial properties in 1882 [91]. However, to reduce side effects associated to the use of elemental iodine, it is generally bounded into carrier molecules. The two most exploited iodophors are povidone-iodine and cadexomer iodine. Although animal studies provided controversial data on the potential toxicity of povidone-iodine, human studies revealed its effectiveness in reducing the bacteria bio-burden [92,93]. For instance, Thorn et al. *in vitro* investigated the capability of iodine-loaded dressings to face preformed biofilms compared to silver-loaded ones [94]. The authors reported that both wound care products exerted an anti-bacterial activity, but those releasing iodine were more effective. These results were also in accordance with Schultz et al. who demonstrated the higher capability of iodine to penetrate biofilms with respect to silver or PHMB [95]. However, both mode of action and biofilm management of povidone-iodine remain unclear. Nevertheless, many iodine-based products, such as Iodosorb gel (0.9% w/w iodine, Smith & Nephew, UK), Iodoflex (Smith & Nephew, UK), IodoFoam (Progressive Wound Care Technologies) are commonly used in clinics.

Antibiotics represent another strategy to strongly face infections and they have become particularly popular with the introduction of *in situ* delivering systems. Indeed, this mode of administration has led to a remarkable reduction in the required dosages compared to intravenous or oral administration routes. During the years, a wide variety of antibiotics has been loaded in many different drug delivery systems. For instance, Sinha et al. encapsulated ciprofloxacin in poly(ethylene glycol)/chitosan scaffolds [96], meanwhile Unnithan et al. loaded the same antibiotic in electrospun poly(urethane)/dextran nanofibers [97]. Moreover, streptomycin was embedded in polyox composite films [98] and polyox/carrageenan and polyox/sodium alginate wafers [99], while vancomycin loading has been reported in chitosan sponges [100]. More recently, Mirani et al. developed a smart wound dressing able to concurrently exert therapeutic and diagnostic functions [101]. Specifically, the authors combined the release of gentamicin sulphate as therapeutic agent and the capability to measure fluid pH in order to detect infections. However, this wide literature has not been successfully translated into commercial products and clinical data because the prolonged use of antibiotics easily leads to bacteria resistance. Thus, irrespective of their mode of administration, the use of antibiotics should be strictly limited.

### 3.2.2. Wound dressings releasing anti-inflammatory drugs

Pain is a common problem affecting all patients suffering from chronic skin wounds. Pain can be caused by physical trauma, but it can also be attributed to dressing changes, debriding, wound cleansing and prolonged inflammatory response. Hence, together with infections, pain management is another important issue to face to improve patients' quality of life. Moreover, pain-related stresses weaken the immune system response, thus leading to delayed wound healing. Ibuprofen, lidocaine and opioids are the most exploited anti-inflammatory and analgesic drugs for the treatment of chronic wounds and, thus, they are widely investigated by researchers. For instance, Djekic et al. reported the preparation and preclinical characterization of crosslinker-free chitosan-based hydrogels for the sustained release of ibuprofen [102]. Aycan et al. designed a conductive polymeric film highlighting the improved effectiveness of ibuprofen-releasing dressings compared to not drug loaded ones [103]. Mohammadpoor et al. studied ibuprofen release kinetics from nanofibers electrospun starting from different poly(vinyl alcohol)/hyper-branched poly(ethyleneimine) volume ratios [104]. Boffito et al. designed an injectable poly(ether urethane)-based hydrogel able to co-currently load and *in situ* release ibuprofen and copper ions [105]. Furthermore, Arapoglu et al. carried out an RCT involving patients affected from different types of chronic wounds to evaluate the analgesic activity exerted by released ibuprofen from foam dressings [106]. The authors reported that patients treated with ibuprofen-loaded dressings showed significantly improved pain-relief compared to those traditionally treated. Thus, they highlighted the remarkably higher effectiveness provided by wound dressings locally releasing ibuprofen compared to systemic administration. Similar results were also obtained by Romanelli et al. [107], who evidenced the superior effectiveness of Biatain Ibu (Coloplast, Denmark) foam dressing compared to traditional local treatments. In a more recent RCT involving 120 patients suffering from exuding and painful venous leg ulcers, Fogh et al. investigated the effects of released ibuprofen on several aspects of wound treatment [108]. Results confirmed the clinically relevant capability of ibuprofen to immediately reduce pain. However, concerning the other wound-related parameters, no statistically significant differences were registered in terms of either healing process, wound area reduction and peri-wound skin conditions or adverse response compared to the control group.

### 3.2.3. Wound dressings embedding biological factors

With the aim of developing wound dressings capable to more actively participate to the wound healing process, more recently the attention has been focused on the investigation of the role exerted by released biological factors. Among them, growth factors (GFs) are biomacromolecules physiologically involved in the wound-healing pathway as they mediate the interactions between cells and the local environment [109]. Specifically, RCTs evidenced that four GFs exert the greatest potential in wound closure, i.e., granulocyte-macrophage colony-stimulating factor (GM-CSF), basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) [110]. Hence, many different delivery systems have been engineered to locally release GFs both in free form or encapsulated within nanoparticles [111]. Furthermore, research papers also reported the combination of GFs with electrospun membranes through their encapsulation within the fibers [112] or their conjunction on nanofiber surface [113]. An alternative approach to preserve GF bioactivity and tune their release kinetics was proposed by Kulkarni et al., who described the preparation of layer-by-layer wound dressings embedding VEGF [114]. However, a thorough investigation revealed that improved results could be achieved through the administration of multiple GFs according to specific time sequences. To this aim, Lai et al. described the preparation of a complex electrospun collagen and hyaluronic acid membrane encapsulating all the aforementioned GFs [115]. Specifically, to tune their sequential release over a period of 1 month, GFs were partially dispersed within the nanofibers and partially loaded into gelatin-based nanoparticles. Results evidenced that the different loading mechanisms led to a temporal control over combined GF release, which in turns significantly improved wound healing in diabetic rats.

An alternative strategy to the administration of multiple GFs could be represented by platelet lysate, i.e., a platelet-derivative, which is involved in the wound healing process. Moreover, the use of allogenic platelet lysate compared to patient's derivatives (e.g., platelet-rich plasma (PRP) and platelet-rich fibrin (PRF)) turned out to minimize individual variability [61]. Platelet lysate has been successfully released from sponge-like dressings [116], mucoadhesive gels [117] and nanoparticles [118]. Studies have also been reported on the co-current release of platelet lysate and vancomycin for the treatment of chronic skin ulcers [119].

However, despite the great interest devoted to the development of wound dressings mimicking the biological environment, a huge gap still exists between research platforms and commercial products. Regranex® Gel (Becaplermin 0.01%, Smith & Nephew, UK) is the only commercial Food and Drug Administration (FDA) approved GF-loaded wound dressing to treat chronic foot ulcers [120,121]. Indeed, although this patch is effectively able to improve wound closure, its use is expensive and requires frequent changes [121]. Moreover, data have highlighted that it could be probably associated to higher cancer risk [121].

In general, the low clinical translation of these platforms could be attributed to different issues [26]: (i) high costs of GFs; (ii) easy loss of GF therapeutic activity during storage and/or application in an aggressive environment; (iii) increased cancer risks; (iv) fast GF degradation in the presence of matrix metalloproteinases; and (v) high costs associated to several changes/day of GF-loaded wound dressings.

To overcome some of these limitations, one promising strategy researchers are working on lies in the combination of GFs with extracellular matrix proteins. Such approach could potentially improve the resultant outcomes, by preserving GFs from degradation, and reduce the costs associated to wound dressing daily changes by lowering the total required dosages and prolonging their release kinetics [122]. Concerning the degradation-induced reduction of released GF effectiveness, an alternative approach could consist of the stimulation of the cells present in the wound bed to directly secrete the required growth factors. To this purpose, two different routes could be identified, i.e., gene therapy or gene medicine and direct release of stem cells in the wound site. The former approach consists of the introduction of exogenous DNA into host cells to stimulate the production of GFs in the wound bed [123]. However, the high costs of production and the low safety associated to their delivery have limited their application to almost exclusively research studies [61]. The latter approach consists of the release of stem cells in the wound site, as they are able to physiologically modulate the healing response in chronic wounds [61]. Among the different available sources of stem cells, bone marrow-derived stem cells (BMSCs) showed their usefulness in promoting tissue regeneration in several clinical studies [124,125]. However, invasive procedures are required to harvest this type of stem cells, thus limiting their exploitation. Adipose-derived stem cells (ADSCs) represent an interesting alternative as they can be extracted through mini-invasive procedures. Irrespective of their origin, both BMSCs and ADSCs showed promising regenerative properties in tests performed using wound models [126,127].

## 4. Advanced wound dressings

Advanced wound dressings refer to a class of smart systems able to release their payload in response to external stimuli, such as temperature, pH, oxygen and moisture composition, with the aim to further increase their therapeutic efficacy while reducing the released dosages (Fig. 5). Based on their mode of operation, stimuli-responsive wound dressings can be classified in: (i) self-responsive wound dressings, (ii) externally-triggered wound dressings, and (iii) automated wound dressings. Specifically, in the case of self-responsive systems, the diffusive payload release is enhanced by structural changes in the wound dressing

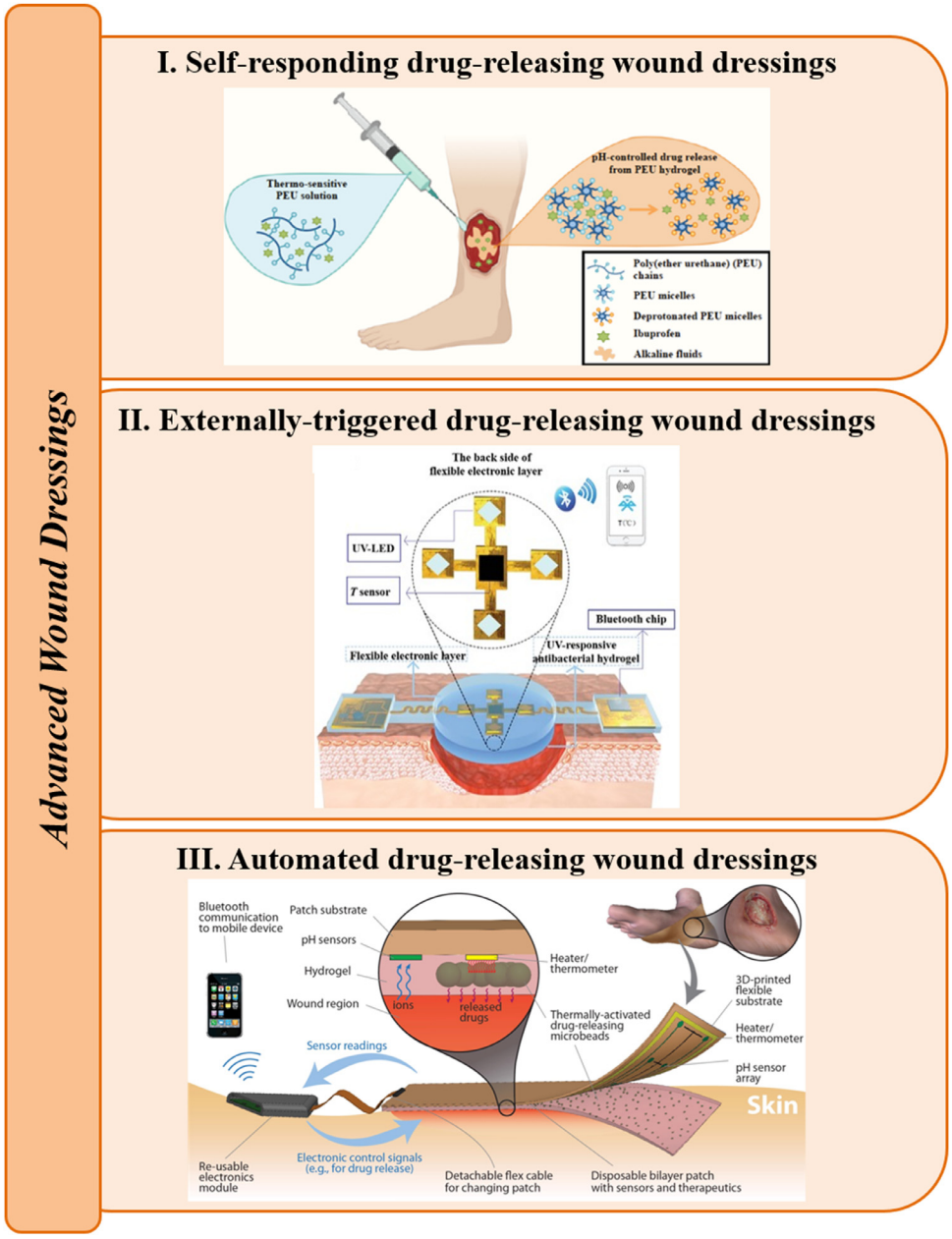


**Fig. 5.** Examples of advanced wound care products. (I) Self-responding wound dressing: the release of drug from a multi-responsive poly(ether urethane)-based hydrogel dressing is triggered by the alkalinity of wound exudate. Reproduced from [140]. Copyright 2021, Publisher KeAi Communications Co. Ltd. (II) Externally-triggered wound dressing: the on-demand release of therapeutic agents is activated through UV irradiation of an UV-responsive anti-bacterial hydrogel. Reproduced from [159]. Copyright 2020, Publisher John Wiley and Sons. (III) Automated wound dressing: smart patch composed by pH sensors, thermo-responsive drug-loaded microbeads, heating activators, electronics to monitor measured parameters and on-request triggered drug release. Reproduced with permission from [161]. Copyright 2018, Publisher John Wiley and Sons.

