ADVANCED THERAPIES IN WOUND MANAGEMENT

CELLS AND TISSUE-BASED THERAPIES, PHYSICAL AND BIO-PHYSICAL THERAPIES, SMART AND IT-BASED TECHNOLOGIES

HEALTH ECONOMICS AND REGULATORY ISSUES

JWC journal of wound care

EWMA

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The document is supported by an unrestricted grant from: Aurealis Pharma, MiMedx, Organogenesis, Klox Technologies, Reapplix, Urgo

This article should be referenced as: **Piaggesi** A, **Läuchli** S, **Bassetto** F et al. Advanced therapies in wound management: cell and tissue based therapies, physical and bio-physical therapies smart and IT based technologies J Wound Care, 2018; 27(6), Suppl 6

Published on behalf of EWMA by MA Healthcare Ltd.
Editor: Rachel Webb
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Designer: Lindsey Kitley
Managing Director: Anthony Kerr
Published by: MA Healthcare Ltd, St Jude's Church, Dulwich Road, London, SE24 0PB, UK
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Note: this final draft version is subject to minor changes before publication. The final document will be available shortly.

Abbreviations

- ADSC: Adipose derived stem cell
- ALU: Arterial leg ulcers
- APFP: The autologous leucocyte and platelet-rich fibrin patch
- Advanced therapies: For the purpose of this document, advanced therapies have been defined as therapies based on novel principles and technologies, or in reference to a novel application of consolidated principles and technologies, including either a singular mechanism of action or a strategy with different levels of action, given that some evidence has been produced in a measurable and comparable way by the manufacturers/developers.
- ATMP (advanced therapy medicinal product): A term used by regulators that describes a class of medicines for human use that are based on genes, tissues or cells.
- AM: Adrenomedullin
- ISBF: the International Society for Biofabrication
- DF: Diabetic foot
- DFU: Diabetic foot ulceration
- dHCAM: dehydrated human amnion-chorion membrane
- EMF: Electromagnetic fields
- ESWT: Extracorporeal shock waves therapy
- EWMA: European Wound Management Association
- FDA: Food and Drug Administration
- GTMP: gene therapy medicinal products (GTMP)
- 'Internet of things' (IoT): The network of physical devices, vehicles, home appliances and other items embedded with electronics, software, sensors, actuators and connectivity, which enables these objects to connect and exchange data. Each thing is uniquely identifiable through its embedded computing system but is able to interoperate within the existing Internet infrastructure.
- LST: Local standard treatment
- MVTR: Moisture vapour transmission rate
- NOSF: Nano-oligosaccharide factor
- NT: Nanotechnologies
- PEG: Poly-ethilen glycole
- PBM: Photo bio-modulation
- PEMF: Pulsatile electro-Magnetic Fields
- PTU: Post traumatic ulceration
- PU: Pressure ulcer
- RCT: Randomised controlled trial
- SSD: Silver Sulfadiazine
- sCTMP: somatic cell therapy medicinal products (),
- STSG: Split-thickness skin graft
- TLC: Tissue Lipid-Colloidale
- TEP: Tissue-engineered products
- TMR: Therapeutic Magnetic Resonance
- VLU: Venous leg ulcer

Introduction

Background and aims

With this document, the European Wound Management Association (EWMA) aims to investigate the barriers and possibilities of advanced therapies in next-generation wound management, including technologies based on cellular therapies, tissue engineering and tissue substitutes, which are all associated with the clinical discipline of regenerative medicine. The document also describes new treatments based on physical therapies and the potential of sensors, software and internet technologies. EWMA wishes to be on the forefront of the development of new, sustainable, cost-effective advanced therapies and to examine further how these measures may support the continuous improvement of wound management with regard to patients' quality of life, while also providing a more effective and efficient approach to wound management.

The objectives of this document are to:

- Review and discuss clinical experiences and the scientific evidence where it is available;
- Provide an objective and exhaustive overview of the available therapies and their potential roles in clinical practice, and make recommendations for the implementation of these therapies in the different areas of wound management;
- Analyse and debate cost-effectiveness issues related to the included therapies; and
- Discuss the regulatory framework for advanced therapies in Europe, providing a point of referral for future discussions and negotiations with healthcare providers and payers.

Due to the general lack of scientific documentation for many of these emerging therapies, this document is based on the available literature and experts' opinions. It includes an evaluation of the potentials for future use in clinical practice and a call for research in recommended areas.

Definition of advanced therapies

The group of authors responsible for this document agreed on the following definition for the term 'advanced therapies'. It has been adopted as a basis for selecting relevant technologies for inclusion in this publication.

The therapies related to chronic wound management can be defined as 'advanced' when they are based on novel principles and technologies or when they refer to a novel application of consolidated principles and technologies, including either a singular mechanism of action or a strategy with different levels of action, given that some evidence has been produced in a measurable and comparable way by the manufacturers/developers. For the sake of this document, advanced therapies will be grouped according to their nature into four different categories: materials, cell and tissue engineering, physical and biophysical, and sensors and IT-related measures.

Method

To define relevant literature, the search strategy presented in Table 1 was conducted. A literature search was performed in Pubmed and Embase for each topic included in the document. The search covered the period of 2007–2017. The authors responsible for the included topics were asked to evaluate the search results and select relevant literature based on the agreed upon definition of advanced therapies defined for this document. Additional literature is included by the authors if relevant in order to describe theory and concepts behind each identified technology. This additional literature may fall outside the time period covered in the search.

The literature was evaluated with reference to the GRADE methodology.[1] Tables providing an

Table 1: Search strategy

Search by titles and abstracts
1. Wound management (and related words with OR)
2. NOT Trauma OR emergency OR heart surgery OR neurosurgery
Combined with the following search terms in separate searches:
Cells
3. AND cells (OR stem cells, skin cells, staminal cells, mesenchymal cells, adipose cells, adipose-derived cells, blood cells, stromal cells, platelets, leucocytes, fibroblasts, monocytes, keratinocytes, endotheli-al cells
Materials
3. AND materials OR dressings, biomaterials/bio-materials, matrices, de-cellularized matrices, acellular matrices, dermal substitutes, delivery systems, carriers, scaffolds, hydrogel, foam, hydrocolloids, films, hydrofibers
Tissues
3 AND engineered tissue OR living tissues, skin equivalents, skin substitutes, composite tissues, bilayered tissues, skin analogues, cryo-preserved tissues, bank tissues
Physical therapies
3. AND physical therapy OR light, electric, magnetic, shock waves, negative pressure, irrigation, oxygen, pressure, HBOT OR exercise OR exergame OR balance training
Sensors
3. AND Sensors OR software, internet technology, communication technology, temperature sensors, pressure sensors, PH sensors, oxygen sensors, telemedicine, wearable, internet of things, telehealth, smart insoles, smart socks, smart shoes, smart mat
Health economy
3. AND (ALL of the above sections/search strings with OR in between)
4. AND health economics, costs, cost-effectiveness, cost-utility, cost-benefit, budget impact, economic resources, economic analysis, eco-nomic implications, cost of illness

overview of the evaluation of evidence supporting the technologies are inserted after each document section with descriptions.

Structure of the document

This document is organised into six different sections. Four of them deal with the different types of advanced therapies and are, in order of position in the document, dedicated to: materials, cells and tissues, physical means, and smart technologies. Each of these sections include:

1. A text describing and summarising the current status and possible evolutions within the field;

2. Tables outlining available relevant studies (indicating number of subjects, main findings, etc)

3. A table outlining the available of evidence and the strength of recommendations for using the different therapies with the related indications.

The document also includes two sections dedicated to the economic and regulatory aspects of advanced therapies in wound management.

The aim of these sections is to provide a different perspective on this complex and fast-evolving field that bridges the gap between the technologies and their inception in the real world of wound healing.

The document is concluded with a 'wish list'; a separate and concise section including ten points that highlight crucial aspects that should be addressed with regard to supporting proper evaluation and potential implementation of relevant advanced therapies in wound management. This final section is included as a potential tool for addressing future issues and controversies in this challenging and promising field. This tool targets healthcare professionals as well as administrators, decision makers and regulators. The list is followed by a paragraph in which EWMA examines the potential role of a European clinical and scientific association with regard to supporting the realisation of the promises that advanced therapies make to wound healing.

The authors hope that reading this document will not only be interesting for scientists and clinicians but also helpful for other stakeholders in the field of wound management by supporting better care for patients with wounds.

Materials/dressings

Introduction

Historically, wounds have been managed with plasters soaked in oil, grease, wine and vinegar after cleansing with astringents or antimicrobial substances, such as honey and resin.[2] The discovery of the antibiotics late in the 19th, beginning of the 20th century, marked a revolution in the medical field and the beginning of the development of modern wound dressings.[3] Up to the mid-1900's, it was confidently believed that wounds should be kept uncovered to dry in order to promote faster healing, but this paradigm was contradicted in the 1980's with the clinical acceptance of new dressings that supported a moist wound environment.[4,5]

Although traditional dressings, which are made of woven and non-woven cotton, rayon, polyester fibres, confer some protection against bacterial infection, they are in general directed for cleaning dry wounds or for use as a secondary dressings.[4] This is because their use in exudating wound situations, even those with slight drainage, are associated with maceration of healthy tissues and adhesion to the wound, which can result in painful removal and delayed healing due to additional trauma to the wound bed.[6] Thus, a new method has risen with the introduction of technologically advanced wound dressings, such as films, foams, hydrocolloids (including hydrofibers), hydrogels, alginates and acellular matrices that are designed to be in contact with the wounds, to act as primary dressing in order to promote healing.

Importantly, the expected enhanced outcome due to the use of advanced wound dressings for healing does not occur if the wound has devitalised tissue, which obstructs granulation of the wound bed and epithelialisation. Autolytic/enzymatic debridement abilities relying on the self-activation of endogenous enzymes for slough degradation to allow for the exposure of well-perfused healthy tissue has been associated with these dressings.[7] However, since autolytic debridement is not as efficient as surgical debridement, it cannot replace surgical debridement.[8] The large number of advanced wound dressings have been divided into different categories, potentially associated with their performance and certain shared features. However, within each group, the various dressings are not identical. In fact, current advanced wound dressings can be successively categorised considering properties that range from general features, such as permeability, absorption and fluid-handling capacity, to more specific features associated with each of the classes and with each of the dressings within that class (Table 2). Hydrocolloids are occlusive dressings that maintain contact of the fluid with the wound during the healing process in a unique way. As with all of the other classes of dressings, they provide a moist environment. Nowadays, this moist environment for healing is well-accepted as advantageous for wound healing.[9] In contrast, hydrocolloids, semi-permeable films and foam dressings permit gases and water-vapour exchange but maintain a barrier against bacteria infiltration mainly due to a layer of acrylic adhesive. While the use of a secondary dressing is hereby avoided, depending on the adhesive strength of the dressing, its use might be contraindicated for patients with friable

Table 2: Wound dressing types and features

General features					
Moist wound bed	Permeability	Absorption capacity	Fluid-handling capacity	Average time in the wound	
Yes	Occlusive • Complete barrier features; • Unable to manage wound fluid since they do not release water vapour	Excellent (form a gel when wet)	Light to moderate	3–7 days	
Yes	Semi-permeable • Permit gases and water vapour exchange but prevent bacteria infiltration; • Adhesive acrylic layer might induce periwound lesions	Little to none	Absent to moderate	up to 7 days	
		Excellent	Moderate to heavy	up to 3–4 days	
	Permeable • Require secondary dressing	Little to none	Absent to moderate	1 day	
		Excellent (form a gel when wet)	Moderate to heavy	up to 7 days	
		N/A	N/A	Unchanged or reapplied up to healing	

	Type of Dressings	Specific features	Limitations
	Hydrocolloids (including hydrofibers)	• The hydrophilicity of the polymer influences the absorption capacity; • Formulations with alginate have increased absorption ability; • Polyurethane-based formulations provide thermal insulation; • Confer a highly hypoxic wound environment; • Hydrofibers are the most mechanically stable.	• Residues can be left in the wound upon dressing removal due to mechanical weakness; • Tissue maceration possible if the fluid overcomes the absorption-handling capacity; • Contraindicated in heavily exuding and infected wounds; • Odour can be mistaken for infection.
	Films	• MVTR varies with properties of the polymer, such as the pore size, density and thickness of the membrane; • Composition determines transparency; • Fluid-handling capacity increases when combined with non/low-adherent absorbent pad	• Contraindicated in infected wounds; • Contraindicated in wounds with friable periwound skin; • Can adhere to the wound in the absence of fluids.
	Foams	• Varied compositions (different types of polyurethanes, silicone, polyvinyl alcohol etc) determine: • Surface hydrophilicity: fluid-handling capacity; atraumatic removal; • Design of the foams: time in the wound • Insulation • Physical properties determine soft character-cushioning • If sufficiently hydrophobic, can entrap bacteria.	• Possible undesirable drying effect on inadequately exudative wounds; • Can adhere to the wound in the absence of fluids.
	Hydrogels	• Hydrogel sheets are more stable than amorphous (hydro)gels and are insoluble in water; • Cross-linking degree determines fluid absorption and amount of moist provided to the wound; • Provide temporary cooling effect;	• Limited absorption ability, thus is indicated for situations where drainage is of secondary concern; • Over-hydration can cause periwound maceration.
	Alginates	• The relative composition in mannuronic and guluronic acid units influences absorption capabilities; • High content in mannuronic acid leads to reduced mechanical stability; • Haemostatic due to ion exchanging properties.	• Might leave residual debris if exudate is not sufficient; • Require moisture to ensure atraumatic removal, thus are contraindicated in wounds with little to no exudate.
	Acellular Matrices	• Artificial acellular matrices potentially have an improved mechanical stability in relation to natural ones; • Collagen is often the main component but the additional components affect 3D structure properties and degradation; • Porosity and pore size of the 3D structure influence cell infiltration; • One-way or two-way approach have different vascularisation and re-epithelialisation outcomes.	• Risk of disease transmission; • The presence of any material that can cause inflammatory/allergic responses.

skin in order to avoid skin tension and periwound lesions.[10] The use of a secondary dressing is required for permeable dressings such as hydrogels, alginates and most of the acellular matrices.