induced by external stimuli; externally-triggered drug-releasing systems refer to wound dressings engineered to monitor specific parameters exploited to manually trigger drug release, while automated wound dressings identify systems able to measure specific parameters, interpret data and release the required amount of encapsulated drugs.

To the best of our knowledge, such advanced research platforms have not reached a clinical phase yet and thus, no clinical trials have been found on the ClinicalTrials.gov database. Conversely, many of these technologies have been already translated into patents (source www.espacenet.com). Some examples are reported in Table 6.

### 4.1. Self-responding drug-releasing wound dressings

Hard-to-close wounds are characterized by specific values of temperature and pH, which are indicative of the wound status. For example, inflammation can cause temperature increase; infections can bring to extremely acid or extremely alkaline wound exudate based on the type of bacteria, while the presence of necrotic tissue leads to locally alkaline pH values. Hence, special efforts have been devoted to the design of smart systems able to respond to these environmental stimuli, triggering the release of encapsulated drugs. Such systems are defined as "self-responding wound dressings" because they are able to autonomously change their structure in response to environmental stimuli, leading to payload release.

Among stimuli-responsive materials, poly(N-isopropylacrylamide) (pNIPAM), triblock copolymers belonging to the Poloxamer® family, gelatin and modified chitosan are the most widely explored polymers for the development of different forms of thermo-responsive systems. For instance, Zhu et al. described the preparation and characterization of layer-by-layer films composed by tannic acid and poly(N-vinylpyrrolidone)-*b*-poly(N-isopropylacrylamide) diblock copolymers able to undergo temperature-driven micellization [137]. Results demonstrated that the therapeutic agent was successfully retained in the micelle hydrophobic core at 37 °C, while temperature decrease up to 20 °C effectively triggered its release. A similar approach was also exploited by Wang et al. for the preparation of amphiphilic nanoparticles based

**Table 6**
Examples of patents on advanced wound dressings.

| Patented advanced wound dressing platforms | | |
|---|---|---|
| Patent ID [Refs.] | Description | Classification |
| CN109152860A [128] | Wound dressing comprising an acid-hydrolyzing oligomer and an acid-activable prodrug which is activated in the presence of acid environment | Self-responding wound dressing |
| CN108524999A [129] | Long-term-healing wound dressing composed by different layers for the prolonged release of drugs: a quick releasing layer for the delivery of anti-bacterial agents and an acid-pH-controlled layer for long-term drug delivery | Self-responding wound dressing |
| US2005159695A1 [130] | Wound dressing sensitive to enzymes (e.g., elastase and collagenase) for the controlled release of therapeutic agents in the infected wound area | Self-responding wound dressing |
| US2006286155A1 [131] | Drug-loaded wound dressing comprising oligopeptide sequences which are cleavable by kallikrein-reach wound fluids for the controlled release of therapeutic agents | Self-responding wound dressing |
| GB2393656A [132] | Wound dressing sensitive to proteases associated with wound fluids for the controlled release of therapeutic agents | Self-responding wound dressing |
| WO03026544A1 [133] | Wound dressing able to release the payload in response to the absorption of fluids present in the wound bed | Self-responding wound dressing |
| CN110665120A [134] | Chitosan-based wound dressing equipped with flexible electronics for remote monitoring and drug release | Externally triggered wound dressing |
| WO2018211458A1 [135] | Fiber-based wound dressing equipped with a sensor element configured to undergo changes in response to a parameter associated to wound fluids and a supply of therapeutic agents | Automated wound dressing |
| CN104114136A [136] | Automated wound dressing equipped with two field reservoirs, an electrokinetic pump and a controller for the delivery of the therapy in the wound area | Automated wound dressing |

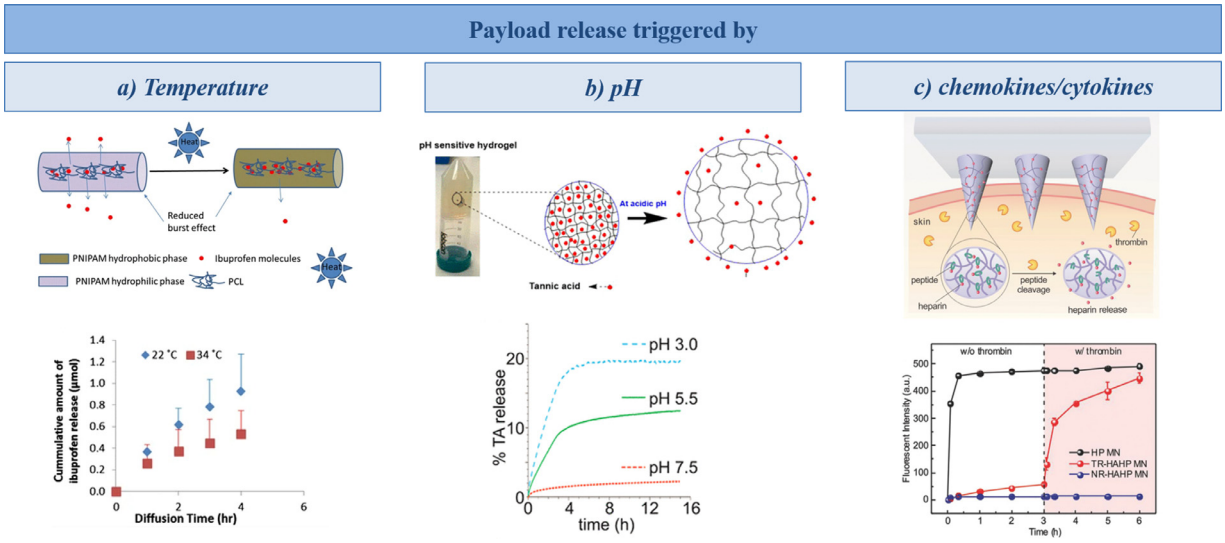


**Fig. 6.** Examples of smart formulations releasing their payload in response to external stimuli. (a) Ibuprofen release from pNIPAM/poly($\varepsilon$-caprolactone) fibers at temperatures below (i.e., 22 °C) and above (i.e., 34 °C) the lower critical solution temperature (LCST) of pNIPAM. Reproduced from [139]. Copyright 2015, Publisher Springer Nature. (b) Increasing tannic acid (TA) release from neutral to acid environments. Reprinted with permission from [141]. Copyright 2016, American Chemical Society. (c) Heparin release from a microneedle-based patch in response to thrombin concentration. Reproduced with permission from [147]. Copyright 2016, John Wiley and Sons.

on N-phthaloylchitosan-g-poly(N-vinylcaprolactam) able to release the loaded analgesic drug upon temperature decrease [138]. Differently, Tran et al. described the design of drug carriers in the form of nanofibrous membranes based on pNIPAM and poly($\varepsilon$-caprolactone) (Fig. 6a) [139]. Results evidenced different ibuprofen release profiles at 22 °C compared to 37 °C.

On the other hand, great interest has been also devoted to the design of systems responsive to pH changes induced by the surrounding medium. These systems are usually based on materials containing pH cleavable sequences or ionizable polymers that are able to change their ionization state based on the external pH. Specifically, polymer protonation or deprotonation leads to the generation of internal repulsive forces, which in turn broaden the system network and enhance drug release. For instance, with this final aim, Laurano et al. recently described the design of a smart injectable drug delivery system for the controlled release of ibuprofen in response to wound alkaline fluid absorption (Fig. 5) [140]. Specifically, the authors functionalized a customized poly(ether urethane) backbone with carboxylic acid groups, being able to generate electrostatic repulsive forces as a consequence of their deprotonation when in contact with a buffer at pH 8. Indeed, hydrogels based on this newly designed polymer showed significantly improved capability to release the encapsulated ibuprofen in an alkaline environment compared to the control (i.e., drug release in an acid milieu). A similar approach to provide hydrogels with pH-responsiveness was reported by Ninan et al. by blending tannic acid as anti-microbial agent with carboxylated agarose [141]. Results highlighted a strong correlation between the amount of released tannic acid and the external pH (Fig. 6b). Moreover, the anti-bacterial activity exerted by tannic acid on *Escherichia Coli* was comparable to that provided by gentamicin.

More recently, the design of polymers responsive to chemokine and cytokine concentrations in the wound exudate has also been explored as it was demonstrated that their production is up-regulated in hard-to-close infected wounds [142–144]. Hence, their concentration could be exploited as trigger stimulus for the controlled release of

therapeutic agents. Specifically, this purpose was achieved by binding drug molecules to protease cleavable peptides, resulting in drug release rate proportional to the amount of targeted chemicals. For example, hyaluronic acid (HA) capped-mesoporous particles were engineered to release drugs upon HA degradation by *in situ* produced hyaluronidase-1 [145]; growth factor-loaded dextran systems were able to expose the encapsulated protein molecules upon degradation by $\alpha$-amylase [146], while thrombin-sensitive microneedles were designed to respond to thrombin up-regulation thus releasing the encapsulated heparin (Fig. 6c) [147].

Therefore, irrespective of the triggering stimulus, all the above-described stimuli-responsive systems showed significantly increased drug-releasing rates in response to specific external conditions. However, such increased releasing capability is not governed by an on/off mechanism triggered by external stimuli: regardless external conditions, drug release takes place through diffusive mechanisms, thus partially limiting the advantages ascribed to stimulus-responsiveness capability.

### 4.2. Externally-triggered drug-releasing wound dressings

Externally-triggered wound dressings are smart drug-loaded systems equipped with sensors able to monitor characteristic wound parameters, such as temperature, pH or oxygen concentration. Specifically, these patches show on/off drug releasing mechanisms, being on-demand triggered by external stimuli, such as heating or light irradiation. The design of such systems has been guided by the need for a precise temporally controlled release of encapsulated drugs. Indeed, conversely to self-responding wound dressings, these smart patches are able to completely overcome drawbacks associated to unnecessary payload release through diffusive mechanisms. Therefore, they are particularly suitable for the controlled release of antibiotics and anti-inflammatory drugs, which should be administered only when strictly required. Within this context, Mauro et al. designed an innovative patch based on electrospun poly($\varepsilon$-caprolactone) fibers covered with graphene oxide for the controlled release of a mixture of therapeutic agents [148]. Specifically, the on-demand release of ibuprofen, ketoprofen and vancomycin was triggered by irradiation with near infrared light. Moreover, *in vitro* results also evidenced the capability of graphene oxide to significantly promote fibroblast adhesion. A similar approach was also recently reported by Ballesteros et al., who described the controlled release of silver nanoparticles from a poly($\varepsilon$-caprolactone) mesh decorated with photosensitive nanogels [149]. Specifically, the release kinetics of the antibacterial agent was tuned by system irradiation with visible light at 405 nm. *In vitro* analyses evidenced a remarkable and strong anti-bacterial activity against *Staphylococcus Aureus* and *Escherichia Coli*.