Independently of this classification in terms of permeability, the dressing's absorption capacity varies from little to none (films and hydrogels) to excellent (foams, hydrocolloids, and alginates). This absorp-tion capacity can be directly translated into wound exudate-handling capability, respectively ranging from absent to moderate for moderate to heavy exudate.[6] An exception must be highlighted for hydrocolloids, which, although being able to absorb a high amount of fluid, cannot manage it on a higher level since it is unable to release water vapour through the occlusive layer. This duality is often linked with tissue maceration and can be balanced by a cost-effective increase in the number of changes of the dressing.[10] In contrast, if there is too little wound fluid, films and foams can adhere to the wound surface, which can result in a painful and traumatic removal of the dressing.[10] This is prevented by the use of hydrocolloids, hydrogels and alginates that, upon contact with fluids, form a gel, which provides a wet and low-adherence interface with the wound bed.[6]

Acellular matrices (collagen dressings), although permeable and capable of providing a moist environ-ment, can be considered another type of dressing primarily because they are primed for intrinsic wound healing and tissue regeneration. These dressings are prepared from allogeneic or xenogeneic tissue from which viable cells are removed. Thus, the risk of disease transmission and the presence of material that can cause inflammatory/allergic responses is not totally absent. Moreover, awareness regarding the religious, cultural and social context of the patients should be raised due to potential objections to the use of animal products.[11]

These common general features represent a challenge for healthcare professionals in the selection of an appropriate dressing for a particular wound. Thus, the choice has to rely on the specific properties of each dressing and the knowledge that these will influence the healing process in different ways. In the following sections, the rationale and expectations regarding the mechanism of action of each type of dressings and the achieved level of clinical evidence will be discussed in order to provide comprehensive information that will allow for a better understanding of which type of dressing can/should be used for different wounds. Importantly, no considerations will be made regarding non-debrided wounds in order to only focus on the healing process itself, which can only happen after a proper preparation of the wound bed.

Films

The first reported use of a film dressing occurred in 1945, when cellophane was used to treat burns.[12] Film dressings are thin membranes of synthetic polymers, originally nylon-based, that evolved to become stronger and more resistant to stretching and to shrinking as compared with the polyurethane ones, which were mostly backed by an adhesive layer for fixation. The moisture vapour transmission rates (MVTR) among the different polyurethane dressings varies with the properties of the polymer, such as the pore size, density and thickness of the membrane,[13] which allows for a tailoring of their fluid-handling capacity and, therefore, can assist in avoiding excessive wound moisture and tissue maceration.[14] Additionally, adhesive films can include the use of a non/low-adherent absorbent pad that is capable of managing a larger, but still light, amount of exudate, which might be sufficient for exudate management.[14] The material of the film also determines if the dressing is transparent. This is an advantage with regard to monitoring the wound without disturbing the healing process.[12]

One proposed mechanism listed as a beneficial effect of semi-permeable film dressings is the accumulation of healing mediators within the wound fluid. This type of dressing is directly in contact with the wound bed, which leads to faster re-epithelialisation, increased healing rates and restoration of the skin barrier.[15] Scientific evidence has shown that the fluid obtained from acute wounds covered with a film dressing can stimulate *in vitro* keratinocytes proliferation,[16] which has recently been associated with an enhanced synthesis of laminin 5.[17] This is a major component of the anchoring filaments in epithelial cells, by playing a role in their adhesion and migration. Although there were speculations that the enhanced keratinocytes proliferation was caused by augmented inflammatory cytokines and growth factors, such as TGF-a, TGF-b1 and TNF-a, their presence in the wound fluid were not confirmed. Several works have identified some of these molecules and others in wound fluids obtained under different clinical conditions but not from wounds covered with film dressings.[18–20] Accelerated epithelialisation in this setting was also associated with the presence of a gelatinous co-agulum containing fibrin (ogen) and fibronectin onto which keratinocytes could migrate.[21] However, a subsequent work showed that keratinocytes do not interact with fibrinogen because they lack the αVβ3 receptor.[22] Thus, the exact mechanism behind re-epithelialisation is not yet known. Interestingly, acute wound fluids were shown to elevate, in a healing time-dependent manner, the levels of plasminogen activators both in fibroblasts and in keratinocytes cultured *in vitro*.[23] These are mediators of an enzymatic cascade involved in the control of fibrin degradation, matrix turnover and cell invasion, which are indicative of a highly proteolytic wound environment. Although enhanced collagen synthesis, possibly associated with increased proteases activity, has been attributed to healing with a film dressing, this proteolytic activity is known to vary based on the type of wound.[24–26]

Fluids recovered from skin graft wounds covered with film dressings also revealed chemotactic properties towards endothelial cells *in vitro* and angiogenic properties in an *in vivo* assay.[27] This was potentially due to the action of FGF-2 although the detected levels were comparable to normal serum. This analysis reflects the early wound environment (24 hours post-wounding), which suggests a rapid proangiogenic stimulus in acute wounds that are dressed with films. Nonetheless, a parallel analysis of fluids derived from burn injuries showed a much less immediate angiogenic activity, which suggests that the overall environment of burns seems to be generally non-angiogenic.

When these observations are evaluated together, wound fluid components seem to be a key factor of the healing cascade, but the sparse clinical results obtained so far about the environment of the different wounds is a major limitation for a better understanding of their pathophysiology.

Films are mostly used to dress superficial wounds with minimal to moderate exudates, such as surgical wounds and split-thickness skin graft donor sites. The suitability of film dressings to cover light to moderately exuding acute and chronic wounds by providing a moist environment without compromising periwound skin, even at a lower changing frequency, was also demonstrated.[28] Randomised controlled trials compared film dressings with traditional[29,30] and other advanced film dressings[31–33] to manage split-thickness skin graft donor sites. When films were compared with paraffin gauze, one of the trials showed no significant differences in terms of the healing rate, which was up to 14 days,[29] but in the most recent study,[30] the film groups showed significantly shorter healing times of less than 12 days as compared to the gauze group, which

had an average healing time of 14.76 days. Film dressings caused less pain and discomfort than paraffin gauze and were also easier to remove[29,30] (Table 3).

Randomised controlled trials with other advanced dressings confirmed no significant differences in terms of the healing rate[32,33] except for hydrocolloids[32] and alginate[31] dressings. Pain scores were also lower but not significantly different when films were used.[31–33] In a large trial of 289 patients randomised (of whom 288 were analysed) who had either alginate (45 patients), film (49 patients), gauze (50 patients), hydrocolloid (49 patients), hydrofiber (47 patients) or silicone (48 patients) dressings, patients who had a film dressing were the least satisfied with their overall scar quality.[32] Overall, there is no clinical evidence that supports an improved healing of split-thickness skin graft donor sites with film dressings.

A recent systematic review analysed the clinical evidence on the effectiveness of semi-permeable dressings, films and foams, to treat radiation-induced skin reactions related to radiation therapy in cancer patients with a focus on pain, discomfort, itchiness, burning and the overall effect on their daily life activities. From the 181 randomised, controlled trials conducted between 2010 and 2015, six concluded that semi-permeable dressings are beneficial in the management of skin toxicity related to radiation therapy.[40]

Foams

The concept of foam was first introduced in the 1970s. Silastic foams were prepared in the clinic by mixing two components, the polymer and a catalyst, which reacted in situ releasing heat and expanding to form a more solid structure that conforms to the shape of a cavity. Then, this concept evolved in to a dressing composed of an absorbent, hydrophilic layer in contact with the wound that would expand when moist, and a hydrophobic outer layer that reduced water vapour loss and acted as a barrier against bacteria.[41] Foam dressings are nowadays associated with multi-layered dressings composed of a hydroconductive, wound-contacting portion that allows wound fluid passage. This is backed by a hydrophobic, highly absorbent, porous structure that draws the fluid into the air spaces and uniformly retains it away from the wound bed. Foam dressings are often combined with a semi-permeable, adhesive, outermost layer, that provides a barrier against bacteria infiltration and an interface for controlling water vapour loss.[6] Although mainly made of polyurethane, foam dressings have varied compositions, such as different types of polyurethanes, silicone, polyvinyl alcohol etc, which determine the surface hydrophilicity of the foams and consequently their capacity for handling wound fluids.[42,43] Therefore, this results in differences in the design, as well as the number and type of layers of each one of the available foam dressings[44] and, potentially, the time that they can safely be left on a wound. The composition of the polyurethane foams also influence their thermal insulation properties,[43] which is one of their most advertise features and highly relevant considering that healing players, like cells and enzymes, act optimally at physiological temperature, and temperature drops can cause vasoconstriction.

Due to their physical soft character, foam dressings offer additionally cushioning. Due to the hydrophilic and non-adherent character of the layer that is in contact with the wound, they also provide moisture and can be removed with a minimal amount of pain.[6] However, this hydrophilic feature might cause desiccation in wounds with eschar or wounds that are not draining due to the absence of fluid to absorb. If the absorbent layer of the foam dressings is sufficiently hydrophobic, they should have enough capacity to entrap bacteria before they reach the wounds.[10]

Table 3: Randomised controlled trials evaluating wound dressings' efficiency for the treatment of split-thickness skin graft (STSG) donor site

Author	Year	Type of material	No. of patients	Compared conditions	Follow up (days)	Results
M. Kazanavičius et al.[34]	2017	Film	98	Polyurethane-based foam vs polyamide-based film vs cotton gauze dressing (control)	21	• Similar mean healing time • Higher proportion of healed wounds for polyamide-based film (66.7% by postoperative day 9)
S. Läuchli et al.[33]	2013		38	Polyurethane-based film vs calcium alginate	Until re-epithelialisation was achieved	• Similar time to epithelialisation
P.J. Terrill[31]	2007		40	Polyurethane-based film vs calcium sodium alginate (control)	30 days	• Higher proportion of complete healed wounds (79% vs 16%) • Faster mean healing time (14 vs 21 days)
Kaiser et al.[33]	2013	Alginate	30	Calcium alginate vs polyurethane-based film vs gauze (control)	Day 1 postoperative; Day 5-7 postoperative; and after full epithelialisation of the donor site, approx. 14-21 days after surgery	• Similar full epithelialisation time (median: 16 days); higher than the control (median: 14 days)
M. Brenner et al.[35]	2015	Foam	57 (children)	Polyurethane-based foam vs carboxy-methylcellulose based hydrocolloid vs calcium sodium alginate	until re-epithelialisation was reached	• Higher median time for healing: 9.5 days (foam) vs 8 days (hydrocolloid) vs 7.5 days (alginate)
L. Higgins et al.[36]	2012		36	Polyurethane-based foam vs calcium sodium alginate	14	• Similar time for wound re-epithelialisation.
M. Karlsson et al.[37]	2014	Hydrocolloid	67	Carboxymethyl cellulose-based hydrocolloid vs polyurethane foam vs natural acellular xenograft	21	• Faster re-epithelialisation in hydrocolloid and acellular dressing vs polyurethane foam
Brolmann et al.[38]	2013		289	Alginate vs film vs gauze vs polysaccha-ride hydrocolloid vs carboxymethyl cellulose-based hydrocolloid vs silicone foam	28 (adverse events and scarring after 84 days)	• Significantly shorter re-epithelialisation time of polysaccharide hydrocolloid (compared to any other dressings)
U. Dornseif-er et al.[39]	2011		50	Carboxymethyl cellulose-based hydrocolloid vs polyurethane film (control)	10	• Lower re-epithelialisation (54.5% vs 86.4%)

While the moist environment provided by film dressings depends on its ability to keep the wound fluid directly in contact with the wound bed, the hydrophilic layer of the dressing is the layer playing that role in wounds dressed with foams. Wound exudate is kept away from the wound bed, and the interchange between the absorbent and the non-adherent layer (many of the dressings imply that fluid is kept away from the wound) will determine the availability of healing mediators at the wound bed. Foam dressing materials are not inert materials and are not only in contact with the cellular players in the wounds, but they can also react with the biochemical mediators through several chemical interactions that also vary with the chemistry of the materials.[14] These, together with the diffusion properties of the hydrophilic layer, greatly influence the diffusivity of the molecules, such as nutrients, electrolytes, cytokines and growth factors, and proteases, that can move into and from the dressing, respectively, and from and to the wound bed. Additionally, as wound fluid content varies along with the healing time as well as the type of wound, the dressing changing time is another critical factor when studying the mechanism of action of foam dressings. The studies aiming to unravel the mechanisms that can support foam dressings benefits in wound healing are typically *in vitro* studies. These studies hardly considered these aspects and instead, mainly focus on the material's capacity to bind and inhibit proteases.[45–47] Other studies have used animal models to look at the effect of foam dressings over the wound bed by analysing the formation of granulation tissues,[48] the synthesis of ECM components, such as hyaluronan,[49] and the level of cytokines[50] in the wound exudate. However, they do not include any considerations regarding an effect of the composition of the foam on the obtained results. This demonstrates that the specific mode of action of foam dressings is poorly studied.