Another example of externally-triggered wound dressing consists of the combination of thermo-sensitive drug carriers and small flexible heaters, which can be manually activated, thus triggering drug release. For instance, the smart patch designed by Bagherifard et al. was composed by an alginate-based hydrogel embedding drug-loaded pNIPAM particles and flexible heaters (Fig. 7a) [150]. Drug release resulted from pNIPAM nanoparticle disaggregation triggered by the on-demand activation of the heating components. Based on the same materials, Mostafalu et al. engineered a nanofibrous membrane able to release controlled amounts of drugs [151]. Specifically, this aim was achieved exploiting the possibility to independently trigger payload release from each fiber. The effectiveness of this advanced patch to improve vascularization and wound closure was assessed both *in vitro* and in animal models.

Beside the use of thermo-responsive carriers, iontophoretic drug releasing systems could represent a promising alternative. Specifically, these wound dressings are composed by two electrodes able to move charged drug molecules through the tissue thickness upon the application of an external electrical field. Hence, this technology allows a very precise control over the drug releasing site. For instance, Lemos et al. developed a silk-fibroin based wound dressing loaded with neurotensin and stimulated by iontophoresis to tune the inflammatory response and prevent microorganism colonization [152]. The *in vitro* characterization revealed that the anodic iontophoresis resulted in a strong bacteriostatic activity against Gram-positive bacteria without affecting fibroblast viability after 30 min of application. More recently, Kazemi et al. explored iontophoresis to improve the delivery of piroxicam-loaded nanoethosomes in the treatment of chronic wounds [153]. Permeation studies performed in *ex vivo* rat skin proved the successful combination of iontophoresis and ethosome-mediated drug delivery showing a remarkably higher drug permeability compared to its traditional administration without iontophoresis. With a similar final aim, Kanaan et al. developed a semi-interpenetrating chitosan/ionic liquid network as iontophoretic biomaterial for the sustained release of lidocaine [154]. Under an external electrical stimulus the developed system showed significantly higher diffusion coefficients compared to that obtained from passive release, thus proving the remarkable iontophoresis contribution in improving drug release kinetics.

More recently, Near Infrared Ray (NIR) irradiation has also been explored as external stimulus to control payload release. For instance, Sun et al. engineered an MXene-integrated microneedle patch loaded with adenosine to improve angiogenesis and thus, accelerate wound healing (Fig. 7b) [155]. *In vivo* results evidenced that NIR-mediated adenosine release successfully promoted fibrosis, matrix production and angiogenesis, thus accelerating wound closure.

Beside the release of therapeutic agents, these advanced wound dressings have also been exploited to engineer systems for the delivery of oxygen in the wound bed as high level of tissue oxygenation could significantly improve tissue healing [156]. To this aim, Ochoa et al. recently developed a wound dressing composed by a hydrophobic substrate coated with catalyst particles and a hydrophobic poly(dimethylsiloxane) (PDMS) membrane [157]. Specifically, this membrane was engineered to show a network of microchannels on-demand filled with $H_2O_2$ through an external pump. Oxygen delivery resulted from catalyst particle-mediated $H_2O_2$ breaking into water molecules and oxygen, which could successfully pass through the hydrophobic PDMS barrier reaching the wound bed. Alternatively, Zhang et al. developed a microneedle patch loaded with black phosphorus quantum dots (BP-QDs) and hemoglobin for the controlled delivery of oxygen [158]. Specifically, taking advantage of the excellent BP-QDs photothermal effect and hemoglobin reversible binding properties, the authors were able to control oxygen release through a NIR-mediated local temperature increase.

However, despite the great beneficial effects potentially deriving from the application of this technology, this class of wound dressings is relatively new and thus, still under investigation.

### 4.3. Automated drug-releasing wound dressings

Compared to the previously described externally-triggered devices, self-governed wound dressings represent a step forward in the design of smart wound care products. Indeed, automated drug-releasing dressings are equipped with specific measuring systems able to continuously monitor crucial parameters to define the wound state, algorithms to interpret data and actuators to trigger the release of the required drug amount. These smart systems could significantly accelerate the healing process of chronic hard-to-close wounds, thus resulting in potentially improved patients' quality of life and reduced costs for the healthcare systems. Moreover, being these patches able to independently work, minor participation should be required by healthcare providers, thus reducing the costs associated with medical staff. However, although drug delivery systems designed to automatically control the level of blood sugar and accordingly release insulin are already on the market, only few examples in the literature report the design of automated drug-releasing wound dressings. For instance, Pang et al. developed a flex-

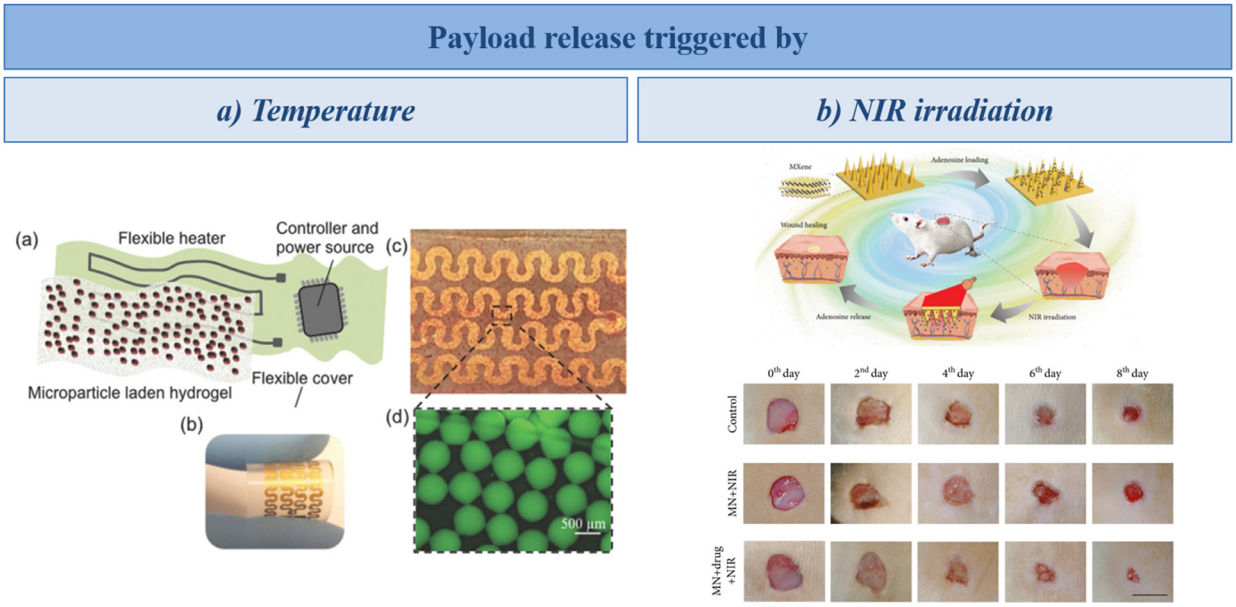


**Fig. 7.** Examples of smart formulations releasing their payload according to an externally controlled mechanism. (a) Dermal patch based on a hydrogel layer loaded with thermo-sensitive pNIPAM drug microcarriers. The patch is connected to a flexible heater with an integrated electronic heater control unit. Payload release is activated by pNIPAM microcarrier disaggregation due to heater-controlled temperature increase. Reproduced with permission from [150]. Copyright 2015, Publisher John Wiley and Sons. (b) Adenosine release from a microneedle patch by means of NIR irradiation and adenosine-enhanced wound contraction. Reproduced from [155]. Copyright 2021, Publisher American Association for the Advancement of Science.

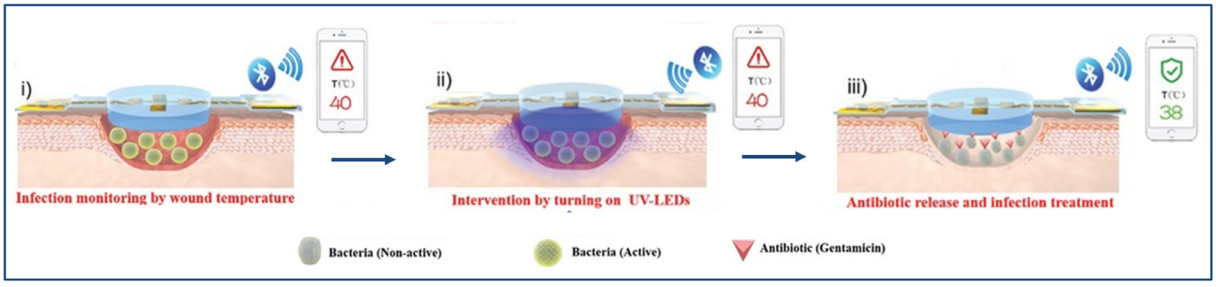


**Fig. 8.** Automated wound dressing able to continuously monitor and on-demand treat chronic wounds: infection monitoring by measuring wound temperature (i), drug release by turning the UV-LED on (ii), and assessment of treatment effectiveness through temperature control (iii). Reproduced from [159]. Copyright 2020, Publisher John Wiley and Sons.

ible electronic-integrated patch for the self-standing treatment of hard-to-close skin wounds (Fig. 5) [159]. The engineered wound dressing was composed of an upper PDMS layer embedding electronics, temperature sensors and light-emitting diodes and a lower photo-responsive hydrogel-based layer loaded with anti-bacterial agents. Temperature sensors were demonstrated to effectively and promptly diagnose infections in animal models, through wound temperature monitoring; infections were then automatically treated via a light-controlled payload delivery mechanism (Fig. 8).

Differently, Mostafalu et al. explored temperature as triggering stimulus to deliver drugs in the wound bed from thermo-responsive microparticles encapsulated in a hydrogel layer [160]. Specifically, payload release was induced by temperature rising, which was governed by heaters activated by a driver and connected to a microcontroller for the on-demand drug delivery. Few years later, an updated version of the previous engineered smart patch has been proposed [161]. Specifically, the authors equipped the automated bandages with a flexible array of pH sensors for the continuous monitoring of infection through the pH measurement of wound fluids. Such information was then recorded and shared with an electronic module which in turns activated the heaters when necessary thus, triggering the release of drugs from thermo-responsive carriers.