Features of foam dressings, such as improved wound management, reduced tissue maceration, non-adherence to the wound, and atraumatic application and removal of foam dressings, are unquestionable.[51] Its use in a large range of applications has been reported. However, the performance of the different foam dressings in relation to other advanced dressings for a particular type of wound has yet to be clearly documented. As described for films, sufficient clinical evidence regarding improved healing with foam dressings for split-thickness skin graft donor sites[32,36,52] and radiation-induced skin reactions related to radiation therapy[40] has yet to be provided. Foam dressings, when compared with traditional gauze dressings, do reduce the healing time for acute wounds.[53] However, further research to provide level A evidence is still needed (Table 3, STSG). The main feature of foam dressings is their fluid-handling ability with improved periwound tissue quality. This has been clinically demonstrated in acute (surgical and trauma) and chronic (pressure ulcers, diabetic foot ulcers, leg ulcers, fungating tumours) wounds although there have been a low number of patients considered in each category.[42,54] A randomised, controlled trial of 118 patients using two different foam dressings showed completely different abilities of management, ranging from excellent to poor, for the exudate of lower leg ulcers.[55]

A recent systematic review with meta-analysis of 12 randomised, controlled trials, selected from 4117 publications, showed no statistically significant differences between foam and other advanced wound dressings with regard to achieving complete diabetic foot ulcer healing.[56] This conclusion is backed by another systematic review which included 157 participants, a meta-analysis of two studies. Foam dressings do not promote the healing of diabetic foot ulcers compared to gauze (RR 2.03, 95% CI 0.91 to 4.55), and healing was not significantly different than

what was observed for alginate dressings (RR 1.50, 95% CI 0.92 to 2.44). Moreover, no statistically significant difference in the number of wounds healed was observed when comparing foam and hydrocolloid dressings.[57] Another systematic review that evaluated 15 eligible studies, foam dressings were shown to increase healing in comparison to basic wound contact materials but not when compared with other advanced wound dressings.[58] Despite these observations, all included studies were small and/or had limited follow-up times with a high risk of bias. Therefore, there is still no clinical evidence available regarding the use of foam dressings to heal diabetic foot ulcers.

The same review with a meta-analysis of randomised, controlled trials for diabetic foot ulcers, selected 19 trials that used foam dressing for venous leg ulcers, confirmed equivalent dressing efficacies in terms of their ability to promote complete ulcer healing.[56] Another systematic review that included twelve randomised, controlled trials (1023 participants) reported an absence of difference in the healing outcomes between two types of foam dressings based on three separate trials. Additionally, healing in the foam group was not statistically different from the healing observed when using paraffin gauze in two trials and film in one trial and in the proportion of ulcers healed at twelve to sixteen weeks (RR 1.00, 95% CI 0.81 to 1.22).[59] Nonetheless, the generated evidence is of low quality, and the analysed trials did not have an overall low risk. Thus, nothing suggests that foams are more effec-tive in the healing of venous leg ulcers when compared to other dressings (Table 4, VLU).

Recently, a randomised, controlled trial comparing the efficiency of a foam and a film in the outpatient treatment of partial thickness burns in paediatric and adult patients showed similar time to re-epithelialisation (12 days; P = 0.75), but improved overall scar quality in the tissue dressed with the film (Film: 2; Foam: 4.5; p< 0.001).[73] Despite this, no further clinical evidence has been reported.

Hydrocolloids

The term 'hydrocolloid' was devised in the 1960s at the time of the development of mucoadhesives and then introduced to practitioners to the use of a hydrocolloid dressings as an occlusive choice, which is virtually impermeable to water vapour and air. These can be described as dressings in which a hydrophilic adhesive mass that contains a dispersion of carboxymethylcellulose, polyvinyl alcohol, gelatine or pectin, which jellifies upon contact with the wound fluids that is then combined/applied to a flexible occlusive film and/or foam. Respectively, this provides the barrier and the mechanical protection described for these types of dressings.[6] Hydrocolloids are capable of providing moisture to the wound since they form a gel after contact with the wound fluid.[74] However, the type of polymer and its hydrophilic characteristics used and its crosslinking degree, which determines mechanical properties, influences the absorption capacity for the dressings (the higher the crosslinking, the lower the fluid uptake) and potentially their performance.[75] Some formulations contain an alginate to increase absorption capabilities. Additionally, because waterproof backing is often made of polyurethane, hydrocolloids also provide insulation to the wound bed. Although it is very appealing due to its adaptability to various body shapes, residues of the gel can be left in the wound when the dressing is removed,[75] and can be mistaken for infection due to its colour and odour.

Hydrofiber dressings are hydrocolloids produced in the shape of hydrophilic, non-woven flat sheets. These dressings have the same ability to form a gel when they come in contact with the exudate but have improved mechanical properties. These properties may overcome the problem of residue

Table 4: Randomised controlled trials evaluating wound dressings' efficiency for the treatment of VLU and mixed aetiology wounds

Author	Year	Material	No. of patients	Compared conditions	Follow up (days)	Results	Comments
Dini et al.[60]	2013	Foam	46	Cellulose-based foam vs polyurethane foam (control)	84	• Higher healing rate	
Alvarez et al.[61]	2012		50	Cellulose-based foam vs gauze (control)	Up to 84	• Faster achievement of >50% re-epithelisation (36 vs 50 days)	
Kelechi et al.[62]	2012		71	Polyurethane foam vs film	Up to 140	• Lower proportion of completely healed ulcers (45% vs 86.4%)	
Wild et al.[63]	2010		40	Cellulose-based foam vs carboxymethyl-cellulose hydrocolloid (control)	28	• Higher reduction in ulcer size after 28 days (45.53% vs 17.94%)	
Andriessen et al.[64]	2009		12	Polyurethane-based foam vs collagen-based foam vs paraffin gauze (control)	28	• Higher reduction in ulcer size for collagen-based foam, followed by polyurethane-based foam and lastly by paraffin gauze	Low number of patients
Franks et al.[65]	2007		156	Polyurethane PEG-based foam vs polyurethane-based foam (control)	Up to 365	• Similar ulcer closure	
Meaume et al.[66]	2017		187	Foam impregnated with TLC-NOSF vs the same foam without NOSF (nano-oligosaccharide fac-tor). Double blind RCT	56 days	• Accelerated wound healing but not significantly different rate of wound closure	
Meaume et al.[67]	2014	Hydro-colloids	156	Polyacrylate hydrocolloid vs carboxymethyl-cellulose hydrocolloid (control)	42	• Similar impact in reduction of wound surface area (34.1% vs 34.4%)	
Dereure et al.[68]	2012		143	Hyaluronic acid impregnated-gauze vs polysaccharide-based hydrocolloid (control)	Up to 60	• Similar efficiency in the reduction of wound area	VLU and mixed aetiology

Meaume et al. (69)	2008		125	Hyaluronic-based hy-drocolloid vs hydrocol-loid (control)	Up to 42	• Similar efficiency in the reduc-tion of wound area	VLU and mixed aetiology
Schmutz et al. (69)	2008		138	Contact layer im-pregnated with TLC-NOSF vs ORC (Promogran)	96	• Wound area reduction (61.4% vs 7.7%)	VLU and mixed aetiology
Nelson et al.[70]	2007		124	Hydrocolloid vs film (control)	168	• Similar proportion of healed wounds	
Romanelli et al.[71]	2010	Acellular Matrices	50	Natural acellular xen-ograft vs gauze	Up to 56	Higher proportion of complete healing (80% vs 65%); p<0.05 Higher area of granulation tis-sue (65% vs 38%); p<0.05	VLU and mixed aetiology
Romanelli et al.[72]	2007		54	Natural acellular xen-ograft vs artificial acellular matrix	112	Higher proportion of complete healing (82.6% vs 46.2%)	
VLUs: venous leg ulcers							

being left in the wound and provide an enhanced, faster absorption capacity that is capable of handling high exudate.[14]

One of the key features of hydrocolloid dressings is their occlusive nature that is responsible for a highly hypoxic wound environment. The analysis of the oxygen tensions of chronic wounds dressed with hydrocolloids and semi-permeable film dressings confirmed values very close to zero.[76] While hypoxia around the wound is one of the critical factors that enhances the progression of chronic wounds, it is also positively correlated with epithelialisation[16] and angiogenesis.[77] However, as for the film, the anticipated mechanism of action of occlusive hydrocolloid dressings has primarily been associated with what has been revealed about the healing process under moist conditions.[78] Therefore, the content of the wound fluids, in this case generated under the hypoxic conditions, is again a major factor to be considered. So far, in addition to the oxygen tension and the pH levels, which are acidic due to the chemical nature of the dressing,[76] not much further is known. The pH drop has also been correlated with a reduced probability of wound infection. However, when the effectiveness of hydrocolloid and permeable dressings to control burn infections were compared, the occlusive dressings were found more susceptible to microbial contamination and infections.[79] A similar tendency was also confirmed for the treatment of autogenous skin donor sites with hydrocolloids.[80]

Safety and technical performance of hydrocolloids based on their ability to absorb and retain exudate with healthy peri-wound skin and minimised pain has been well-demonstrated.[81–84] The comparison of hydrocolloids, for the treatment of split-thickness skin graft donor site, with other advanced dressings showed a diverse range of results. These confirm the great variability among the trials that do not allow definitive conclusions about the clinical relevance of one dressing over the other

for a specific wound type[32,85,86] (Table 3, STSG). Despite this, hydrocolloid dressings are more com-monly used to treat chronic wounds. Several systematic reviews have analysed the effectiveness of hydrocolloid dressings in the treatment of diabetic foot ulcers. One of the reviews reported that hydrocolloid dressings were suggested to be associated with a higher likelihood of healing compared to other advanced dressings. It did, however, also highlight the very low quality of the studies and conveyed uncertainty concerning this conclusion.[58] In the four studies (511 participants) included in another review, no significant difference between hydrocolloids and traditional wound dressings (RR 1.01; 95% CI 0.74 to 1.38) was observed regarding ulcer healing.[87] The comparison between hydrocolloids and traditional and other advanced wound dressings, which was further analysed in a recent review with meta-analysis, reported that no significant differences were found among the pairwise groups in terms of achieving complete diabetic foot ulcer healing.[56]

This same review found similar results regarding the effectiveness of hydrocolloids in promoting complete healing of venous ulcers.[56] This is in agreement with what was reported by another systematic review and meta-analysis of 42 studies.[88] From eight selected trials, it was concluded that hydrocolloid dressings were not more effective than the low adherent dressings (RR 1.02, 95% confidence interval 0.83 to 1.28) in the control group.[88] More recently, other reviews[89] concluded that lim-ited quality data is available regarding randomised, controlled trials using hydrocolloids to treat venous leg ulcers to be able to confidently make comparisons (Table 4, VLUs).

Regarding the effectiveness of hydrocolloid dressings in the healing of pressure ulcers, based on 646 identified studies, 69 were evaluated, nine were selected, and four were used for the meta-analysis that showed no significant difference between the hydrocolloid and the foam groups (RR 1.06, CI 95% 0.61-1.86; p value=0.84). A slight inferiority was observed for the hydrocolloid dressings, but the collected evidence was not sufficient to either confirm or deny superior/inferior effectiveness.[90] This is in agreement with another review that also reported a lack of evidence to support conclusions about different performance in relation to other dressings in the management of category III and category IV pressure ulcers, including in seniors in long-term care.[91] Two older systematic reviews reported that hydrocolloid dressings were superior in comparison to traditional gauze dressings in terms of complete healing of pressure ulcers and venous leg ulcers.[53,92] However, the lack of evidence supporting that hydrocolloids are better than any other advanced dressing was also confirmed.[53]

Recent trials have also demonstrated the possibility of using hydrocolloid dressings in the manage-ment of partial thickness burns. A randomised, controlled trial (50 patients/group) that compared hydrocolloid and film dressings showed significantly increased comfort for patients when a hydrocolloid dressing was used. However, no difference in healing time was found.[93] Another randomised study that included 70 patients and compared the effectiveness of hydrocolloid dressing versus standard of care treatment for partial-thickness burns, showed shorter time for healing in the hydrocolloid group (10 +/- 3 versus 13.7 +/- 4 days, $P < 0.02$).[94] Although these are interesting outcomes, it must be highlighted that the tested dressings contained silver. Thus, clear clinical evidence on the benefit of using hydrocolloid dressings without additional antimicrobial components for burns management has not yet been examined.

Hydrogels

Hydrogels have been used in a wide range of medical applications. Originally proposed in

the 1950s; they were only explored in wound management in the 1980s. Hydrogels are gels, which contain more than 99.9% water. Gels are materials composed of a three-dimensional crosslinked polymer network, usually soft and weak, immersed in a fluid. The degree of chemical interactions within this network can be changed by promoting its crosslinking or enhancing interactions, which make it harder and tougher.[95] Hydrogel dressings comprise both amorphous gels and sheets, which can be similar in terms of polymer composition but are physically very different. Contrary to the amorphous gels, sheets have a higher crosslinking degree and are insoluble in water. Depending on this crosslinking, the different sheets have different fluid exchange properties that provide moisture to the wound bed or absorb wound exudate.[6] In addition, as the hydration of the hydrogels dressings is high (inconsistent data are available about the exact value which accounts for the differences among them) the amount of exudate that can be absorbed is relatively low. Therefore, these dressings are useful in sit-uations where drainage is of secondary concern. The unprecedented amount of water in hydrogels is also responsible for their unique ability to immediately cool the wound surface, which provides a soothing effect.[96] Importantly, this cooling effect should be temporary since prolonged reduced temperatures may delay healing due to the temperature's dependence on key biochemical and cellular elements.[97] Because hydrogels are dressings with high water content, and autolytic debridement has been highly associated with moist environments allowing natural enzymatic reactions to take place, hydrogels dressings have long been recognised as the standard treatment for necrotic and sloughy wounds.[98]

The mode of action of hydrogel dressings is not known beyond the general considerations regarding the effect of a moist wound environment in wound healing. From the high number of studies reporting the potential application of hydrogels in a wide range of areas, it can be extrapolated that the 'bioactivity' of hydrogels depends on microstructural parameters, such as the chemical composition, crosslinking density, and mesh size, and on the macroscopic properties, such as mechanical stiffness and degradation rates, which may directly and indirectly affect cells and may be important for remodelling within the host tissue.[99] *In vivo* works, both in murine[100–104] and pig[101, 105] models of burns, it was shown that hydrogels can be tailored to modulate different stages of the wound healing, for example inflammation, which consequently affects neovascularisation within the granulation tissue. This leads to the progression of the healing and re-epithelialisation. Nonetheless, these types of analyses have not been the focus of attention for the different hydrogel dressings, but they are critical due to the dependence of the response on the materials that are being tested.