## 5. Towards personalized medicine

Despite the advancements in the development of wound dressings able to more effectively treat hard-to-close wounds through the combined and timely controlled release of different therapeutic agents (e.g., anti-inflammatory drugs, anti-microbial agents, growth factors, antibiotics), great attention has been recently devoted to dressing shape personalization. This challenging issue derives from the need to better accomplish one requirement of the moist wound healing theory, namely the one recommending the use of wound dressings able to perfectly fill the ulcer cavity without causing pressure to the surrounding tissues. Indeed, to promote tissue regeneration the ideal wound dressing should be completely in contact with the entire wound bed maintaining a moist environment and acting as a barrier, but, at the same time, it should not damage the peri-wound tissues, potentially leading to delayed healing. Currently, commercial wound dressings are produced in different sizes and shapes to offer healthcare providers the possibility to select the most befitting one. However, wounds are characterized by extremely different morphologies and thus, nowadays available wound dressings can only be adapted to the lesion, thus limiting their effectiveness. Therefore, exploiting the progress achieved in the fields of biomaterials, advanced processing technologies and diagnostic, great efforts have been concen-

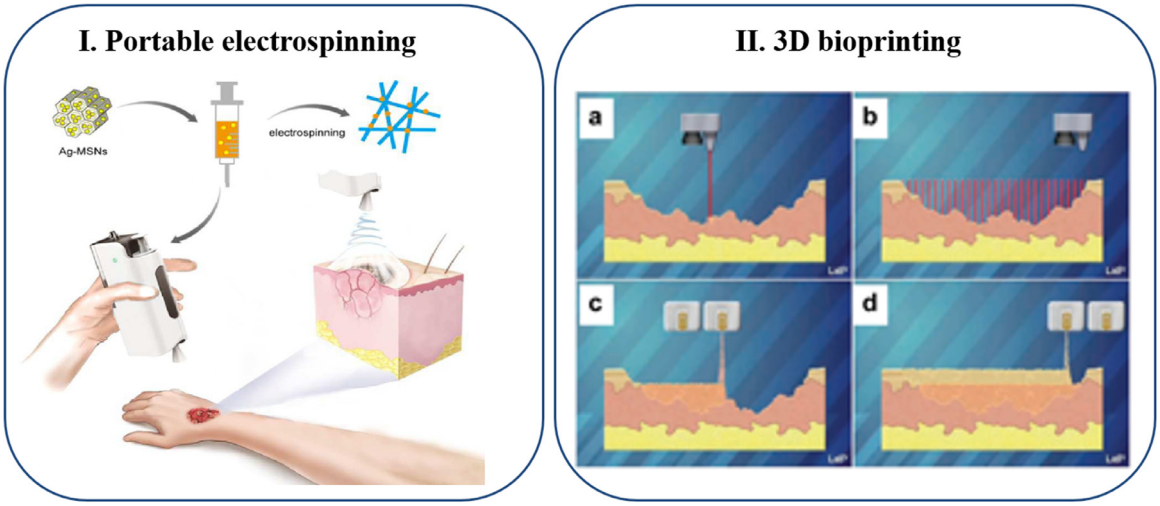


**Fig. 9.** Representation of the main strategies recently exploited to fabricate personalized wound dressings. (I) Portable electrospinning: poly(ε-caprolactone) solution loaded with silver-doped mesoporous silica nanoparticles electrospun in the wound cavity to form a nanofibrous membrane. Reproduced with permission [162]. Copyright year, Publisher. (II) 3D bioprinting: skin wound scanning (*a* and *b*) to reconstruct the CAD cavity model and cell/drug-loaded hydrogel deposition according to acquired information of wound topography (*c* and *d*). Reproduced from [163]. Copyright 2019, Publisher Springer Nature Limited.

trated toward the development of patient-specific wound dressings. Indeed, the launch to the market of shape-personalized wound dressings could potentially open a new era in the field of wound care treatments, offering to healthcare providers a powerful tool to significantly improve patients' quality of life. Consequently, they could also reduce the severe costs associated to the management of hard-to-close wounds.

Two different processing technologies are mainly reported in the literature for the design of personalized wound dressings, namely electrospinning and 3D printing (Fig. 9). Specifically, the former allows the deposition of nanofibrous membranes directly onto the wound site by exploiting portable electrospinning devices; the latter consists of 3D printing wound dressings according to personalized Computer-Aided Design (CAD) models deriving from ulcer cavity reconstruction.

For instance, in 2014 Lau et al. investigated the possibility to electrospin poly(D,L-lactide-*co*-glycolide) (PLGA)-based solutions on wound models to form waterproof nanofibrous membranes [164]. Specifically, to assess the feasibility of this approach, the authors electrospun the nanofibrous mesh directly on *ex vivo* pig skin wounds and a live human hand, revealing that this device was able to quickly cover the wound bed. Moreover, the fabricated membranes perfectly adapted to the wound shape, maintained their waterproofing capability after being held under 30 s of running water and could be easily removed. In the same year, Jiang et al. described the exploitation of the *in situ* electrospinning technique to fabricate thin and homogeneous nanofibrous membranes as hemostatic dressings [165]. The investigation was performed on both *in vitro* and *in vivo* pig liver and lung resections evidencing promising results in terms of precise nanofiber deposition, membrane strength and flexibility, and low dosages, thus paving the way for further applications, such as in wound healing. Few years later, Haik et al. studied the potential improvements deriving from the use of a manual electrospinning to fabricate wound dressings by comparing the wound healing process of pig wounds treated with traditional gauzes or with electrospun membranes [166]. Although no significant healing rate improvements were registered compared to the control, electrospun dressings were more conformable and easier to remove without causing damages to the newly formed tissues. Moreover, their direct deposition to the wound bed could further contribute to cross-contamination avoidance. A more advanced study was reported by Dong et al., who investigated the effectiveness of *in situ* deposited electrospun membranes in terms of healing rate [162]. Specifically, the authors compared the healing capability and the anti-microbial activity provided by three different wound dressings, i.e., gauzes, poly (ε-caprolactone) nanofibrous membranes and poly (ε-caprolactone) nanofibers embedding silver-doped mesoporous silica nanoparticles (Ag-MSNs). Both *in vitro* and *in vivo* studies performed on Wistar rat wounds revealed significantly more rapid wound closure with the application of nanofibrous membranes compared to traditional gauzes. Moreover, the release of silver ions induced a strong anti-bacterial activity against *Staphylococcus Aureus* and *Escherichia Coli*. Furthermore, the authors registered an easy application and painless removal of the nanofibrous membranes together with an improved protection against bacteria contamination.

On the other hand, additive manufacturing and biofabrication have been initially explored to develop wound dressings with controlled and reproducible macro- and micro-architectures, which in turn could enhance gas exchange, cell attachment and migration [167]. For instance, Hafezi et al. reported the fabrication of chitosan-based films through additive manufacturing techniques [167]. Their *in vitro* characterization showed high mucoadhesive properties and enhanced water absorption capability. In another work, Liu et al. investigated the effectiveness of alginate- and gelatin-based 3D matrices in promoting wound healing in *in vivo* murine models [168]. Results evidenced an accelerated healing rate (i.e., 16 ± 1 days *vs.* 14 ± 1 days) and improved healing performances in mice treated with 3D printed matrices compared to the control (i.e., wounds treated with vaseline and covered with gauzes). More recently, the wide versatility provided by rapid prototyping techniques in fabricating constructs with extremely different morphologies has been exploited to develop shape-personalized wound dressings. Indeed, being modeled through a CAD software, these constructs could achieve a high degree of personalization in terms of area, pore size and thickness. Moreover, these patches could also show a personalized drug content, both in terms of drug type and quantity. To this final purpose, Long et al. recently reported the fabrication of 3D printed chitosan/pectin matrices loaded with lidocaine hydrochloride as analgesic drug [169]. Results evidenced that such matrices were characterized by high flexibility, capability to absorb exudate while maintaining a moist wound environment and self-adhesion properties. Specifically, the bioadhesion strength was measured to vary between 86.5 and 126.9 g, making these matrices comparable with commercial products. On the other hand, lidocaine hydrochloride release turned out to occur by erosion (as assessed by analyzing drug release data through the Korsmeyer Peppas model), thus suggesting the reduction of the adhesion strength over time, which could facilitate dressing removal with no tissue damages. However, to the best

of our knowledge, the first proof of concept towards the fabrication of shape-personalized dressings was reported by Mawaffak Hassan et al. in 2017 [170]. Specifically, the authors explored the feasibility of wound dressing customization by exploiting 3D scanning processes as starting point for the preparation of CAD models. Moreover, they developed anti-microbial inks by enriching poly ($\varepsilon$-caprolactone)-based solutions with different metal ions (i.e., silver, zinc and copper). *In vitro* characterization evidenced good anti-microbial properties exerted by silver and copper ions and good ink printability according to complex geometries. Thus, this work thoroughly assessed the feasible combination of 3D scanning and 3D printing processes (i.e., hot melt extrusion) to develop patient specific patches, thus paving the way to further investigations. Lastly, Lau et al. described the design of a mobile skin bioprinting system to treat extensive wounds directly at the patient's bed [164]. Specifically, this integrated system was equipped with a mobile scanner to acquire the required information for the wound cavity reconstruction, which was immediately converted into the code used by the print-heads to deposit cell-loaded hydrogels. *In vivo* characterization performed on murine and porcine full thickness wounds proved the superior healing capability provided by cell-printed wound dressings compared to traditional cell spraying technique. Moreover, the released cells were able to form a new tissue with dermal structure and composition similar to healthy skin.

## 6. Discussion on practical concerns limiting the quick market entry of advanced and personalized solutions

Due to the high pressure that these pathologies exert on the healthcare system worldwide, the research community has devoted great efforts towards the development of smart and more effective wound dressings as proved by the wide literature available on this topic. However, despite promising *in vitro* and *in vivo* results, such advanced systems, to our knowledge, have not been translated into the clinic yet.

Concerning external stimuli-responsive wound dressings, irrespective of their working mechanism (i.e., self-responding, externally triggered or automated systems), this delayed translation could be probably ascribed to several key limitations:

(i) The performed material modifications to impart specific responsiveness lead to new materials requiring validation by the US FDA and/or an European Union Notified Body (according to the Medical Device Regulation MDR 2017/745) and thus, a further step is needed to assess the material safety and quality prior to technology validation.

(ii) The identification of standardized procedures to prepare and characterize advanced wound dressings is extremely complex. Indeed, the characterization techniques commonly exploited to investigate the effectiveness of traditional wound dressings could not be sufficiently adequate to clearly assess the superior capability of such advanced platforms in enhancing wound healing. In detail, characterization tests should be able to simultaneously investigate tissue regeneration rate, effectiveness of drug release in wound treatment, and capability to electronically control on-demand drug releasing mechanisms, while avoiding result dependence on boundary conditions.

(iii) The wound dressing effectiveness is strongly dependent on the external tested conditions and thus, collected data are not always sufficient to clearly prove their superiority compared to traditional medications.

(iv) The cost/benefit ratio is not remarkably advantageous thus reducing the attractiveness of these wound care devices by the healthcare systems and limiting their accessibility to patients.

Concerning personalized wound dressings, great efforts have been devoted to move from one-size-fits-all treatments towards patient-specific approaches under the pressure of governments aiming at developing sustainable healthcare systems. In Europe, this recent trend has also been supported by huge long-term investments in favor of universities and research centers through the FP7 and Horizon 2020 programs [171]. However, although relevant research achievements have been reached, their clinical translation has suffered a setback. Both for advanced wound dressings and for patient-specific patches, the main reason lies in the complex regulatory path leading to their approval. Indeed, although for patient-specific patches the assessment of conformity and CE marking is not required [172], safety-related issues and wound dressing effectiveness must be verified and the protocols to achieve these goals cannot be of general purpose being the devices patient-customized. Advanced and personalized wound dressings are composed of sensors, drivers, biomaterials, therapeutic agents, and show diagnostic and therapeutic functions, providing multicomponent and/or multifunctional systems. Therefore, such remarkable complexity leads to difficulties in device classification and validation processes in terms of functionality, safety and proof of superior effectiveness. The main guideline adopted up to now for system classification lies in the identification of the wound dressing primary mode of action [173]: if they primarily work as drug delivery systems they are classified as a drug and thus, they should require authorization by the US FDA or the European Medicines Agency (EMA). Moreover, much attention should be paid to the type of encapsulated payload (i.e., drugs or biological factors). On the other hand, if the primary mode of action does not involve drug release, such wound dressings are classified as devices and thus, they should accomplish the regulation on medical devices. However, due to the combined mode of action of many wound care products, it is sometimes unclear which function is responsible for wound dressing primary mode action. Moreover, irrespective of their working mechanism, the majority of these products should be validated both as drug and device. Concerning personalized patches (e.g., 3D printed patches replicating the wound cavity topography of a specific patient) an additional issue should be considered, i.e., the identification of the worst case, based on available literature and clinical data, to define the worst operative conditions and assess if the device is safe and effective also in this unfavorable scenario. This is required to avoid the validation of each patient-fabricated construct. As a result, complexity of multicomponent and multifunctional wound dressings is generally the main cause for delayed validation and, thus, delayed clinical translation and market entry. In addition, another important concern is the need to get funds for technological readiness level (TRL) increase, requiring preclinical animal tests and then clinical studies. In this regard, the development of *in vitro* human skin models for preclinical validation of drug and therapies could help in reducing time- and cost-to the market of products for wound treatment/healing. During the last years, researchers' efforts towards this direction have been proved through the engineering of several advanced 3D *in vitro* skin models [174–176], and some of them have already reached the market (e.g., EpiDerm™ (MatTek, Slovak Republic), EpiSkin™ and SkinEthic™ (EPISKIN, France)). Indeed, such interest has been significantly triggered by the EU full ban on animal testing of cosmetics and their ingredients since 11 March 2013 [177]. Based on these models, new preclinical validation methods have been developed and other are in progress, as underlined in the periodic reports by the EU Reference Laboratory for alternatives to animal testing [178].

## 7. Conclusion

The treatment of hard-to-close wounds represents a challenging issue to face due to the huge and growing incidence of this pathology in the society: only in Europe, 4 million patients suffer from chronic wounds every year. Moreover, chronic wound management is associated with severe costs for the healthcare systems and low patients' quality of life. Therefore, based on the "moist wound healing theory" postulated by Prof Winter in 1962 [7], many different wound dressings have been developed during the years aimed at becoming the ideal wound dressing for chronic ulcer treatment.

Traditional wound dressings, such as gauzes, transparent films, foams, hydrogels, hydrocolloids and hydroconductive bandages were first developed to efficiently absorb wound exudate, providing a moist environment and protecting from external pathogens. Moreover, these bandages were designed to be easily applied and removed without causing mechanical debridement. However, all these traditional wound dressings were only able to support the physiological healing pathway, without actively participating to the process.

A step forward was achieved with the introduction of drug-eluting wound dressings. Indeed, in most cases chronic wounds are associated to infections and biofilm formation, both factors that contribute to hinder wound closure. Therefore, bioactive wound dressings were fabricated to exert anti-bacterial pro-regenerative activities through the selection of naturally-derived macromers [54–59] and the addition of anti-microbial peptides [60–71]. Among intrinsically bioactive agents, Manuka honey found widespread application as anti-microbial agent in the preparation of impregnated gauzes, film dressings, foams and hydrocolloids [72–75]. Regardless bioactive wound dressings, wound care products were also engineered to *in situ* release drugs with the aim to more effectively manage delayed lesion healing, while avoiding systemic administration of high drug dosages. Specifically, silver ions were widely exploited as anti-microbial agent able to exert a strong activity against a wide spectrum of bacteria [82–89], while ibuprofen and lidocaine were selected as anti-inflammatory and analgesic drugs to reduce pain [102–107]. Moreover, to further improve tissue regeneration, medicated wound dressings were also loaded with biological factors, such as growth factors and cells [112–115]. Compared to traditional wound dressings, the introduction of drug-eluting wound dressings resulted in significantly improved tissue regeneration capability and patients' quality of life.

However, aiming at further increasing the effectiveness of drug releasing wound dressings, a new era in the field of medicated bandages has started, resulting in the introduction of advanced wound dressings. Indeed, exploiting characteristic wound parameters (e.g., temperature, pH, cytokine concentration) as triggering stimulus, these devices have been designed to release drugs in response to environmental changes. Specifically, based on their operation mode, they have been defined as self-responding [137–141,145–147], externally-triggered [150,157] and automated wound dressings [159–161].

More recently, to further accomplish the moist wound healing theory, researchers have focused their efforts toward the design of shape-personalized wound dressings through electrospinning [162,164,166] and 3D printing [163,167,169] techniques, thus fabricating patches able to perfectly fill the ulcer cavity. Hence, these technologies, together with advancements in the fields of imaging and biomaterials, could potentially lead to the fabrication of highly effective wound dressings and thus, the reduction of costs associated to hard-to-close wound treatments.

As thoroughly analyzed in this critical survey of the state-of-the-art, the available wound dressings in the market are broad in nature, mechanisms of action and targeted applications. However, there is an urgent need of alternative and more efficient wound care products, overcoming the key limitations of currently available wound bandages and progressing toward the implementation of the "ideal" wound dressing to treat chronic wounds. In this quite dynamic scenario, which is currently at low TRL, device developers should pay attention to the regulation pathway their products should follow to accordingly plan their design and characterization activities. This represents a great but useful effort that will ultimately shorten the time required to achieve the market-entry of newly-developed advanced platforms.

## Declaration of Competing Interest

The authors declare no competing financial interest.

## CRediT authorship contribution statement

**Rossella Laurano:** Conceptualization, Writing – original draft. **Monica Boffito:** Conceptualization, Writing – review & editing. **Gianluca Ciardelli:** Supervision, Writing – review & editing. **Valeria Chiono:** Supervision, Writing – review & editing.

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## Supplementary materials

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## References

[1] A. Sood, M.S. Granick, N.L. Tomaselli, Wound dressings and comparative effectiveness data, Adv. Wound Care 3 (2012) 511–529.
[2] T.A. Mustoe, K. O'Shaughnessy, O. Kloeters, Chronic wound pathogenesis and current treatment strategies: a unifying hypothesis, Plast. Reconstr. Surg. 117 (2006) 35–41.
[3] L.G. Ovington, Bacterial toxins and wound healing, Ostomy Wound Manage 49 (2003) 8–12.
[4] R.D. Galiano, T.A. Mustoe, *Wound Care*, Grabb and Smith's Plastic Surgery, Wolters Kluwer, Lippincott Williams & Wilkins, Philadelphia, USA, 2007.
[5] Wound closure products market share 2018. Industry analysis, growth and forecast to 2022, fortunebusinessinsight.com, accessed: March, 2020.
[6] Advanced wound care market size, share & industry analysis, by product (advanced wound dressings, wound care devices, active wound care), by indication (diabetic foot ulcers, pressure ulcers, surgical wounds, and others), by end users (hospitals, clinics, homecare settings, and others), and regional forecast, 2020-2027, fortunebusinessinsight.com, accessed: September, 2020.
[7] G.D. Winter, Formation of the scab and the rate of epithelialisation of superficial wounds in the skin of young domestic pig, Nature 193 (1962) 293–294.
[8] J.C. Lawrence, What materials for dressings? Injury 13 (1982) 500–512.
[9] Z.T. Piskozub, The efficiency of wound dressing materials as a barrier to secondary bacterial contamination, British. J. Plast. Surg. 21 (1968) 387–401.
[10] S. Thomas, Surgical dressings and wound management, Medetec Publications, Cardiff, UK, 2010.
[11] J.C. Lawrence, Dressings and wound infection, American J. Surg. 167 (1994) 21–24.
[12] J.J. Hutchinson, Prevalence of wound infection under occlusive dressings: a collective survey of reported research, Wounds 1 (1989) 123–133.
[13] J.J. Hutchinson, A prospective clinical trial of wound dressings to investigate the rate of infection under occlusion, in: K. Harding (Ed.), Proceedings of the first European Conference on Advances in Wound Management, MacMillan, London, 1993.
[14] L.G. Ovington, Hanging wet to dry dressings out to dry, Home Healthcare Nurse 19 (2001) 477–483.
[15] S. Wei, Y. You, Y. Ma, W. Huang, X. Liang, A. Zhang, Y. Lin, Bi-layer supramolecular polydimethylsiloxane elastomer film: Synthesis, characterization, and application in wound dressing on normal and diabetic rat, React. Funct. Polym. 141 (2019) 21–32.
[16] S. Seaman, Dressing selection in chronic wound management, J. Am. Podiatr. Med. Assoc. 92 (2002) 24–33.
[17] N. Namviriyachote, V. Lipipun, Y. Akkhawattanangkul, P. Charoonrut, G.C. Ritthidej, Development of polyurethane foam dressing containing silver and asiaticoside for healing of dermal wound, Asian J. Pharmac. Sci. 14 (2019) 63–77.
[18] G.W. Oh, S.Y. Nam, S.J. Heo, D.H. Kang, W.K. Jung, Characterisation 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 (2020) 1565–1573.
[19] E. Call, C. Oberg, I. Streit, L.M. Rappl, Comparing fluid handling and microclimate conditions under superabsorbent polymer and superabsorbent foam dressings over an artificial wound, World Council of Enterostomal Therapists J 39 (2019) 11–23.
[20] K.E. Anderson, C.P.M. Franken, P. Gad, A.M. Larsen, J.R. Larsen, P.A.F.A. Van Neer, J. Vuerstaek, J. Wuite, H.A.M. Neumann, A randomised, controlled study to compare the effectiveness of two foam dressings in the management of lower leg ulcers, Ostomy Wound Manage 48 (2002) 34–44.
[21] H.C. Gwak, S.H. Han, J. Lee, S. Park, K.S. Sung, H.J. Kim, D. Chun, K. Lee, J.H. Ahn, K. Kwak, H.J. Chung, Efficacy of a povidone-iodine foam dressing (Betafoam) on diabetic foot ulcer, Int. Wound J. 17 (2020) 91–99.
[22] M. Choucair, T.J. Phillips, *Wound dressing*, Fitzpatrick's Dermatology in General Medicine, McGraw-Hill Book Co., New York, USA, 2000.
[23] L.G. Ovington, The well-dressed wound: an overview of dressing types, Wounds 10 (1998) 1–8.
[24] S.L. Darkovich, M. Brown-Etris, M. Spencer, Biofilm hydrogel dressing: a clinical evaluation in the treatment of pressure sores, Ostomy Wound Manage 29 (1990) 47–60.