The comfort in the use of hydrogel dressings, associated with the easy adaptation to the wound, reduced pain and resulting in an absence of trauma has been well-demonstrated.[106, 107] Moreover, their soft tissue-like properties, together with the cooling effect potentially provided by the high-water content, make them prime dressings for the treatment of burns. From a total of 30 randomised, controlled trials covering the treatment of partial and full-thickness burns systematically analysed with different dressings, three trials showed that wounds treated with hydrogels appeared to heal faster than those treated with standard care.[108] A meta-analysis was, however, not conducted due to the poor quality of the results or the heterogeneity of the studies.

A series of systematic reviews also showed moderate quality-level of evidence that hydrogels were more effective in healing diabetic foot ulcers

as compared to traditional gauze dressings.[56,58,109] However, no difference was found when one hydrogel was compared to a different hydrogel[109] or another advanced wound dressings.[56,58] Meta-analysis also revealed that hydrogel dressings are more effective (RR 1.80, 95% CI 1.27 to 2.56) in healing (lower grade) diabetic foot ulcers than basic wound contact dressings. However, this finding is uncertain due to risk of bias in the original studies.[56,109]

A review with meta-analysis of randomised, controlled trials for venous leg ulcers showed that hydrogels dressings have equivalent efficacies, in terms of promoting complete ulcer healing, to traditional and other advanced wound dressings.[56]

From the results of a systematic review that evaluated eleven studies that involved a total of 539 participants, it was not possible to confirm that the healing of pressure ulcers was faster with hydrogel dressings as compared to healing with traditional dressings or any other advanced wound dressing.[110]

Alginates

Although alginate has been explored in the wound management context early in the 1940s, it was not until 1983 that the first alginate wound dressing was commercially available. Alginate dressings are made of sodium and calcium salts, usually in a ratio of 80:20, or of only calcium alginate salt obtained from a family of brown seaweed.[111] Upon contact with the wound, an exchange of ions between the dressing, the calcium ions, and the fluid, the sodium ions, occurs, which slowly converts the calcium alginate in the dressing into sodium alginate. This is water-soluble and, thus, forms a gel.[112] Originally, alginate dressings were available as a loose fleece formed from calcium alginate fibers, but current products are often made of woven and non-woven fibers that provided a more cohesive structure improving the handling of the hydrated dressings. Most of the alginate dressings are produced in the form of sheets, but other shapes, such as ribbons or rope, which are suitable for deep, cavity wounds, are also available.[6]

Alginate dressings have the capacity to absorb fluid 15 to 20 times their weight. This makes them very useful in highly exuding wounds and contraindicated in wounds with little to no exudate due to their adhesive nature, which can cause pain and damage healthy tissue upon removal. Nonetheless, the relative composition of the alginate in mannuronic and guluronic acid units influences the amount of exudate that can be absorbed due to the gelling properties of the alginates. Alginate dressings with high content of mannuronic acid are less stable and more gelatinous and need to be washed off from the wound, while those with a high content of guluronic acid can be removed in one piece.[112] Due to the ion exchanging properties, alginate dressings are useful haemostatic agents. The released calcium, factor IV in the haemostasis cascade, activates thrombocytes and serine proteases that lead to fibrin formation and clotting.[113]

As for all of the dressings that form a gel upon contact with the wound fluids, it has been assumed that the mode of action of alginate dressings relies on their capacity to provide moisture to the wound. In fact, very little is known about the specificities of each alginate dressing, but several studies have highlighted the biological activity of alginates. Alginates high in mannuronic acid are 10 times more potent compared to those with a high content in guluronic acid with regard to stimulating *in vitro* monocytes to produce pro-inflammatory cytokines, such as TNF-alpha, IL-6 and IL-1.[114] This occurs through a common receptor to pro-inflammatory lipopolysaccharides, potentially the beta 1-4-glycosidic linkage of the guluronic acid.[115]

Interestingly, this activity can be eliminated by the selection of the different oligomers present in the raw material.[116] In addition, *in vitro* tests showed increased proliferation of fibroblasts and decreased proliferation of endothelial cells and keratinocytes in the presence of alginates.[117] However, a confirmation of these observations in wounds dressed with alginates is still lacking. Thus, there is a need for further understanding about the mechanism of action of these dressings.

Confirmation that alginate dressings are comfortable in use and can be removed with no trauma, without pain and discomfort to the patient, has been delivered.[118] Moreover, the suitability of alginate dressings to manage low to moderate levels of exudate was demonstrated.[119]

Randomised, controlled trials have shown a potentially improved healing of split-thickness skin graft donor sites treated with alginate dressings in comparison to those treated with paraffin gauze.[33,118] Nonetheless, a recent systematic review with meta-analysis that assessed randomised, controlled trials on diabetic foot ulcers (12 trials) and venous leg ulcers (19 trials) treated with alginate dressings did not show statistically significant differences in terms of achieving complete healing when compared with other advanced wound dressings.[56]

Regarding the use of alginates in the treatment of pressure ulcers, a review of 54 randomised controlled trials (RCTs) evaluating absorbent wound dressings found one in which calcium alginate dress-ings improved healing (mean wound surface area) when compared with a dextranomer paste.[120] A more recent review that also considered the former one and aimed at analysing the effectiveness of commonly used dressings in the management of category III and category IV pressure ulcers, including seniors in long-term care, reported that there is no evidence to support consistent superiority of one dressing over the other.[91] Interestingly, a randomised, controlled trial (110 patients) that compared the sequential (alginate followed by hydrocolloid) and non-sequential (hydrocolloid) treatment of stage III or IV pressure ulcers showed an accelerated healing in the sequential group in comparison with the control group.[121] In relation to burn wounds, a trial with 65 patients that compared the effectiveness of an alginate dressing with a standard of care cream in partial-thickness burns showed a significantly shorter healing time for the alginate dressing.[122]

Despite the common use of alginate dressings in the clinic, there are few reports of trials that provide significant evidence to justify their use for a specific wound type. As for the other studies, the low number of patients involved, the relatively high risk of bias, the heterogeneity of the studies as well as the poor quality of the results prevent an analysis with a higher level of significance.

Acellular matrices

The recognition of the key role of the extracellular matrix (ECM) in wound healing has steered the development of products that aim at replacing and/or promoting the deposition of the ECM. These products, comprising natural or artificial tri-dimensional matrices, provide a substrate for host cell migrate acting as a template or temporary scaffold that gradually degrades when new tissue is formed.[123]

Natural acellular matrices are derived from animal or human tissue from which cells are removed while artificial man-made manufactured matrices are made from purified biological molecules and derived from cells after the onset. Although intended to work as ECM mimickers, acellular matrices are different from native tissue. Natural acellular matrices are derived from animal sources (porcine, equine)-xenografts or human skin (cadaver)-allografts and developed by processing

the animal tissues (dermis, small intestine submucosa, pericardial) to remove the cells and deactivate or destroy pathogens. Although this processing (decellularisation and/or dehydration) intends to eliminate only the cellular content of the tissue, the ECM is also affected, which results in a loss of components (decellularised products are often mainly composed of collagen) and structural integrity (absence of basement membrane, additional chemical crosslinking required), which then may impact their biological performance.[124]

Artificial acellular matrices have been proposed that aim at targeting those limitations by combining multiple animal-derived (bovine, shark, calf) ECM components such as collagen (types I, III and V), elastin, and glycosaminoglycans (GAGs – hyaluronic acid), and at promoting their crosslinking to increase mechanical stability. Collagen is often used as the main component. Other artificial acellular matrices can also combine those animal-origin components with synthetic ones, usually identified as bio-composites, to facilitate processing and tailoring of the properties of the product. However, they do not mimic native dermis in its entirety.[123]

Independently of the type of acellular matrix, materials are mostly processed in a 3D porous structure using technologies that allow for the controlling of the amount and the size of the pores, which is known to influence host cells infiltration.[125] Additionally, the composition, the type and amount of materials, of the products are tailored together with the form (chemical or physical) and degree (exposure conditions) of crosslinking. All of these are directly linked to the degradation and, consequently, to the matrix remodelling rate during the healing process of the wound. These differences are likely to affect multiple wound healing aspects since microarchitecture (porosity and pore size), mechanical features (elasticity) and chemistry (reactive groups, surface charge) are known to affect cell adhesion, migration and differentiation.[126–129] In fact, the treatment of acute cutaneous wounds with autografts or acellular matrices resulted in variable fibrotic (amount and organisation of collagen I) outcomes with reduced scarring in relation to wounds healed by secondary intention.[130]

Acellular matrices are not intended to directly replace dermal collagen. They are used as a way to achieve an environment that cells sense as native to promote a faster and better healing. Thus, if the degradation is not controlled to avoid a major foreign body reaction and an exacerbated inflammatory process, which are hallmarks of burns and chronic wounds, scarring or impaired healing are likely to occur.[123] Due to the permeable nature of these products, several of them comprise an outer silicone layer, which works as a temporary epidermis and serves to control moisture loss from the wound. These allow vascularisation of the dermis under a protective layer, which can then be re-moved and replaced by an autologous split skin graft.[131] On the other hand, in the absence of the outer layer, earlier wound closure is achieved but onto an avascular dermis.[132] Independently of the strategy, vascularisation will depend on the composition (some materials or degradation products are intrinsically angiogenic) and properties of the materials to allow an influx of cells and the formation of a capillary network. Improved vascularisation before the application of a split skin graft has been shown to lead to better take rates, reduced wound contraction and good aesthetic outcomes.[123,133,134]

Natural skin-derived acellular matrices, in opposition to artificial ones, usually contain parts of the native basement membrane. This has been shown to promote the adhesion and further *in vitro* differentiation of keratinocytes due to the presence of laminin and collagen

IV.[135] In fact, a significantly higher proportion of completely healed wounds was attained with a natural acellular matrix as compared with an artificial one.[72]

Acellular matrices have been tested as an option for burn and chronic wound management with growing evidence for the use in diabetic and venous leg ulcers. A recent systematic review included seven studies (205 patients) to compare the efficiency of acellular dermal matrices and or split-thickness skin grafting in burns, focusing on the graft take, infection rate and scar quality.[136] Similar wound coverage was reported, but four out of the seven trials included did not show a significant difference in scar quality, which does not provide conclusive evidence about the effectiveness of the acellular dermal dressing.[137] Another systematic review that analysed six trials, using a different acellular dermal dressing to treat partial thickness burns in children, concluded that the acellular dressing performed better than the standard of care regarding the epithelialisation rate. However, hardly any of the studies assessed long-term performance, such as scar quality[138] (Table 5).

Regarding the use of acellular dressings to treat chronic/non-healing wounds, a recent randomised controlled trial (60 patients were included and 46 selected) compared the performance of an artificial acellular dermal matrix with a traditional gauze dressing in diabetic foot ulcers up to six weeks based on epithelialisation and granulation tissue formation. The results with the acellular dermal matrix were significantly superior (86.95% versus 52.17% complete healing in the total 69.56%; p=0.001) with lower amputation (p=0.0019) and re-hospitalisation (p=0.028) rates.[147] Another randomised, controlled trial (168 patients, 36 withdrew due to either an adverse event or significant noncompliance) compared two different acellular allografts, one or two applications, with standard of care treatment (patients randomised at a ratio of 2:1:2) for diabetic foot ulcers up to 16 weeks. This trial showed that the two acellular dressings performed differently, but not at significant levels (67.9% versus 47.8%; P = 0.1149), although the one with the higher proportion of completely healed ulcers had a significantly better result than the standard of care group (67.9% versus 48.1%; p=0.0385) and independent of the number of applications. Interestingly, the two acellular grafts showed a significantly different average percent of reduction of the wound area (91.4% vs 73.5%; p=0.0762).[148]

The performance of acellular matrices in the treatment of diabetic foot ulcers was also recently compared with other advanced wound dressings. In a small trial (17 patients) that compared a natural acel-lular xenograft with a foam, the incidence of wound healing was 90% and 100% versus 33% and 83.3% (p=0.062) respectively, at 12 and 16 weeks. Additionally, the mean time for healing was 62.4 days for the acellular matrix in opposition to the 92.8 days in the foam group (p=0.031). Importantly, the incidence of ulcer recurrence at one year was 10% (1/11) in the acellular matrix group and 50% (3/6) in the control group.[149] A larger multicentred trial (307 patients) evaluated the safety and efficacy of an artificial acellular matrix in comparison to a sodium chloride gel for the treatment of nonhealing diabetic foot ulcers. Complete ulcer closure was significantly greater with the acellular matrix (51%) than with the control (32%; p=0.001) treatment at 16 weeks. The median time for complete closure was 43 days for the experimental group and 78 days for control in the wounds that healed. Moreover, the rate of wound size reduction was 7.2% (acellular matrix) versus 4.8% (control) per week (p=0.012).[150]

Two different randomised, controlled trials compared the ability of a natural acellular xenograft (natural acellular matrix) with a

Table 5: Randomised, controlled trials evaluating wound dressings' efficiency in the treatment of skin burns

Author	Year	Type of material	N. of patients	Compared conditions	Follow up (days)	Results
Hundeshagen et al. [139]	2017	Foam	60	Polyurethane foam vs lactic acid-based film	Day 1, every 3-7 days up to 30	• Similar median reepithelialisation time (12 days) • Re-epithelialisation time greater than 21 days (20% vs 7%) • Reduced scar quality: 4.5 (foam) vs 2 (film); p< 0.001).
Li et al.[140]	2015	Acellular Matrices	60	Natural acellular allograft vs STSG (control)	180 and 900	No scar formation in natu-ral acellular allograft group
Lagus et al.[141]	2013		10	• Artificial acellular matrix vs STSG (control) • Artificial acellular matrix vs cellulose-based foam	Punch biopsy at days 3, 7, 14, and 21; assess-ment at 90 and 365 post-injury	• Lower number of neu-trophils, histiocytes, and lymphocytes at days 7 and 14 in artificial acellular matrix vs cellulose-based foam • Later vascularisation for artificial acellular matrix vs control and vs cellu-lose-based foam • Less myofibroblasts on day 14 (artificial acellular matrix vs control)
Wood et al.[142]	2012		13 (children)	Artificial acellular matrix vs LST	Reassessment for further sur-gical need at 10 days post-burn. Follow up until 180 days	• Reduced time for complete healing (16 vs 36.5 days)
Bloemen et al.[143]	2012		86	Artificial acellular xenograft vs STSG (control)	4 to 7 days after surgery; weekly up to 90; 365	• Similar graft takes • Scar surface roughness scores at 12 months were lower for acellular dressing but without significant differences
Ryssel et al.[144]	2008		10	Artificial acellular matrix vs STSG (sheet and mesh; control)	90–120 after surgery	• No differences for the necessity of regrafting • Significant improvement in VBSS measurements (score of 3 and 5 vs 6 and 7 for sheet and mesh, respectively)
Branski et al.[145]	2007		20 (children)	Artificial acellular matrix vs STSG (control)	At admission, on discharge, and at post-burn months 180, 365, 540, and 720	• Similar graft takes • aesthetically improved scar at 12 months and 18–24 months post-injury. • Reduction in Hamilton scoring (5.4 vs 7.7 at 12 months and 4.3 vs 6.6 at 18-24 months) post-injury.