[25] A.Z. Kaya, N. Turani, M. Akyuz, The effectiveness of a hydrogel dressing compared with standard management of pressure ulcers, J. Wound Care 14 (2005) 42–44.
[26] J. Koehler, F.P. Brandl, A.M. Goepferich, Hydrogel wound dressings for bioactive treatment of acute and chronic wounds, European Polym. J. 100 (2017) 1–11.
[27] W.R. Lee, J.H. Park, K.H. Kim, S.J. Kim, D.H. Park, M.H. Chae, S.H. Suh, S.W. Jeong, K.K. Park, The biological effects of topical alginate treatment in an animal model of skin wound healing, Wound Rep. Regen. 17 (2009) 505–510.
[28] C. Wiegand, T. Heinze, U.C. Hipler, Comparative in vitro study on cytotoxicity, antimicrobial activity, and binding capacity for pathophysiological factors in chronic wounds of alginate and silver-containing alginate, Wound Repair Regen 17 (2009) 511–521.
[29] M. Cannavo, G. Fairbrother, D. Owen, J. Ingle, T. Lumley, A comparison of dressings in the management of surgical abdominal wounds, J. Wound Care 7 (1998) 57–62.
[30] S. Thomas, C.A. Tucker, Sorbsan in the management of leg ulcers, Pharm. J. 243 (1989) 706–710.
[31] L.A. Pirone, L.L. Bolton, K.A. Monte, R.J. Shannon, Effect of calcium alginate dressings on partial-thickness wounds in swine, J. Invest. Surg. 5 (1992) 149–153.
[32] K. Azuma, R. Izumi, T. Osaki, S. Ifuku, M. Morimoto, H. Saimoto, S. Minami, Y. Okamoto, Chitin, chitosan, and its derivatives for wound healing: old and new materials, J. Funct. Biomater. 6 (2015) 104–142.
[33] L.I.F. Moura, A.M.A. Dias, E.C. Leal, L. Carvalho, H.C. Sousa, E. Carvalho, Chitosan-based dressings loaded with neurotensin – an efficient strategy to improve early diabetic wound healing, Acta Biomater 10 (2014) 843–857.
[34] V.W.L. Ng, J.M.W. Chan, H. Sardon, R.J. Ono, J.M. Garcia, Y.Y. Yang, J.L. Hedrick, Antimicrobial hydrogels: a new weapon in the arsenal against multi-drug resistant infections, Adv. Drug Deliv. Rev. 78 (2014) 46–62.
[35] S. Fu, A. Thacker, D.M. Sperger, R.L. Boni, S. Velankar, E.J. Munson, L.H. Block, Rheological evaluation of inter-grade and inter-batch variability of sodium alginate, AAPS Pharm. Sci. Tech. 11 (2010) 1662–1674.
[36] T. Phaechamud, K. Yodkhum, J. Charoenteerabon, Y. Tabata, Chitosan-aluminum monostearate composite sponge dressing containing asiaticoside for wound healing and angiogenesis promotion in chronic wound, Mater. Sci. Eng. C. 50 (2015) 210–225.
[37] T. Kean, M. Thanou, Biodegradation, biodistribution and toxicity of chitosan, Adv. Drug Deliv. Rev. 62 (2010) 3–11.
[38] S. Chattopadhyay, R.T. Raines, Review collagen-based biomaterials for wound healing, Biopolymers 101 (2014) 821–833.
[39] L.I. Moura, A.M.Dias Dias, E. Suesca, S. Casadiegos, E.C. Leal, M.R. Fontanilla, L. Carvalho, H.C. Sousa, E. Carvalho, Neurotensin-loaded collagen dressings reduce inflammation and improve wound healing in diabetic mice, Biochim. Biophys. Acta 1842 (2014) 32–43.
[40] C. Helary, A. Abed, G. Mosser, L. Louedec, D. Letourneur, T. Coradin, M.M. Giraud-Guille, A. Meddahi-Pelle, Evaluation of dense collagen matrices as medicated wound dressing for the treatment of cutaneous chronic wounds, Biomater. Sci. 3 (2015) 373–382.
[41] J.J. Willard, J.W. Drexler, A. Das, S. Roy, S. Shilo, O. Shoseyov, H.M. Powell, Plant-derived human collagen scaffolds for skin tissue engineering, Tissue Eng. A. 19 (2013) 1507–1518.
[42] V. Natarajan, N. Krithica, B. Madhan, P.K. Sehgal, Preparation and properties of tannic acid cross-linked collagen scaffold and its application in wound healing, J. Biomed. Mater. Res. B. 101 (2013) 560–567.
[43] Y. Qin In, Woodhead Publishing Series in Textiles, Smart Textiles for Medicine and Healthcare, Woodhead Publishing, 2007, pp. 27–49. ISBN 9781845690274.
[44] G. Schoukens, In Woodhead Publishing Series in Textiles, Advanced Textiles for Wound Care, Woodhead Publishing, 2009, pp. 114–152. ISBN 9781845692711.
[45] M. Chvapil, H. Holubec, T. Chvapil, Inert wound dressings is not desirable, J. Surg. Res. 51 (1991) 245–252.
[46] M.C. Varghese, A.K. Balin, D.M. Carter, D. Caldwell, Local environment of chronic wounds under synthetic dressings, Arch. Dermatol. 122 (1986) 52–57.
[47] C. Gokoo, K. Burhop, A comparative study of wound dressings on full-thickness wounds in micropigs, Decubitus 6 (1993) 42–48.
[48] R. Truhan-Ortiz, L.T. Molfatt, M.C. Robson, M.H. Jordan, J.W. Shupp, In vivo and in vitro evaluation of the properties of Drawtex LevaFiber wound dressing in an infected wound model, in: Symposium of investigators, innovations for wound bed preparation: the role of Drawtex hydroconductive dressings, Tampa, Florida, 2012.
[49] D. Ochs, M.G. Uberti, G.A. Donate, M. Abercrombie, R.J. Mannari, W.G. Payne, Evaluation of mechanism of action of a hydroconductive wound dressing, Drawtex, in chronic wounds, Wounds 24 (2012) 3–7.
[50] R.D. Wolcott, S. Cox, The effects of a hydroconductive dressing on wound biofilm, in: Symposium of investigators, innovations for wound bed preparation: the role of Drawtex hydroconductive dressings, Tampa, Florida, 2012.
[51] G. Schoukens, Bioactive dressings to promote wound healing in: *Adv. Textile for Wound Care*, Woodhead Publishing Series in Texties, 2009, pp. 114–152.
[52] R. Laurano, V. Chiono, C. Ceresa, L. Fracchia, A. Zoso, G. Ciardelli, M. Boffito, Custom-design of intrinsically antimicrobial polyurethane hydrogels as multifunctional injectable delivery systems for mini-invasive wound treatment, Engineered Regeneration 2 (2021) 263–278.
[53] M.C. Straccia, G.G. D'Ayala, I. Romano, P. Laurienzo, Novel zinc alginate hydrogels prepared by internal setting method with intrinsic antibacterial activity, Carbohydr. Polym. 125 (2015) 103–112.
[54] A.E. Poor, U.K. Ercan, A. Yost, A.D. Brooks, S.G. Joshi, Control of multi-drug-resistant pathogens with non-thermal-plasma-treated alginate wound dressing, Surg. Infect. 15 (2014) 233–243.
[55] K. Murakami, H. Aoki, S.I. Nakamura, M. Takikawa, M. Hanzawa, S. Kishimoto, H. Hattori, Y. Tanaka, T. Kiyosawa, Y. Sato, M. Ishihara, Hydrogel blends of chitin/chitosan, fucoidan and alginate as healing-impaired wound dressings, Biomaterials 31 (2010) 83–90.
[56] C. Chen, L. Liu, T. Huang, Q. Wang, Y. Fang, Bubble template fabrication of chitosan/poly(vinyl alcohol) sponges for wound dressing applications, Int. J. Biol. Macromol. 62 (2013) 188–193.
[57] A. Nacer Khodja, M. Mahlous, D. Tahtat, S. Benamer, S. Larbi Youcef, H. Chader, L. Mouhoub, M. Sedgelmaci, N. Ammi, M.B. Mansouri, S. Mameri, Evaluation of healing activity of PVA/chitosan hydrogels on deep second degree burn: pharmacological and toxicological tests, Burns 39 (2013) 98–104.
[58] M.G. Neuman, R.M. Nanau, L. Oruna-Sanchez, G. Coto, Hyaluronic acid and wound healing, J. Pharm. Sci. 18 (2015) 53–60.
[59] T.V. Anilkumar, J. Muhamed, A. Jose, A. Jyothi, P.V. Mohanan, L.K. Krishnan, Advantages of hyaluronic acid as a component of fibrin sheet for care of acute wound, Biologicals 39 (2010) 81–88.
[60] K.V. Reddy, R.D. Yedery, C. Aranha, Mini-review: antimicrobial peptides and enzymes as promising candidates to functionalise biomaterial surfaces, Biofouling 30 (2004) 483–499.
[61] J. Boateng, O. Catanzano, Advanced therapeutic dressings for effective wound healing, J. Pharm. Sci. 104 (2015) 3653–3680.
[62] V. Patrulea, G. Borchard, O. Jordan, Characterization of a new homologous anti-lipopolysaccharide factor SpALF7 in mud crab Scylla paramamosain, Pharmaceutics 12 (2020) 840–879.
[63] M. Carretero, M.J. Escámez, M. Carcía, B. Duarte, A. Holguín, L. Retamosa, J.L. Jorcano, M. Del Río, F. Larcher, In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37, J. Invest. Dermatol. 128 (2008) 223–236.
[64] L. Steinstraesser, T. Hirsch, M. Schulte, M. Kueckelhaus, F. Jacobsen, E.A. Mersch, I. Stricker, N. Afacan, H. Jenssen, R.E.W. Hancock, J. Kindrachuk, Innate defense regulator peptide 1018 in wound healing and wound infection, Plos ONE 7 (2012) 1–7.
[65] T. Hirsch, M. Spielmann, B. Zuhaili, M. Fossum, M. Metzig, T. Koehler, H.U. Steinau, F. Yao, A.B. Onderdonk, L. Steinstraesser, E. Eriksson, Human beta-defensin-3 promotes wound healing in infected diabetic wounds, J. Gene Med. 11 (2009) 220–228.
[66] D. Gopinath, M. Senthil Kumar, D. Selvaraj, R. Jayakumar, Pexiganan-incorporated collagen matrices for infected wound-healing processes in rat, J. Biomed. Mater. Res. Part A 1 (2005) 1–12.
[67] J. Tang, H. Liu, C. Gao, L. Mu, S. Yang, M. Rong, Z. Zhang, J. Liu, Q. Ding, R. Lai, A small peptide with potential ability to promote wound healing, Plos ONE 9 (2014) 1–10.
[68] T. Nishikawa, H. Nakagami, A. Maeda, R. Morishita, N. Miyazaki, T. Ogawa, Y. Tabata, Y. Kikuchi, H. Hayashi, Y. Tatsu, N. Yumoto, K. Tamal, K. Tomono, Y. Kaneda, Development of a novel antimicrobial peptide, AG-30, with angiogenic properties, J. Cell. Mol. Med. 13 (2009) 535–546.
[69] C.G. Starr, W.C. Wimley, Antimicrobial peptides are degraded by the cytosolic proteases of human erythrocytes, Biochim. Biophys. Acta 1859 (2017) 2319–2326.
[70] A. Martin-Serrano, R. Gómez, P. Ortega, F. Javier de la Mata, Nanosystems as vehicles for the delivery of antimicrobial peptides (AMPs), Pharmaceutics 11 (2019) 1–24.
[71] F. Costa, C. Teixeira, P. Gomes, M. C. L. Martins, in *Advances in experimental medicine and biology*, (Ed. K. Matsuzaki) Springer Nature Singapore Pte Ltd. 2019.
[72] S.E. Blair, N.N. Cokcetin, E.J. Harry, D.A. Carter, The unusual antibacterial activity of medical-grade leptospermum honey: antibacterial spectrum, resistance and transcriptome analysis, Eur. J. Clin. Microbiol. Infect Dis. 28 (2009) 1199–1208.
[73] S. Gulati, A. Qureshi, A. Srivastava, K. Kataria, P. Kumar, A. Balakrishna Ji, A prospective randomized study to compare the effectiveness of honey dressing vs. Povidone iodine dressing in chronic wound healing, Indian J. Surg. 76 (2014) 193–198.
[74] G. Gethin, S. Cowman, Manuka honey vs. hydrogel – a prospective, open label, multicentre, randomised controlled trial to compare desloughing efficacy and healing outcomes in venous ulcers, J. Clin. Nurse 18 (2009) 466–480.
[75] S.S. Gupta, O. Singh, P.S. Bhagel, S. Moses, S. Shukla, R.K. Mathur, Honey dressings versus silver sulfadiazine dressing for wound healing in burn patients: a retrospective study, J. Cutan Aesthet Surg. 4 (2011) 183–187.
[76] P. Woodhouse, Managing a breast wound, Nursing Times 88 (1992) 74–76.
[77] M. Boardman, K. Mellor, Treating a patient with a heavily exudating malodorous fungating ulcer, J. Wound Care 2 (1993) 74–76.
[78] T. Young, The challenge of managing fungating wounds, Nursepre-scriber/Community nurse 3 (1997) 41–45.
[79] J. Dissemond, V. Gerber, A. Kramer, G. Riepe, R. Strohal, A. Vasel-Biergans, T. Eberlein, A practice-oriented recommendation for the treatment of critically colonised and locally infected wounds using polihexanide, J. Tissue Viabil. 19 (2010) 106–115.
[80] T. Eberlein, G. Haemmerle, M. Signer, U. Gruber Moesenbacher, J. Traber, M. Mittlboeck, M. Abel, R. Strohal, Comparison of PHMB-containing dressing and silver dressings in patients with critically colonised or locally infected wounds, J. Wound Care 21 (2012) 14–16.
[81] N. Tomaselli, The role of topical silver preparations in wound healing, J. Wound Ostomy Cont. Nurse 33 (2006) 367–378.
[82] K. Neibert, V. Gopishetty, A. Grigoryev, I. Tokarev, N. Al-Hajaj, J. Vorstenbosch, A. Philip, S. Minko, D. Maysinger, Wound-healing with mechanically robust and biodegradable hydrogel fibers loaded with silver nanoparticles, Adv. Healthcare Mater. 1 (2012) 621–630.
[83] T.D. Nguyen, T.T. Nguyen, K.Loan Ly, A.H. Tran, T.T.N. Nguyen, M.Thuy Vo, H.Minh Ho, N.T.N. Dang, V.Toi Vo, D.H. Nguyen, T.T. Hoai Nguyen, T.Hiep Nguyen, In vivo study of the antibacterial chitosan/polyvinyl alcohol loaded

with silver nanoparticle hydrogel for wound healing applications, Int. J. Polym. Sci. 1 (2019) 1–10.
[84] N. Masood, R. Ahmed, M. Tariq, Z. Ahmed, M.S. Masoud, I. Ali, R. Asghar, A. Andleeb, A. Hasan, Int. J. Pharmac. 559 (2019) 23–36.
[85] E. Torre, D. Giasafaki, T. Steriotis, C. Cassinelli, M. Morra, S. Fiorilli, C. Vitale-Brovarone, G. Charalambopoupou, G. Iviglia, Silver decorated mesoporous carbons for the treatment of acute and chronic wounds, in a tissue regeneration context, Int. J. Nanomedicine 14 (2019) 10147–10164.
[86] H. Haidari, S. Garg, K. Vasilev, Z. Kopecki, A.J. Cowin, Silver-based wound dressings: current issues and future developments for treating bacterial infections, Wound practice & research 28 (2020) 173–180.
[87] S. Meaume, D. Vallet, M.N. Morere, L. Téot, Evaluation of a silver-releasing hydroalginate dressing in chronic wounds with signs of local infection, J. Wound Care 14 (2005) 411–419.
[88] K.C. Munter, H. Beele, L. Russell, A. Crespi, E. Grochenig, P. Basse, N. Alikadic, F. Fraulin, C. Dahl, A.P. Jemma, Effect of a sustained silver-releasing dressing on ulcers with delayed healing: the CONTOP study, J. Wound Care 15 (2006) 199–206.
[89] L.G. Ovington, The truth about silver, Ostomy Wound Manage 50 (2004) 1–10.
[90] K.K. Tsang, E.W.Y. Kwong, T. Shin-Shun To, J. Wai-Yee Chung, T. Kwok-Shing Wong, A pilot randomized, controlled study of nanocrystalline silver, Manuka honey, and conventional dressing in healing diabetic foot ulcer, Evidence-based Compl. Alternative Med. 2017 (2017) 1–15.
[91] E. Vallin, Traite des desinfectants et de la desinfectations, Masson, Paris, 1882.
[92] R.I. Burks, Povidone-iodine solution in wound treatment, Phys. Ther. 78 (1998) 212–218.
[93] A. Drosou, A. Falabella, R.S. Kirsner, Antiseptics on wounds: an area of controversy, Wounds 15 (2003) 149–166.
[94] R.M. Thorn, A.J. Austin, J. Greenman, J.P. Wilkins, P.J. Davis, In vitro comparison of antimicrobial activity of iodine and silver dressings against biofilms, J. Wound Care 18 (2009) 343–346.
[95] G. Schultz, P.L. Phillips, Q.P. Yang, E.M. Sampson, Microbiocidal effects of wound dressings on mature bacterial biofilm on porcine skin explants presented at European Wound Management Association (EWMA), Finland (2009).
[96] M. Sinha, M.R. Banik, C. Haldar, P. Maiti, Development of ciprofloxacin hydrochloride loaded poly(ethylene glycol)/chitosan scaffold as wound dressing, J. Porous Mater. 20 (2013) 799–807.
[97] A.R. Unnithan, N.A. Barakat, P.B. Pichiah, G. Gnanasekaran, R. Nirmala, Y.S. Cha, C.H. Jung, M. El-Newehy, H.Y. Kim, Wound dressing materials with antibacterial activity from electrospun polyurethane-dextran nanofibre mats containing ciprofloxacin HCl, Carbohydr. Polym. 90 (2012) 1786–1793.
[98] H.V. Pawar, J. Tetteh, J.S. Boateng, Preparation, optimisation and characterisation of novel wound healing film dressings loaded with streptomycin and diclofenac, Coll. Surf B Biointerf. 102 (2013) 102–110.
[99] H.V. Pawar, J.S. Boateng, I. Ayensu, J. Tetteh, Multifunctional medicated lyophilised wafer dressing for effective chronic wound healing, J. Pharm. Sci. 103 (2014) 1720–1733.
[100] D.J. Stinner, S.P. Noel, W.O. Haggard, J.T. Watson, J.C. Wenke, Local antibiotic delivery using tailorable chitosan sponges: the future of infection control? J. Orthop. Trauma 24 (2010) 592–597.
[101] B. Mirani, E. Pagan, B. Currie, M.A. Siddiqui, R. Hosseinzadeh, P. Mostafalu, Y.S. Zhang, A. Ghahary, M. Akbari, An advanced multifunctional hydrogel-based dressing for wound monitoring and drug delivery, Adv. Healthcare Mater. 6 (2017) 1700718–1700733.
[102] L. Djekic, M. Martinović, A. Ćirić, J. Fraj, Composite chitosan hydrogels as advanced wound dressings with sustained ibuprofen release and suitable application characteristics, Pharm. Develop. Tech. 25 (2019) 332–339.
[103] D. Aycan, B. Selmi, E. Kelel, T. Yildirim, N. Alemdar, Conductive polymeric film loaded with ibuprofen as a wound dressing material, Europ. Polym. J. 121 (2019) 109308–109320.
[104] S.A. Mohammadpoor, S. Akbari, M. Sadrjahani, P. Nourpanah, Fabrication of electrospun ibuprofen-loaded poly(vinyl alcohol)/hyper-branched poly(ethylenimine) fibers and their release behaviours, J. Biomater. Sci. 31 (2020) 261–275.
[105] M. Boffito, C. Pontremoli, S. Fiorilli, R. Laurano, G. Ciardelli, C. Vitale-Brovarone, Injectable thermosensitive formulation based on polyurethane hydrogel/mesoporous glasses for sustained co-delivery of functional ions and drugs, Pharmaceutics 11 (2019) 501–521.
[106] V. Arapoglou, K. Katsenis, K.N. Syrigos, E.P. Dimakakos, N. Zakopoulou, K. Gjodsbol, C. Glynn, E. Schafer, B. Petersen, D. Tsoutos, Analgesic efficacy of an ibuprofen-releasing foam dressing compared with local best practice for painful exuding wounds, J. Wound Care 20 (2011) 319–325.
[107] M. Romanelli, V. Dini, R. Polignano, P. Bonadeo, G. Maggio, Ibuprofen slow-release foam dressing reduces wound pain in painful exuding wounds: preliminary findings from an international real-life study, J. Dermatolog. Treat. 20 (2009) 19–26.
[108] K. Fogh, M.B. Andersen, M. Bischoff-Mikkelsen, R. Bause, M. Zutt, S. Schilling, J.L. Schmutz, J. Borbujo, J.A. Jimenez, H. Cartier, B. Jørgensen, Clinically relevant pain relief with an ibuprofen-releasing foam dressing: results from a randomized, controlled, double-blind clinical trial in exuding, painful venous leg ulcers, Wound Rep. Reg. 20 (2012) 815–821.
[109] F.M. Chen, M. Zhang, Z.F. Wu, Toward delivery of multiple growth factors in tissue engineering, Biomaterials 31 (2010) 6279–6308.
[110] S. Barrientos, H. Brem, O. Stojadinovic, M. Tomic-Canic, Clinical application of growth factors and cytokines in wound healing, Wound Repair Regen 22 (2014) 569–578.
[111] K. Ulubayram, A. Nur Cakar, P. Korkusuz, C. Ertan, N. Hasirci, EGF containing gelatin-based wound dressings, Biomaterials 22 (2001) 1345–1356.
[112] Y. Yang, T. Xia, W. Zhi, L. Wei, J. Weng, C. Zhang, X. Li, Promotion of skin regeneration in diabetic rats by electrospun core-sheath fibers loaded with basic fibroblast growth factor, Biomaterials 32 (2011) 4243–4254.
[113] J.S. Choi, K.W. Leong, H.S. Yoo, In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilised with human epidermal growth factor (EGF), Biomaterials 29 (2008) 587–596.
[114] A. Kulkarni, W. Diehl-Jones, S. Ghanbar, S. Liu, Layer-by-layer assembly of epidermal growth factors on polyurethane films for wound closure, J. Biomater. Appl. 29 (2014) 278–290.
[115] H.J. Lai, C.H. Kuan, H.C. Wu, J.C. Tsai, T.M. Chen, D.J. Hsieh, T.W. Wang, Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing, Acta Biomater 10 (2014) 4156–4166.
[116] S. Rossi, A. Faccendini, M.C. Bonferoni, F. Ferrari, G. Sandri, C. Del Fante, C. Perotti, C.M. Caramella, Sponge-like" dressings based on biopolymers for the delivery of platelet lysate to skin chronic wounds, Int. J. Pharm. 440 (2013) 207–215.
[117] G. Sandri, M.C. Bonferoni, S. Rossi, F. Gerrari, M. Mori, C. Del Fante, C. Perotti, L. Scudeller, C. Caramella, Platelet lysate formulations based on mucoadhesive polymers for the treatment of corneal lesions, J. Pharm. Pharmacol. 63 (2011) 189–198.
[118] M.C. Barsotti, R. Di Stefano, F. Chiellini, A. Lisella, C. Errico, R. Feriani, S. Buechielli, A. Balbarini, G. Soldani, Controlled delivery of platelet lysate by polymer nanoparticles in ischemic tissue, Europ. Heart J. 34 (2013) 1–10.
[119] M. Mori, S. Rossi, M.C. Boferoni, F. Ferrari, G. Sandri, F. Riva, C. De Fante, C. Perotti, C. Caramella, Calcium alginate particles for the combined delivery of platelet lysate and vancomycin hydrochloride in chronic skin ulcers, Int. J. Pharm. 461 (2014) 505–513.
[120] Smith & Nephew, REGRANEX (Becaplermin) Gel 0.01%, http://www.smith-nephew.com/key-products/advanced-wound-management/regranex-becaplermin-gel/ 2008.
[121] Food and Drug Administration, REGRANEX, http://www.fda.gov/downloads/Drugs/drgSafety/PostmarketDrugSafetyInformationforPatientandProviders/UCM142821.pdf 2008.
[122] Z. Upton, L. Cuttle, A. Noble, M. Kempf, G. Topping, J. Malda, Y. Xie, J. Mill, D.G. Harkin, O. Kravchuk, D.I. Leavesley, R.M. Kimble, Vitronectin: growth factor complexes hold potential as a wound therapy approach, J. Invest. Dermatology 128 (2008) 1535–1544.
[123] L.K. Branski, C.T. Pereira, D.N. Hemdon, M.G. Jeschke, Gene therapy in wound healing: present status and future directions, Gene Ther 14 (2007) 1–10.
[124] M.M. Yolanda, A.V. Maria, F.G. Amaia, P.B. Marcos, P.L. Silvia, D. Escudero, O.H. Jesus, Adult stem cell therapy in chronic wound healing, J. Stem Cell Res. Ther. 4 (2014) 1000162–1000168.
[125] T. Yoshikawa, H. Mitsuno, I. Nonaka, Y. Sen, K. Kawanishi, Y. Inada, Y. Takakura, K. Okuchi, A. Nonomura, Wound therapy by marrow mesenchymal cell transplantation, Plast. Reconstr. Surg. 121 (2008) 860–877.
[126] D. Seol, M.J. Magnetta, P.S. Ramakrishnan, G.L. Kurriger, H. Choe, K. Jang, J.A. Martin, T.H. Lim, Biocompatibility and preclinical feasibility tests of a temperature-sensitive hydrogel for the purpose of surgical wound pain control and cartilage repair, J. Biomed. Mater. B. 1 (2013) 1–8.
[127] Y.X. Dong, W.U. Hassan, R. Kennedy, U. Greiser, A. Pandit, Y. Garcia, W.X. Wang, Performance of an in situ formed bioactive hydrogel dressing from a PEG-based hyperbranched multifunctional copolymer, Acta Biomater 10 (2014) 2076–2085.
[128] A. Arshi, K. Hemmrich, C. Schulze, CN109152860A, 2019.
[129] H. Duan, J. Gao, H. Guo, L. Wang, J. Zhang, CN108524999A, 2018.
[130] B. M. Cullen, D. W. Silcock, J. Warrick, US2005159695A1, 2005.
[131] B. M. Cullen, P. J. Trotter, US2006286155A1, 2006.
[132] D. W. Silcock, P. J. Trotter, GB2393656A, 2002.
[133] B. M. Cullen, A. J. Kirkwood, D. W. Silcock, J. Warrick, WO03026544A1, 2003.
[134] Q. Fang, W. Ren, W. Tan, X. Wang, Z. Wang, W. Zhao, B. Zheng, CN110665120A, 2020.
[135] M. Akbari, A. Ghahary, B. Mirani, M. A. Siddiqui, WO2018211458A1, 2018.
[136] C. S. Bryant, K. R. Hencken, R. B. Lewis, Q. Mai Tuan, K. H. Nip Kenneth, D. S. C. Shieh, J. L. Strohmann, CN104114136A, 2014.
[137] Z. Zhu, N. Gao, H. Wang, S.A. Sukhishvili, Temperature-triggered on-demand drug release enabled by hydrogen-bonded multilayers of block copolymer micelles, J. Controlled Rel. 171 (2013) 73–80.
[138] Y. Wang, J. Wang, H. Han, J. Liu, H. Zhao, M. Shen, Y. Xu, J. Xu, L. Li, X. Guo, Self--assembled micelles of N-phthaloylchitosan-g-poly(N-vinylcaprolactam) for temperature-triggered non-steroidal anti-inflammatory drug delivery, J. Mater. Sci. 51 (2016) 1591–1599.
[139] T. Tran, M. Hernandez, D. Patel, E. Burns, V. Peterman, J. Wu, Controllable and switchable drug delivery of ibuprofen from temperature responsive composite nanofibers, Nano Conv 2 (2015) 15–22.
[140] R. Laurano, M. Boffito, M. Abrami, M. Grassi, A. Zoso, V. Chiono, G. Ciardelli, Dual stimuli-responsive polyurethane-based hydrogels as smart drug delivery carriers for the advanced treatment of chronic skin wounds, Bioactive Mater 6 (2021) 3013–3024.
[141] N. Ninan, A. Forget, V.P. Shastri, N.H. Voelcker, A. Blencowe, Antibacterial and anti-inflammatory pH-responsive tannic acid-carboxylated agarose composite hydrogels for wound healing, ACS Appl. Mater. Interf. 8 (2016) 28511–28521.
[142] B. Law, R. Weissleder, C.H. Tung, Peptide-based biomaterials for protease-enhanced drug delivery, Biomacromolecules 7 (2006) 1261–1265.
[143] C. Wang, W. Sun, G. Wright, A.Z. Wang, Z. Gu, Inflammation-triggered cancer immunotherapy by programmed delivery of CpG and anti-PD1 antibody, Adv. Mater. 28 (2016) 8912–8920.