Cassidy[146]	2005		72 (children)	Artificial acellular matrix vs poly-saccharide-based hydrocolloid	Not available	• Similar time to healing • Similar mean time for complete re-epitheliali-sation (12.24 (±5.1) vs 11.21 (+/-6.5) days)
Verbelen et al.[93]	2014	Hydrocol-loid	100	Carboxymethyl-cellulose-based hydrocolloid vs polyester poly-ethylene-based film	Every 3 days up to 21 days or until wound healing	• Similar mean healing time (15.06±3.42 vs 16.16+/-7.19 days)
Muangman et al.[94]	2010		70	Carboxymethyl-cellulose-based hydrocolloid vs LST (SSD)	Day 1, every 3 days until wound healing	• Time-to-wound closure significantly shorter (10±3 days vs 13.7±4 days) • Pain scores at days 1, 3 and 7 lower (4.1, 2.1, 0.9 vs 6.1, 5.2, 3.3)
Opasanon et al. [122]	2010	Alginate	65	Calcium alginate vs LST (SSD)	Until healing occurred	- Lower mean time to heal (7 vs 14 days)
STSG: split-thickness skin graft donor sites; LST: local standard treatment; SSD silver sulfadiazine						

traditional dressing (54 patients)[71] and an artificial acellular matrix (50 patients)[72] for the treatment of mixed arterial/venous leg ulcers. Faster achievement of complete healing was observed for the natural acellular xenograft compared to the artificial acellular matrix (82.6% versus 46.2%; $p<0.05$), in a significantly shorter time (5.4 versus 8.3 weeks; $p=0.02$)[72] and as compared to the traditional dressing (80% versus 65%; $p<0.05$).[71]

Despite these results, two previous systematic reviews[89,153] concluded that limited data were available regarding RCTs with acellular dressings. In particular, sufficient evidence to draw meaningful conclusions regarding the treatment of diabetic foot and arterial ulcers was lacking[153] while low-strength evidence was found for venous leg ulcers.[89,153]

Future perspectives

As mentioned before, the performed analysis of the healing effectiveness of the available dressings was based on the premise that wounds have to be cleaned and the wound bed should be well-prepared for healing to proceed. Nonetheless, infection is a major issue in wound healing. Each type of dressing discussed is also available with different antimicrobials, such as silver, betaine, chitosan, polyhexamethylene biguanide and honey, for preventing and treating wound infection. Dressings that provide a sustained release of silver, in sufficient concentrations, is one of the newer approaches tak-ing advantage of nanocrystalline silver.[93,154] Physical approaches that rely on dressings that irreversibly bind bacteria due to their outer chemistry have been presented as alternatives, which do not include the risk of inducing bacteria resistance and avoid bacteriolysis and pro-inflammatory endotoxin being released into the wounds. While prophylaxis is limited for these types of dressings, current clinical results can be considered encouraging and a good basis for further development.[155] Despite all these possibilities, wound infections and biofilms, which represent a physical barrier to healing and an extension of the inflammatory phase, are still

Table 6: Randomised controlled trials evaluating wound dressings' efficiency for the treatment of diabetic foot ulceration (DFUs)

Author	Year	Type of material	No. of patients	Compared conditions	Follow up (days)	Results
Zhang et al.[151]	2014	Foam	50	Polyurethane-based foam vs gauze (control)	84	• Reduced wound area and time of healing (49.9 vs 65.5 days)
Alvarez et al.[152]	2017	Acellular	17	Natural acellular xenograft vs polyurethane-based foam (control)	84; 112; 365	• Reduced time of healing (62.4 vs 92.8 days) • Reduced incidence of ulcer recurrence at one year (10% vs 50%)
Campitiello et al. [147]	2017		46	Artificial acellular matrix vs gauze	42	• Greater wound closure (86.95% vs 52.17%) (p=0.001)
Walters et al.[148]	2016		168	Natural acellular allograft I vs standard of care Natural acellular allograft I vs natural acellular allo-graft II	112	• Reduction of wound area (91.4% acellular I vs 80.3% standard of care; p=0.0791) (91.4% acellular I vs 73.5% acellular II – p=0.0762) • Proportion of healed wounds (67.9% acellular I vs 48.1% standard of care; p= 0.0385) (67.9% acellular I vs 47.8% standard of care)
Driver et al.[150]	2015		307	Artificial acellular matrix vs sodium chloride-based hydrogel (control)	112 or until confirma-tion of wound closure	• Greater ulcer closure (51% vs 32%) • Wound size reduction (7.2%/ week vs 4.8% /week)

DFU: diabetic foot ulcers

a major challenge.[156] Many antibiotic-containing topical formulations have also been developed, but the routine administration of these has not led to better outcomes. Instead, it has often resulted in patient discomfort along with the possibility of anti-biotic resistance and contact dermatitis.[157] These results added to a general consensus that topical antibiotics should be used for clearly infected wounds and not for prophylaxis.[158]

In what concerns the outcome of the performed analysis referring to the healing efficiency of the cur-rent wound dressings, it is consensual and common to all dressings that high-level evidence of the benefits of one over the other has yet to be demonstrated. This highlights the urgent need to better understand the pathophysiology of each wound as well its progression under specific dressing considering their particular properties. In most cases, these depend on their composition and/or processing methodology. New methods have been employed to create innovative matrices with intrinsic features such as pH-sensitivity[159] envisioning their application as controlled

Fig 1: Porous matrix of fibers of cross-linked bovine tendon collagen and glycosaminoglycan (chon-droitin-6-sulfate)

Fig 2: Acellular dermal matrix

release dressings, or calcium chelating ability[160] to modulate keratinocytes behaviour. In line with this, new research on novel molecules of interest in wound healing such as adrenomedullin (AM) and its binding protein-1 (AMBP-1),[161] astragaloside IV[162] as nitric oxide[163] has shown interesting results. However, complementary clinical research on the presence and activity of these molecules in the different wounds is still required. Although numerous case reports and non-controlled trials on the use of therapeutic molecules in different dressings have reported issues associated with cytokines and boarder growth and pleiotropic

Fig 3: Porcin dermal matrix

action that are determined by the wound environment. In addition to the need to achieve faster and better healing, particularly for chronic wounds, scarring associated with burns is another major concern in the field, particularly because wounds heal by a reparative rather than a regenerative process. While the differences between scarless foetal and adult healing are currently under study, the knowledge generated so far has not been profoundly explored and mostly relies on taking advantage of isolated factors that are up- or down-regulated in foetal wound healing based on all of the associated limitations that were already discussed.

Table 7: Evaluation of evidence

No.	Therapy	Indication for use	Level of evidence (for each indication)	Comments
1	Hydrocolloids (including hydrofibers)	STSG donor sites	2c	Likely to perform equal to other approaches; great variability among the trials
		DFUs	2c	Low quality results; Likely to perform equal to other approaches; great variability among the trials; hydrofibers are less cost-effective than other non-adherent dressings
		VLUs	2c	Low quality results; likely to perform equal to other approaches; great variability among the trials
		Pus	2c	Low quality results; likely to perform equal to other approaches; great variability among the trials
		Burns	2c	Potential benefits lack systematic analysis; RCT performed used dressing-containing silver
2	Films	STSG donor sites	1b	Moderate quality evidence
3	Foams	STSG donor sites	2c	Any estimation of the effect is uncertain
		DFUs	2c	Low quality results; likely to perform equal to other approaches; great variability among the trials
		VLUs	2c	Low quality results; likely to perform equal to other approaches; great variability among the trials
4	Hydrogels	Burns	2c	Poor quality results; heterogeneity of the studies
		DFUs	2c	Moderate-quality level of evidence in relation to traditional gauze dressing; likely to perform equal to other approaches
		VLUs	2c	Based on RCT results, other alternatives may be equally reasonable; high risk of bias; heterogeneous studies; poor quality of analysis performed
		Pus	2c	
5	Alginates	DFUs	2c	
		VLUs	2c	
		Pus	2c	
6	Acellular Matrices	Burns	2c	Based on RCT results, other alternatives may be equally reasonable; short-term results
		DFUs	2c	Potential benefits lack systematic analysis; RCT performed under differ-ent conditions and different inclusion criteria
		VLUs	2c	

Cell- and tissue-based therapies

Cell therapies

Regenerative medicine has far-reaching origins and is currently considered as a 'multidisciplinary medicine involving life, physical sciences and engineering'. The objective is to develop cells, tissues and functional organs to repair, replace or improve a biological function that has been lost due to congenital anomalies, injuries, illness or ageing. Already in the eighth century BC, Hesiod addressed the liver's ability to regenerate. He described this in the poem, *Theogony,* about the myth of Prometheus. Also, Aristotle speaks about tissue regeneration in a salamander, hypothesising the development of a biological tissue from an 'undifferentiated matter', thus giving rise to what would then be recognised as epigenetic theory.

It is only in 1868 that the academic world, for the first time, learnt about the concept of stem cells. The term was coined by Ernst Haeckel, who indicated progenitor cells of multicellular organisms. Since then, stem cells and their potential use have created great interest in the scientific communi-ties and led to a number of experiments. In 2003, Haseltine identified all of the potential for the development of an adult human being within a single cell. In 2004, the international introduction of the term 'regenerative medicine' took place. On 2 November 2004, the US Federal Government approved Proposition 71, which included funding for a research institute called the 'Californian Institute of Regenerative Medicine'. The purpose of this institute was to carry out scientific research on stem cells.

Today, it is possible to define a stem cell as an undifferentiated cell that is capable of producing both copies of itself and mature cells that are completely differentiated for a particular type of tissue.

Although it was initially believed that only embryonic cells had this potential, worldwide research has, from the late 1990s to the first decade of 2000, shown the presence of stem cells, which come from a different origin. These are defined as multi-potential cells that can differentiate into a specific tissue with which they have come into contact. Such stem cells, called mesenchymal, are also present in adult tissues. For example, adult stem cells can be derived from adipose tissue. However, in order to properly use these cells within so-called cell therapy, the branch of medicine that deals with 'replacing' damaged tissue by injection or application of healthy cells, it has become necessary to define the 'minimum' requirements needed to define a stem cell as well as its ideal features.[164–166]

The ideal stem cells

In order to standardise stem cell detection and at the same time to facilitate a better analysis of sci-entific papers in literature and the correct use of the term 'stem cells' in wound healing, the International Society for Cellular Therapy proposed the 'minimum' criteria needed to define a human 'mesenchymal stem cell' in 2006:

1. The cell must adhere to the surface under standard culture conditions *in vitro*;

2. The cell must express CD105, CD73 and CD90 but not CD45, CD34, CD11b or 14 CD79 alfa or CD1) and HLA DR;

3. The cell must be able to differentiate, *in vitro*, in osteoblastic, adipocyte and chondrocyte.

In light of these criteria, it was also attempted to understand what the ideal characteristics for a therapeutic stem cell could be by identifying the following criteria:

1. The cell must be able to multiply infinite times without increasing the risk of oncogenic mutations in its DNA.

2. Cell differentiation must be controllable.

3. Cellular collection should be easily accessible, abundant and with minimal discomfort and/or morbidity for the patient.

In order to identify the 'ideal' cell, many of the cell lines have been studied and used, but only a few of them are actually usable.

Initially, it was thought that stem cells were present exclusively in embryonic/foetal tissue, where they were able to differentiate in any cell line in order to form the individual. These were known as ESCs or stem cells of embryonic origin. Today's research has shown the presence of numerous stem cells in adult tissues also. These are named mesenchymal stem cells.[167–174]

The stem cells and other therapeutically active cells

Nowadays, many cells are studied for wound healing. Some of them are stem cells, and others are living cells. The most studied and used cells within tissue healing include the types listed in Table 8.