[144] R.D. Wolcott, D.D. Rhoads, S.E. Dowd, Biofilms and chronic wound inflammation, J. Wound Care 17 (2008) 333–341.
[145] Z. Chen, Z. Li, Y. Lin, M. Yin, X. Qu, Bioresponsive hyaluronic acid-capped mesoporous silica nanoparticles for targeted drug delivery, Chem. Eur. J. 19 (2013) 1778–1783.
[146] J. Hardwicke, E.L. Ferguson, R. Moseley, P. Stephens, D.W. Thomas, R. Duncan, Dextrin-rhEGF conjugates as bioresponsive nanomedicines for wound repair, J. Control. Rel. 130 (2008) 275–283.
[147] Y. Zhang, J. Yu, N.J. Wang, Z. Hanne, C. Cui, C. Qian, H. Wang, H. Xin, J.H. Cole, C.M. Gallippi, Y. Zhu, Z. Gu, Thrombin-responsive transcutaneous patch for auto-anticoagulant regulation, Adv. Mater. 29 (2017) 1604043–1604050.
[148] N. Mauro, S.E. Drago, G. Cavallaro, G. Giammona, Near-infrared, light-triggered, on-demand anti-inflammatories and antibiotics release by graphene oxide/electrospun PCL patch for wound healing, J. Carbon Res. 5 (2019) 1–13.
[149] C.A.S. Ballesteros, D.S. Correa, V. Zucolotto, Polycaprolactone nanofiber mats decorate with photoresponsive nanogels and silver nanoparticles: slow release for antibacterial control, Mater. Sci. Eng. 107 (2020) 110334–110342.
[150] S. Bagherifard, A. Tamayol, P. Mostafalu, M. Akbari, M. Comotto, N. Annabi, M. Ghaderi, S. Sonkusale, M.R. Dokmeci, A. Khademhosseini, Dermal patch with integrated flexible heater for on demand drug delivery, Adv. Healthcare Mater. 5 (2016) 175–184.
[151] P. Mustafalu, G. Kiaee, G. Giatsidis, A. Khalilpour, M. Nabavinia, M.R. Dokmeci, S. Sonkusale, D.P. Orgill, A. Tamayol, A. Khademhosseini, A textile dressing for temporal and dosage controlled drug delivery, Adv. Funct. Mater. 27 (2017) 1702399–1702409.
[152] C.N. Lemos, C. Cubayachi, K. Dias, J.N. Mendonça, N. Peporine Lopes, N.A.J. Cardoso Furtado, R.F.V. Lopez, Iontophoresis-stimulated silk fibroin films as a peptide delivery system for wound healing, European J. Pharmac. Biopharmac. 128 (2018) 147–155.
[153] M. Kazemi, R. Mombeiny, S. Tavakol, P. Keyhanvar, K. Mousavizadeh, A combination therapy of nanoethosomal piroxicam formulation along with iontophoresis as an anti-inflammatory transdermal delivery system for wound healing, Int. Wound J. 16 (2019) 1144–1152.
[154] A.F. Kanaan, A.P. Piedade, H.C. de Sousa, A.M.A. Dias, Semi-interpenetrating chitosan/ionic liquid polymer networks as electro-responsive biomaterials for potential wound dressings and iontophoretic applications, Mater. Sci. Eng. 121 (2021) 111113–111798.
[155] L. Sun, L. Fan, F. Bian, G. Chen, Y. Wang, Y. Zhao, MXene-integrated microneedle patches with innate molecule encapsulation for wound healing, Research 2021 (2021) 1–9.
[156] P.G. Rodriguez, F.N. Felix, D.T. Woodley, E.K. Shim, The role of oxygen in wound healing: a review of the literature, Dermatol. Surg. 34 (2008) 1159–1169.
[157] M. Ochoa, R. Rahimi, T.I. Huang, N. Alemdar, A. Khademhosseini, M.R. Dokmeci, B. Ziaie, A paper-based oxygen generating platform with spatially defined catalytic regions, Sensors Actuators B. Chem. 198 (2014) 472–478.
[158] X. Zhang, G. Chen, Y. Liu, L. Sun, Y. Zhao, Black phosphorus-loaded separable microneedles as responsive oxygen delivery carriers for wound healing, ACS Nano 14 (2020) 5901–5908.
[159] Q. Pang, D. Lou, S. Li, G. Wang, B. Qiao, S. Dong, L. Ma, C. Gao, Z. Wu, Smart flexible electronics-integrated wound dressing for real-time monitoring and on-demand treatment of infected wounds, Adv. Sci. 7 (2020) 1902673–1902683.
[160] P. Mostafalu, S. Amugothu, A. Tamayol, S. Bagherifard, M. Akbari, M.R. Dokmeci, A. Khademhosseini, S. Sonkusale, Biomed. Circuits Syst. Conf. (2015) IEEE, Atlanta, GA, USA.
[161] P. Mostafalu, A. Tamayol, R. Rahimi, M. Ochoa, A. Khalilpour, G. Kiaee, I.K. Yazdi, S. Bagherifard, M.R. Dokmeci, B. Ziaie, S.R. Sonkusale, A. Khademhosseini, Smart bandage for monitoring and treatment of chronic wounds, Small 1 (2018) 1703509–1703518.
[162] R.H. Dong, Y.X. Jia, C.C. Qin, L. Zhan, X. Yan, L. Cui, Y. Zhou, X. Jiang, Y.Z. Long, In-situ deposition of personalised nanofibrous dressing via a handy electrospinning device for skin wound care, Nanoscale 8 (2016) 3482–3488.
[163] M. Albanna, K.W. Binder, S.V. Murphy, J. Kim, S.A. Qasem, W. Zhao, J. Tan, I.B. El-Amin, D.D. Dice, J. Marco, J. Green, T. Xu, A. Skardal, J.H. Holmes, J.D. Jackson, A. Atala, J.J. Yoo, In situ bioprinting of autologous skin cells accelerates wound healing of extensite excisional full-thickness wounds, Sci. Reports 9 (2019) 1856–1871.
[164] W.K. Lau, P. Sofokleous, R. Day, E. Stride, M. Edirisinghe, A portable device for in situ deposition of bioproducts, Bioinsp. Biomim. Nanobiomater. 3 (2014) 94–105.
[165] K. Jiang, Y.Z. Long, Z.J. Chen, S.L. Liu, Y.Y. Huang, X. Jiang, Z.Q. Huang, Airflow-directed in situ electrospinning of a medical glue of cyanoacrylate for rapid haemostasis in liver resection, Nanoscale 6 (2014) 7781–7792.
[166] J. Haik, R. Kornhaber, B. Blal, M. Harats, The feasibility of a handheld electrospinning device for the application of nanofibrous wound dressings, Adv. Wound Care 6 (2017) 166–174.
[167] F. Hafezi, N. Scoutaris, D. Douroumis, J. Boateng, 3D printed chitosan dressing crosslinked with genipin for potential healing of chronic wounds, Int. J. Pharm. 560 (2019) 406–415.
[168] J. Liu, J. Chi, K. Wang, X. Liu, J. Liu, Full-thickness wound healing using 3D bioprinted gelatin-alginate scaffolds in mice: a histopathological study, Int. J. Clin. Exp. Pathol. 9 (2016) 11197–11205.
[169] J. Long, A.E. Etxeberria, A.V. Nand, C.R. Bunt, S. Ray, A. Syfoddin, A 3D printed chitosan-pectin hydrogel wound dressing for lidocaine hydrochloride delivery, Mater. Sci. Eng. C 104 (2019) 109873–109882.
[170] Z.M. Hassan, A. Goyanes, V. Clark, A.W. Basit, S.T. Hilton, S. Gaisford, Patient-specific 3D scanned and 3D printed antimicrobial polycaprolactone wound dressings, Int. J. Pharm. 527 (2017) 161–170.
[171] E. Nimmesgern, I. Norstedt, R. Draghia-Akli, Enabling personalized medicine in Europe by the European Commission's funding activities, Per. Med. 14 (2017) 355–365.
[172] C.I. Gioumouxouzis, C. Karavasili, D.G. Fatouros, Recent advances in pharmaceutical dosage forms and devices using additive manufacturing technologies, Drug Disc. Today 24 (2019) 636–643.
[173] S.C. Rizzi, Z. Upton, K. Bott, T.R. Dargaville, Recent advances in dermal wound healing: biomedical device approach, Expert Rev. Med. Devices 7 (2010) 143–154.
[174] F.F. Schmidt, S. Nowakowski, P.J. Kluger, Improvement of a three-layered in vitro skin model for topical application of irritating substances, Front. Bioeng. Biotechnol. 8 (2020) 388–399.
[175] A. Idrees, V. Chiono, G. Ciardelli, S. Shah, R. Viebahn, X. Zhang, J. Salber, Validation of in vitro assays in three-dimensional human dermal constructs, J. Artificial Organs 41 (2018) 779–788.
[176] A. Idrees, I. Schmitz, A. Zoso, D. Gruhn, S. Pacharra, S. Shah, G. Ciardelli, R. Viebahn, V. Chiono, J. Salber, Fundamental in vitro 3D human skin equivalent tool development for assessing biological safety and biocompatibility-towards alternative for animal experiments, 4open 4 (2021) 1–21.
[177] https://ec.europa.eu/growth/sectors/cosmetics/ban-animal-testing_it, accessed: 2021.
[178] V. Zuang, A. Dura, D. Asturiol Bofill, S. Batista Leite, E. Berggren, S. Bopp, D. Carpi, S. Casati, S. Coecke, R. Corvi, P. Deceuninck, A. Franco, L. Gribaldo, M.E. Halder, M. Holloway, A. Kienzler, I. Langezaal, F. Madia, A. Milcamps, S. Munn, A. Paini, M. Peirgiovanni, F. Pistollato, A. Price, M.D.P. Prieto Peraita, J.F. Viegas Barroso, C. Wittwehr, A. Worth, M. Whelan, Non-animal methods in science and regulation, EUR 30553 EN, Publications Office of the European Union, Luxembourg, 2021.