To date, significant shortcomings have been documented with the clinical application of live cell therapies. It has been established that stem cells typically do not survive, engraft, or differentiate long-term following clinical implantation.[175–177] Especially within a harsh wound environment, cells rapidly undergo apoptosis and are cleared by the body within 24 hours to one week after implantation.[176] While stem cell differentiation has been demonstrated *in vitro*, differentiation of implanted mesenchymal stem cell (MSCs) has yet to be definitively shown *in vivo*. Additional concerns persist with maintaining the phenotype of live stem cells during expansion in culture, cryopreservation, and rapid thawing of MSCs prior to implantation.[178,179] Therefore, recent research has largely focused on the signalling properties of MSCs, particularly related to the release of cytokines and modulation of inflammation.[180,181]

Bone marrow stem cells

These cells are already considered the best stem cell reserve, and the cells derived from bone marrow are certainly the class, which has been studied the most extensively. Their ability to differentiate in any cellular line, from bone to cartilage, to muscle,

Table 8: Stem cells and other therapeutically active cells

Type	Pro	Con
Bone marrow stem cells	Ability to differentiate in any cell lines	Complex acquisition, low number of cell obtainable
Keratinocytes and fibroblasts	Derived from skin biopsies	High culture time, friable and easily damage-able skin, cannot be used to cover large areas
Adipose derived stem cells	Easy to acquire; ability to differentiate in any cell lines; regenerative and volumetric effect	Not recommended for use in cancer diseases
Platelets	Rich in growth factors, can be used as support for other cell therapies	Requires at least 20cc/blood sample
Leukocytes	Rich in growth factors, antibacterial potential, immunomodulating, orchestrating wound healing	Requires at least 20cc/blood sample
Monocytes	Accelerates neovascularisation, easy to acquire, *in vitro* differentiation	No differentiation in epithelial cells *in vivo*
Epithelial Stem Cell (Hair follicle)	Easy acquisition	Low number of studies

stromal cells, tendon and fat, has suggested that this cell class could be the most suitable for various uses.

However, a much deeper analysis, though confirming the great potentiality of bone marrow cells, demonstrated how the process of acquiring these cells is complex and rather painful for the patient. An acquisition from the sternum or iliac crest was expected, and it has little efficacy in terms of the number of cells obtainable. Thus, these types of cells fail to comply with two of the criteria previously considered. Most important of these factors are the reduction of the suffering of the patient and the unlimited multiplication.

Keratinocytes and Fibroblasts

Since the late 1990s, keratinocytes and fibroblasts have been widely used in the treatment of chronic wounds and burns. They have been used alone or in association with other cell lines, such as melanocytes. The possibility to use skin biopsies in order to produce new skin for the patient by manipulating the skin biopsy with hyaluronic acid, suggested that the use of keratinocytes and fibroblasts could be a decisive choice. Even in this case, however, experience has shown concern about the potential of this 'artificial' skin, such as: 1) the culture time is too high since it takes at least two weeks to get usable skin; 2) the method cannot be used to cover large areas since large number of biopsies are needed in this case, and this is not always possible, especially in case of large burns; 3) the skin obtained, while being "complete" with both epidermis and dermis, is very friable and, therefore, easily damageable. It has also been found that the extraction is too traumatic for the patient.

Adipose derived stem cells (ADSCs)

Obtained by a lipoaspiration procedure, adult stem cells derived from adipose tissue are multipotent cells. These are very similar to those obtained from the bone marrow, and since the year 2000, they have been used extensively in the field of tissue healing and regenerative medicine. They are currently becoming the most widely-used cellular line.

They are popular thanks to the easy extraction process and the low patient morbidity. ADSCs can

be found in large quantities, representing a fraction of 1/500 to 1/1500 cells for a total of 5000 per cell each gram of fatty tissue extracted, with a stem potential 500 times higher than the medullary equivalent.

These cells have excellent plasticity, are 'self-healing' and can, *in vitro*, differentiate in any other cell line. They also offer the patient the opportunity to obtain not only a regenerative effect, but also a trophic and volumetric effect in the grafting area. These cells are contained in the vascular stromal fraction of the lipoaspirate when they are separated from the remaining cellular parts. These are present in the aspirate by a process known as decantation or centrifugation and can be reinstituted directly in the same patient (lipofilling) during the same session without the need of any delivery system. ADSCs grafting, therefore, induces neoangiogenesis stimulation, partly by promoting paracrine, and their presence can modulate the formation of granulation tissue, extracellular matrix, and immune response, thereby promoting tissue healing. ADSCs also have an antioxidant effect, and by secretion of lymphoangiogenetic factors, they improve tissue lymphedema by stimulating re-absorption. Finally, by use of chemokines, they are able to recruit other endogenous stem cells to the graft site.

In the last decade, the above mentioned characteristics have made the adult stem cell derived from adipose tissue, the ideal cell for use in wound healing.

Other cells

- Platelets

Platelets are a good source of growth factors. They are frequently used in combination with other treatments, such as lipofilling. Once you have a blood test of at least 20cc, it is possible to separate the platelets from other cellular elements, such as white blood cells and blood cells, in order to obtain plasma rich plasma (PRP) or products rich in growth factors derived from the platelets. This happens via a 'filtered centrifugation process. This plasma can be used alone or in combination with other treatments to assist in the healing of wounds, such as ulcers or skin grafts, and for aesthetic purposes, such as a revitalising treatment for the skin, ageing skin or in the treatment of alopecia.

- Leukocytes

Both *in vitro* and *in vivo* studies have proven the key importance of leukocytes in wound healing. Neutrophils are known to be a key part of the innate immune response key in the clearance of bacteria and debris as well as important in transferring the wound from the initial inflammatory phases into the proliferative wound healing phases.[182] In addition, recently both B- and T-cells have shown importance in the resolution of inflammation and, eventually, wound healing.[183] However, the most well described leukocyte to be involved in wound healing is the monocytes.

- Monocytes

In vitro studies have shown that mononuclear fraction is a source of stem cells that can accelerate neovascularisation and differentiate into epithelial, smooth, and endothelial muscle cells. However, epithelial differentiation has not yet been shown *in vivo*.

- Epithelial stem cells collected from a hair follicle

Acquired by biopsy or by scraping of the scalp, these cells were used in vitro for cellular vitality stud-ies. These experiments have shown that it is possible to obtain 0.5 million stem epithelial stem cells from 100 hair follicles, and these cells are positive for cytokeratin K15, thus retaining the potential for transdifferentiation in similar

epithelial corneal cells. This feature could make them readable, not so much for the treatment of chronic wounds, but for the treatment of ocular pathologies, such as Limbal Stem Cell Deficiency, which provides a patient the possibility of ensuring a corneal transplant.

The application modes

In order to promote growth, differentiation of stem cells, and their positioning in the area to be treated, so-called scaffolds and/or vehicle systems are needed. This is especially true if it is impossible to make a direct graft, and the procedure is similar to the previously explained procedure for lipofilling.

Scaffolds

Scaffolds are absolutely necessary to promote proper cell differentiation and above all the construction of a three-dimensional tissue. The ideal scaffold should be characterised by a functional plan, and one that is structurally similar to the native extracellular matrix of the tissue is required. It should also be biodegradable, not stimulate an inflammatory response, have surface properties capable of promoting adhesion, proliferation and cell differentiation, and should be able to mimic the skin *in vitro*, and have effective mechanical properties. Finally, it should be sufficiently plastic to be moulded into various shapes, depending on the receiving area. Biological and synthetic scaffolds can be found on the market today.

Organic scaffolds are characterised by a base consisting of, for example, collagen, glycosaminoglycans, hyaluronic acid or chitosan. They are composed of up to three layers. A category belonging to the biological scaffolds is represented by so-called decellularised scaffolds that are derived from dermic matrix, which are more complex architecturally and in the matrix composition. The latter have found extensive use as the absence of cells would avoid the formation of an inflammatory process. However, the processes necessary to obtain proper decellularisation are very complex and critical because they have to maintain the matrix proteins, the architecture of this and the growth factors in the proteins.

Synthetic scaffolds, on the contrary, are more readily obtainable and customisable as needed. These can also be produced in large quantities. Formulated, for example, by polylacticglycolic acid or even polycaprolactone, they can host fibroblasts, keratinocytes and ADSCs. In the latter case, they support stem cell growth and differentiation in epithelial cells and fibro-vascular components by promoting tissue healing in the event of acute and chronic lesions.

At the moment, 'hybrids' scaffolds made of organic material are designed to provide the ideal environment for cell proliferation and differentiation, and inorganic materials are most useful to facilitate cellular production and quality.

Carrier systems

Carrier systems are useful to convey cells *in vivo*, without the use of three-dimensional scaffolds. Carrier systems include topical sprays, direct grafts or systemic delivery.[100,172,184–187]

Possible uses

Stem cell therapy, as mentioned above, has found widespread use in wound healing, whether acute or chronic. Of the approximately 500 clinical trials currently taking place worldwide, 23 trials are closely related to the use of stem cells in healing wounds. More precisely, trials are in progress for the use of haematopoietic stem cells, ADSCs, BMSCs and MSCs in pressure ulcers, vascular and diabetic ulcers and burns. Finally, trials are being conducted to evaluate the possible use of scaffolds that favour neural proliferation in the treatment of chronic spinal cord injuries (for further details on

all clinical trials, see clinicaltrial.gov).[188–191]

Autologous blood-derived products for wound care

The use of autologous blood-derived biomaterials in the treatment of chronic wounds was introduced in the mid-1980s by Dr David Knighton and his colleagues.[192] They developed a platelet-derived wound healing factor (PDWHF) formula derived from autologous blood. Platelets were first isolated from anticoagulated whole blood and then activated by the addition of thrombin (1U/ml) in a specific buffer. The supernatant from the activated platelets were mixed with 1-gram jar of microcrystalline bovine collagen to generate an acellular salve containing a plethora of growth factors at super-physiological concentrations.

Since the launch of PDWHF, several different platelet-derived or platelet concentrate products have been developed. From the generic platelet-rich plasma (PRP) products, four distinct product catego-ries have evolved.[193] These autologous products are classified according to their specific cell composition and fibrin content as follows:

1. Pure platelet-rich plasma (P-PRP) products consist of platelets without leukocytes in plasma and can be used either as a fluid for injection into orthopaedic injuries or can be activated by calcium and thrombin to release growth factors and polymerise fibrin to form a gel for topical application to skin wounds. The level of fibrin (2–3mg/ml) generally matches that of plasma.

2. Leukocyte- and platelet-rich plasma (L-PRP) products are similar to P-PRPs, but they contain leukocytes in addition to the platelets. Typically, leukocytes are concentrated by 3–5 times as compared with the concentration in whole blood. L-PRPs are administered as a liquid without activation or in the form of a gel entangling cells and platelets after activation.[194]

3. Pure platelet-rich fibrin (P-PRF) preparations constitute activated platelets in a polymerised fibrin matrix. The fibrin content is higher than in P-PRP and L-PRP products and the cohesiveness typically prevents P-PRF products from being injected. Instead, P-PRF products are applied directly to the wound.[195]

4. Leukocyte- and platelet-rich fibrin (L-PRP) preparations are similar to P-PRF products with respect to their high fibrin content. As opposed to P-PRFs, they contain leukocytes apart from the platelets derived from the blood.[196]

Products in groups one, two and three are made from anticoagulated, whole blood in multiple steps. L-PRF products of group four are prepared in two steps without extra chemicals. L-PRF is isolated manually from the fibrin clot that forms after instant centrifugation at a low speed in whole blood where 'most platelet aggregates and leukocytes are concentrated within the end of the PRF clot, close to the border with the base of red blood cells. The way the clot is separated considerably influences the final biologic content of the PRF'.[197] In other words, L-PRF will vary in composition depending on the individual preparing the product.

In general, PRP/PRFs is obtainable by a blood sample of 20–140cc depending on the procedure used. All systems provide platelets that release growth factors, including PDGF, TGF-β, VEGF, IGF-1, FGF, and EGF, thus promoting tissue repair, modulating inflammatory processes and neoangiogenesis and, ultimately, regulation of tissue homeostasis and regenerative processes.

Easily obtainable by the patient without morbidity, PRP/PRFs has recently found employment also in aesthetics in order to treat aging skin and in the trichological field in cases of alopecia. Finally, it is increasingly associated with autologous and

lipofilling grafts, in order to favour its intake and differentiation. It can also be used in the presence of skin substitutes.

5. The autologous leucocyte and platelet-rich fibrin patch (APFP) is a newer advanced therapy without chemical additives. As such, it belongs to the 4th group described above. The APFP, however, has a layered structure and is produced mechanically by use of the 3CP procedure, via a single use closed sterile device. The bedside production is performed in three steps: 1) blood is drawn by venepuncture into a sterile vacuumed device in a process identical to normal blood sampling; 2) the device is positioned in a specially designed centrifuge insert and spun in an automated two-step process at the bedside; and 3) the device is opened and the formed patch is transferred directly to the wound of the patient. The process takes approximately 20 minutes of which the hands-on time is 2–3 minutes including the drawing of the blood.[198]

Platelets and leukocytes are concentrated by 8–18 times as compared with the total quantity of blood.

The APFP is a three-layered patch composed of: 1) a polymerised and cross-linked fibrin matrix; 2) a layer of compacted platelets; and 3) a layer of concentrated leukocytes on the lower surface. In Figure 5, the three-layered structure and the leukocytic accumulation at the surface of this dressing is shown.

Extract analyses of the APFP have shown that high levels of growth factors are released continuously from the patch for up to a week. The addition of chronic wound fluid increased the speed of the growth factor release, and this feature may be relevant in the treatment of chronic wounds.

When comparing the levels of selected growth

Fig 4: Venous ulcer: PRP and autologous skin graft

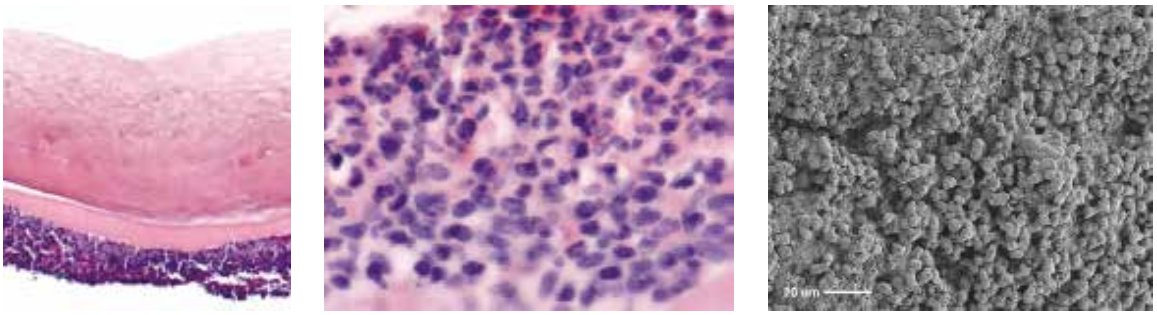


Fig 5: The physical structure of the the autologous leucocyte and platelet-rich fibrin patch (APFP)[199]

factors and cytokines in the APFP to P-PRP generated by standard procedures, higher levels of the platelet-derived growth factors by a factor of three and 10, for PDGF-AB and VEGF, respectively, were found. The leukocyte-derived cytokine IL-8 is more than 280 times higher in the APFP, which demonstrates a clear difference from P-PRP. *In vitro* studies in the culturing of fibroblasts and keratinocytes in the presence of these dressings have shown an enhancing effect on both cell growth and migration of these cell types in response to the APFP.[199] Furthermore, studies done with *Pseudomonas aeruginosa* cultures have shown the ability of these cells to phagocytise and kill bacteria.[200]

Recently, a number of preclinical and clinical studies have been performed to test the safety and effectiveness of the APFP with generally positive results.[201,202] More information is expected from a large multi-centre trial that was conducted in Europe but has not yet been published. No RCT has been published to date.

Clinical evidence for platelet-derived products in wound care

Only a near-physiological concentration P-PRP gel has been tested in a properly conducted, RCTs)

on DFUs. Despite the fact that no statistically significant improvement of healing could be demonstrated in this RCT, the gel was cleared by the FDA for wound management.[203] Meta-analyses of small-sized trials on platelet products indicated a positive effect on the healing of DFUs, and a retrospective analysis of a US database indicated an effect of platelet releasate in healing DFUs.[204–206]

This analysis may be biased due to the differences in treatment regimens among the included trials. This is one reason why neither the National Institute for Health and Care Excellence (NICE) of England nor the Center for Medicare Services (CMS) of the US have recommended nor reimbursed these products for routine use in wound treatment.[207,208]

Other cell therapies

Advanced cell therapies

Defined as new medical products based on genes, cells and tissues, the 'advanced cell therapies' can be used to promote wound healing also in recalcitrant wounds. Nowadays, some novel and very promising cell therapies have been developed.

- The use of safe food-grade lactic acid bacteria

In order to stimulate a 'personalised' production of therapeutically active proteins within the damaged tissues, such as in chronic wounds, it is now possible to use genetically modified lactic acid bacteria as a delivery method. The modified bacteria serve as a local bioreactor in the wounds by producing and secreting certain proteins, such

as FGF-2, IL-4 and CSF-1, which are known to promote wound healing. This approach enables continuous exposure to these therapeutic factors and by producing more than only one factor, these bacteria are able to address several aspects of the aberrant wound healing at the same time, such as fibroblast/keratinocyte proliferation, angiogenesis and anti-inflammation. This method can modulate the local immune system by switching the production of therapeutic proteins on and off directly in the damaged tissues. The method is safe, cost-effective and easy-to-apply. It can be used for ulcers from different aetiologies and also for cancer treatment.[198,200,201,209,210]

Placental-based allografts

Use of amniotic tissue allografts has been cited in clinical literature for over 100 years. Placental-based allografts have surfaced as an effective allograft option for the treatment of chronic ulcerations. Placental-based allografts are derived from multiple tissue types collected from the afterbirth post-delivery of a live baby. These tissue sources include the amniotic sac, the umbilical cord and the placental itself (Fig 6). After placental-based allografts have been processed, they can be configured into many different forms, such as sheets grafts, tissue morsels or 'mini' grafts, and powders or 'micro' grafts. Each configuration has a specific utility. The sheets can cover large areas with minimal clinician effort, morselised tissue can be used to pack tissue voids, and powders can used as a paste or to inject directly into soft tissue.

Modern processing techniques for placental-based allograft membranes have been developed to improve storage and availability of these tissues. For example, dehydration has been used to support storage of allografts in ambient conditions. While dehydrated amniotic tissues do not contain viable cells, the cellular components and regulatory proteins are preserved within the tissues. This diverse array of regulatory proteins that are naturally found within amniotic tissues are able to modulate the activity of endogenous cells, including the patients' own population of stem cells, to promote healing and reduce scar tissue formation. Additionally, improvements in donor screening, aseptic processing, and terminal sterilisation have significantly reduced the risk of disease transmission by allograft tissues. These allografts are available in many forms — cryopreserved, lyophilised, and dehydrated, and are widely available in most countries in Europe from local tissue banks or imported from third country tissue banks.

The ability to effectively screen donors and tissues coupled with new methods to cleanse, preserve, and sterilise placental based allografts have facilitated a dramatic increase in their use over the past few years. A proprietary process utilised for dHACM (a placental-based allograft consisting of a dehy-drated human amnion/chorion membrane) composite allograft[211–217] combines the amnion layer with the chorion layer. There have been 285 regulatory proteins identified in dHACM, including growth factors, specialised cytokines and enzyme inhibitors,[211–218] which deliver clinically relevant bioactive factors and inflammatory mediators to assist in the healing process of acute and chronic wounds.[218] Another proprietary process allows for the preservation of the spongy layer between the amnion and chorion, which has been shown to contain high levels of proteoglycans, glycoproteins and hyaluronic acid as well as a high level of growth factors.[219]

Notably, recent multiple, randomised clinical studies evaluating the treatment of diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs) have been published in peer-reviewed literature on the use of placental allografts (dHCAM), which have demonstrated clinical superiority over the standard of care for the treatment of DFUs[220–222] and for the treatment of VLUs.[223,224]

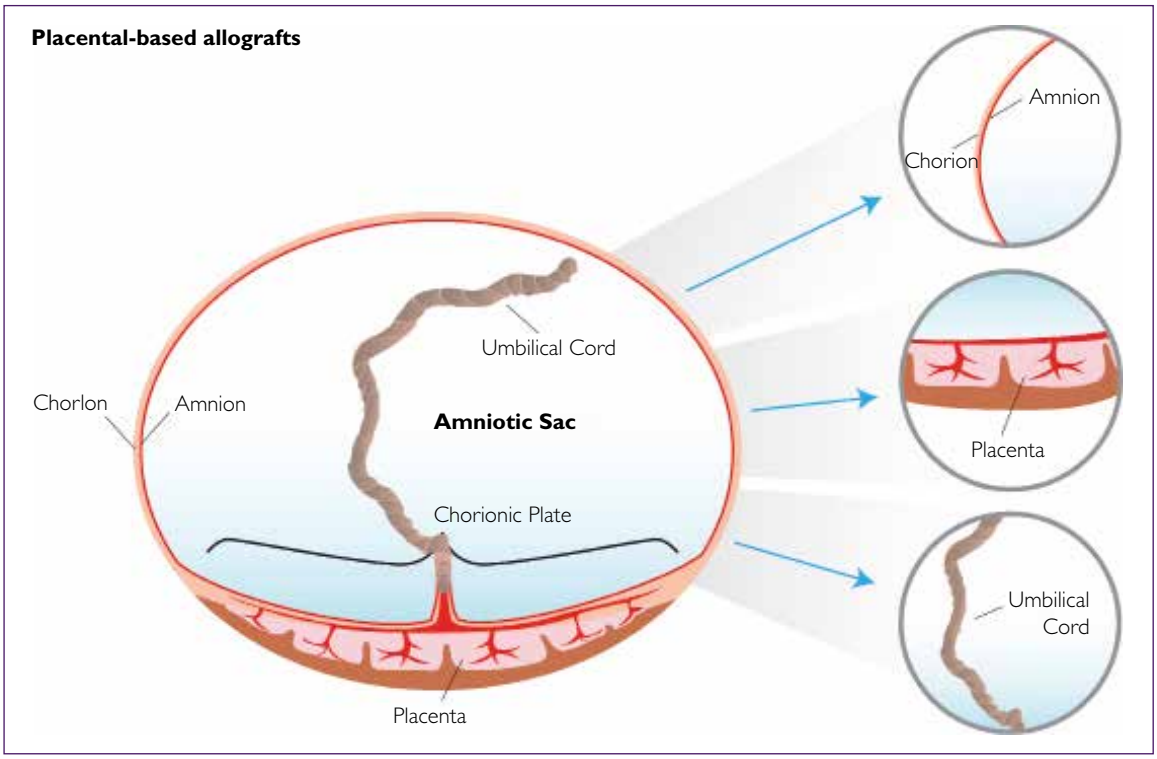


Fig 6: The donated afterbirth obtained from consenting mothers undergoing caesarean section delivery is processed to yield three major tissue allografts, which are the amniotic sac consisting of amnion/chorion, the placenta and the umbilical cord

A randomised and parallel group trial was implemented at eight clinical sites in which patients with DFU received either standard of care (foam dressing) (n = 14) or a dehydrated amniotic membrane (n = 15) until wound closure or six weeks, the first to occur. This showed complete wound closure in 35% and 45.5% of the patients in the experimental group, respectively, in the intent-to-treat (p=0.017 in relation to 0% of standard of care) and per a group population (p=0.0083 in relation to 0% of standard of care).[225]

The efficacy of a dehydrated amniotic membrane as an adjuvant to multilayer compression therapy for the treatment of non-healing full-thickness venous leg ulcers was addressed in a multicentre RCT. The 109 patients were assigned to placental-based allograft plus a compression group (n=52) or a compression therapy alone (n=57). Participants receiving weekly application of the placental-based allograft plus compression were more likely to experience complete wound healing (60% versus 35% at 12 weeks, p=0·0128, and 71% versus 44% at 16 weeks, p=0·0065) and a significantly improved time of healing using the allograft (log-rank p=0·0110), as seen in Table 11.[226]

A multicentre trial also compared the healing effectiveness of a placental-based allograft with a live skin tissue substitute or with an alginate dressing (n=60, 20 per group). The respective proportion of patients that had complete wound closure at four and six weeks was 85% and 95% (p≤ 0·003), as compared to patients receiving standard of care 35% and 45%, or 30% and 35%. Similarly, the respective median time for healing was 13 days

Fig 7: A recalcitrant venous leg ulcer which showed insufficient healing tendency since >10 years (a) was treated with a dehydrated human amnion-chorion membrane (dHACM), resulting in immediate pain reduction and rapid healing after 4 (b) and 10 (c) weeks

(p≤0·001), compared to live skin tissue substitute (49 days) or standard of care (49 days).[220] This study was continued in order to address the rates and the time for closure at a longer time interval, by including at least 100 patients. The proportion of patients achieving complete closure within the 12-week study period were 97% (31/32), 73% (24/33) or 51% (18/35) (p=0.00019). Mean time-to-heal within 12 weeks was 23.6 days (95% CI: 17.0–30.2), 47.9 days (95% CI: 38.2–57.7 or 57.4 days (95%CI: 48.2–66.6) ($p=3.2 \times 10^{-7}$), respectively, as seen in Table 9.[227]

Cultured tissue-based therapies

In this chapter, therapies based on cultured cells and their application as tissue-engineered or bio-engineered skin substitutes are highlighted.[228–232] The use of non-cultured or cultured cells in sus-pension and acellular materials have been described in the previous chapters.

Bioengineered skin substitutes are composed of skin cells or living cells and extracellular matrix components. Over the last 30 years, the industry has presented a large number of skin substitutes that can be applied essentially for two purposes, which are: 1) experimental, such as cellular permeability models or toxicological screening and 2) clinical, as actual skin substitutes bases for autologous grafts or for delivering growth.

Most of the world's need for these substitutes is due to a demand for materials for clinical purposes. It is expected that in 2019 at least 6.4 million people will need a cutaneous substitute.[188,231,233–240]

Skin substitutes for *in vitro* application

These substitutes are used as models to study tissue-healing processes and can also be considered for testing the skin toxicity of chemicals as well as drug permeability (Table 10). The possibility to obtain a correct differentiation of the epidermal layers is fundamental, and a support that works as a skin barrier comparable to the natural barrier in its properties should be obtained.

Table 9: Randomised controlled trials evaluating wound dressings and placental-based allograft efficiency for the treatment of DFU,VLU and mixed aetiology wounds

Author	Year	Type of material	No. of patients	Compared conditions	Follow-up (days)	Results	Ulcer type
Snyder et al.[225]	2106	Non-viable cellular matrices	29	Dehydrated amniotic membrane vs foam dressing	42	• Complete wound closure in 35% of the patients (p=0.017 in relation to 0% for standard of care in the intent-to-treat group • Complete wound closure in 45.5% of the patients (p=0.0083 in relation to 0% for the standard of care) in the per group population	DFU
Zelen et al.[220,227]	2015	Non-viable cellular matrices	100	Dehydrated amnion/chorion membrane vs Live skin substitute vs alginate dressing	Up to 84	• Higher proportion of patients with closed wounds at 4, 6 and 12 weeks (85%, 95% (p≤ 0·003, 97 (p=00019)) vs 35%, 45%, 73% vs 30%, 35%, 51% • Lower mean time to healing within 12 weeks 23.6 days (95% CI: 17.0-30.2) vs 47.9 days (95% CI: 38.2-57.7 vs 57.4 days (95%CI: 48.2-66.6) ($p=3.2x10^{-7}$)	DFU
Bianchi et al.[226]	2017	Non-viable Cellular Matrices	109	Dehydrated amnion/chorion membrane plus compression vs compression	112	Higher probability to complete healing (60% vs 35% at 12 weeks, p=0.0128, and 71% vs 44% at 16 weeks, p=0.0065)	VLU and mixed aetiology

Skin substitutes for *in vivo* application

In general, living cellularised skin substitutes can be divided into the following categories: 1) epidermal, 2) dermal, and 3) bilayered or dermo-epidermal substitutes (Table 11). Further, substitutes can contain autologous (patient's own) or allogenic (from other humans) cells. The skin substitutes that are clinically applied can be permanent remaining on the patient or temporary. The temporary skin grafts need to be replaced or modified by additional techniques at a certain time after application.

Skin substitutes for *in vivo* application are not applicable in the case of infected wounds.

History of tissue-based therapies

One of the main developments in the field of (cellular) skin substitutes was the introduction of cultured epithelial autografts (CEA) in 1975. Rheinwald and Green managed to culture primary epidermal cells that they isolated from human skin samples on a so-called feeder layer of lethally irradiated fibroblasts.[242] They could grow and expand the keratinocytes in serial cultures that made it possible to prepare keratinocyte sheets or CEAs. Although they were already clinically applied onto small burn wounds in the 1980s,[243] the breakthrough for CEAs was in 1983 when two siblings were treated after life-threating large burns with the culture keratinocyte sheets as compassionate therapy.[244] Since they survived because of the CEA application, epidermal sheets have been used ever since for clinical applications.

Regarding dermal substitutes, the first clinically usable acellular dermal grafts were available in the mid-1980s. They consisted mainly of a porous collagen type I matrix.[131] Subsequently, cellularised dermal substitutes evolved from this. They are mostly used as temporary biological dressings in chronic wounds to stimulate wound healing as they contain allogenic fibroblasts.

Once it was realised that a matrix or scaffold provides not only mechanical stability but in addition provides good biological properties by resembling more the normal extracellular microenvironment, the use of collagen for skin substitutes was intensified. In the 1980s, based on a porous collagen type I matrix, the first attempts began to incorporate not only fibroblasts into the porous collagen (the dermis) but also to add keratinocytes onto the fibroblast-populated dermis. This resulted in autologous dermo-epidermal skin substitutes that were first clinically applied in the late 1980s.[246,247]

Further, in another approach, allogenic fibroblasts were mixed with collagen, and after additional days of dermal maturation, allogenic keratinocytes were also seeded onto the formed dermis.[248] These allogenic dermo-epidermal skin substitutes have

Table 10: Skin substitutes for *in vitro* application

Name	Cell involved
Epidermal Skin Test 1000	Human keratinocytes: epidermal model with fully differentiated epidermidis
Advanced Skin Test 2000	Full thickness model with fibroblasts and keratinocytes
Epiderm	Neonatal human-derived epidermal keratinocytes
EpidermFT	Neonatal human-derived epidermal keratinocytes and fibroblasts
Episkin	Human keratinocytes on a collagen base
StrataTest	Skin model from a near diploid keratinocytes cell line
SkinEthic Reconstructed Human Epidermis	Human keratinocytes on a polycarbonate filter in medium

Table 11: Skin substitutes for *in vivo* application

Type	Features
Epidermal	From a small superficial autologous skin biopsy (2-5 cm^2): keratinocytes are isolated, cultured and applied onto a supportive layer
Dermal	Allogenic human fibroblasts are cultured onto scaffolds
Dermo-Epidermal	Composed of autologous or allogenic epidermidis and dermis with the presence of keratinocytes and fibroblasts seeded on extracellular matrix (for example, Collagen type I).

Box 1: Tissue engineering

Definition by Robert S. Langer and Joseph P. Vacanti: 'An interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve [biological tissue] function or a whole organ'.[241]

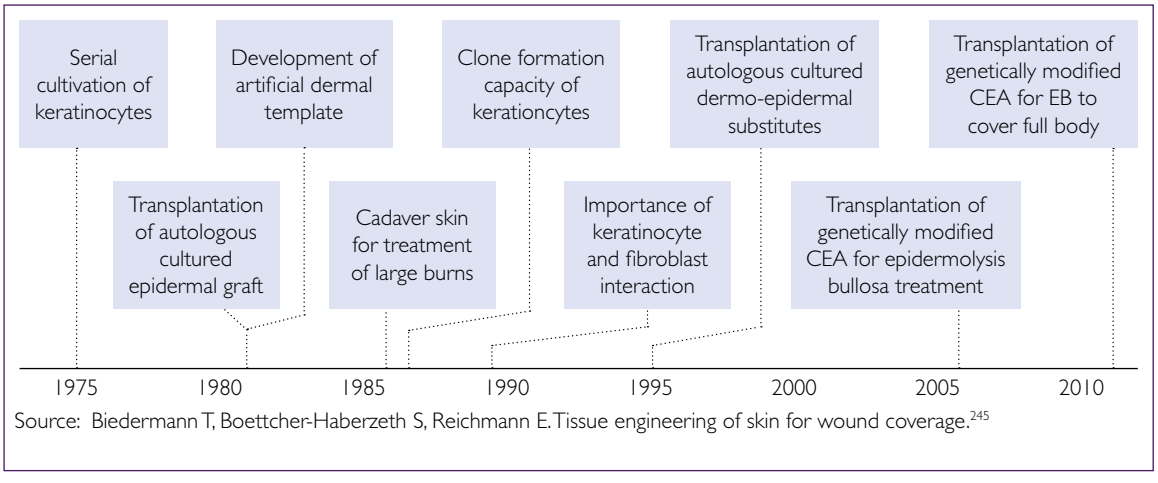
been used clinically for chronic wounds since the 1990s.[249]

In general, although dermo-epidermal skin substitutes resemble normal skin, they still lack skin appendages, such as hair follicles or sweat glands.[250] However, in many cases, the mechanism of ac-tion of skin substitutes in wound healing is not to replace the skin, but it is to deliver growth factors and therefore change the wound environment from chronic to acute.

Tissue-based therapies today

Epidermal substitutes (CEAs)

Today, several commercial suppliers provide epidermal substitutes for clinical use.[239] Most of the cultured epithelial autografts (CEA) are still prepared according to the technique developed by Rheinwald and Green. A small split-thickness skin biopsy or hairs from the eyebrows/scalp are taken from the patient, and keratinocytes are isolated and cultured in the presence of so-called feeder cells. The keratinocytes are propagated to result in some layers representing the epidermis. For better handling properties, as the epidermal sheets are very fragile and thin, they are then applied onto supportive materials (Figure 9, Table 12). Various approaches have been employed for supporting layers such as culturing the keratinocytes on a layer containing inactivated mouse fibroblasts or a membrane of hyaluronic acid which is perforated by laser. Keratinocytes have also been delivered as a spray.



Source: Biedermann T, Boettcher-Haberzeth S, Reichmann E. Tissue engineering of skin for wound coverage.[245]

Fig 8: Timeline of developments of dermal and skin substitutes[245]

As they have been applied clinically, a number of studies have been published regarding the out-comes of CEAs for chronic wounds.[251–253] In particular, an epidermal skin substitute based on au-tologous cultured outer root sheath cells was commercially available in some European countries for a few years.[254,255] This cell type was chosen for the cultures as it displays a high proliferative potential even in elderly persons, who are the largest population with chronic wounds. A prospective randomised trial involving 77 patients with recalcitrant leg ulcers showed an equivalence of this skin substitute with autologous split thickness skin graft with regards to healing time and the number of healed ulcers after 12 and 24 weeks.[256] Due to the logistics and cost of the product, it is currently not commercially available anymore.

Another approach to treat diabetic neuropathic foot ulcers used autologous keratinocytes isolated from split-thickness skin biopsies from the patient's thigh. The isolated basal keratinocytes were cultured and then applied onto a medical grade PVC carrier for clinical handling. Based on the twelve included patients, five displayed complete healing. The remaining patients showed a reduction of the ulcer's size of at least 50%. Although between five to 12 applications of the autologous keratinocytes were needed, the outcome was much more beneficial compared to the control group that was treated with acellular carriers.[257]

Dermal substitutes

Beside the use of pure epidermal substitutes, the application of cellularised dermal substitutes is also used. Human (allogenic) fibroblasts are cultured onto or into supportive materials, such as bioabsorbable scaffolds (Fig 11, Table 13). The dermal substitutes should stimulate wound healing responses as fibroblasts deposit extracellular matrix

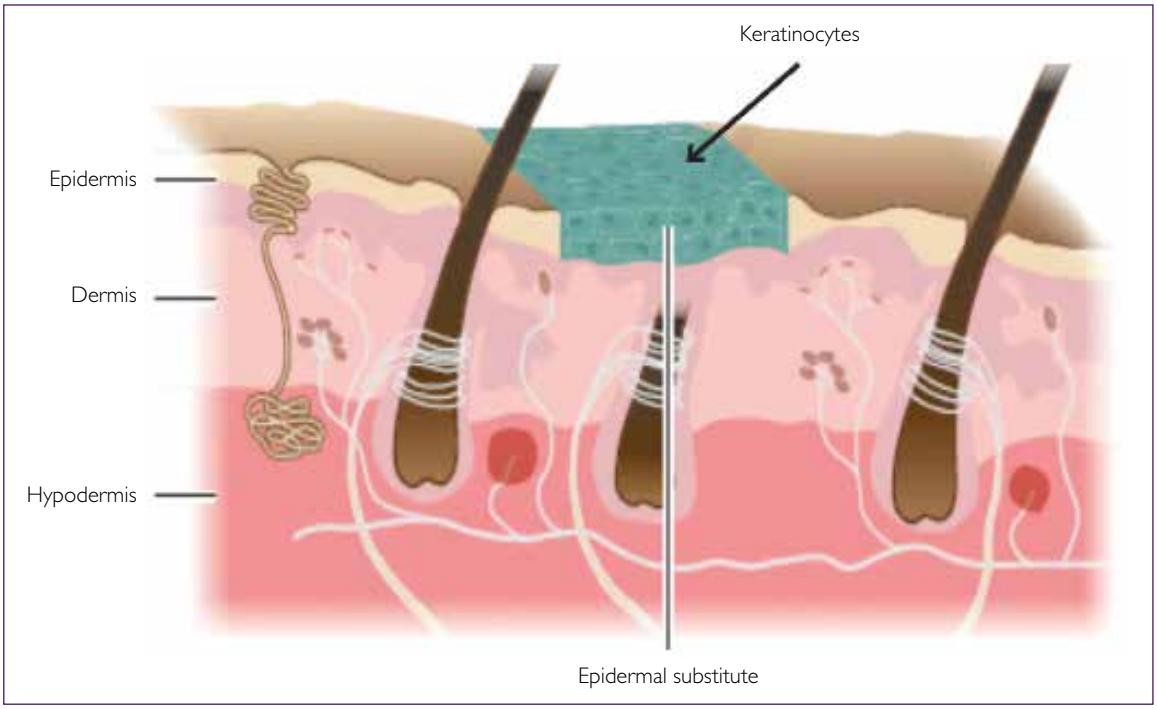


Fig 9: Application of an epidermal substitute (CEA)[228] Source: Berthiaume F, Maguire TJ, Yarmush ML. Tissue engineering and regenerative medicine: history, progress, and challenges. Annu Rev Chem Biomol Eng 2011; 2:403–30

Table 12: Epidermal substitutes

Product (company)	Description	Indications	Approval
Epicel (Genzyme Corp.)	Cultured epidermal autograft: Autologous keratinocytes are cultured in the presence of murine fibroblasts to form cultured epidermal autografts. These are processed into sheets and attached to gauze.	Burn wounds	FDA
Epidex (Euroderm GmbH)	Cultured epidermal autograft: Autologous outer root sheet hair follicle cells are cultured to form epidermal equivalents. These are attached to silicone membranes and can be placed onto the wound bed.	Venous ulcers Diabetic ulcers	Currently under evaluation by SwissMedic
MySkin (Altrika Ltd.)	Cultured epidermal autograft: Autologous keratinocytes are grown in the presence of irradiated murine fibroblasts. It is supplied as a circular disk for application.	Burn wounds Venous ulcers Diabetic ulcers	MHRA (United Kingdom)
Abbreviation: FDA: Food and Drug Administration; MHRA: Medicines and Healthcare Products Regulatory Agency			

proteins and secrete growth/angiogenic factors if applied onto wounds. They show great mechanical stability and might prevent scar contraction.

An allogenic dermal substitute using cultured neonatal human dermal fibroblasts was investigated for patients with diabetic foot ulcers. The neonatal fibroblasts were seeded onto a bioabsorbable polyglactin mesh scaffold and produced a three-dimensional matrix containing several types of collagen. Patients (n=130) were treated with the dermal substitute if the ulcer had not decreased in size by 50% after two weeks of standard therapy. A weekly application for a maximum of eight weeks, if necessary, was performed. The trial revealed a significant increase in healed wounds using the dermal substitute compared to the control group (n=115) after twelve weeks. This study showed that complete wound closure was achieved significantly faster with the group.[258]

Interestingly, another survey suggested that the use of the dermal substitute resulted in a moderate

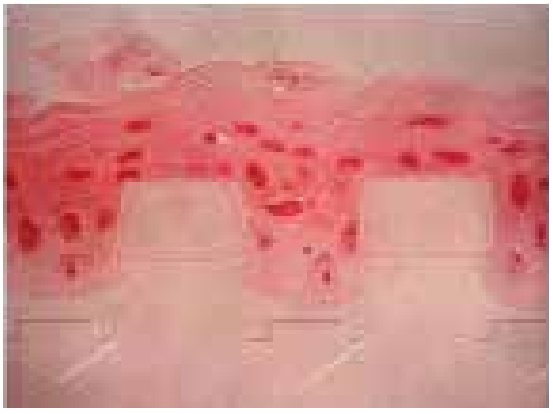
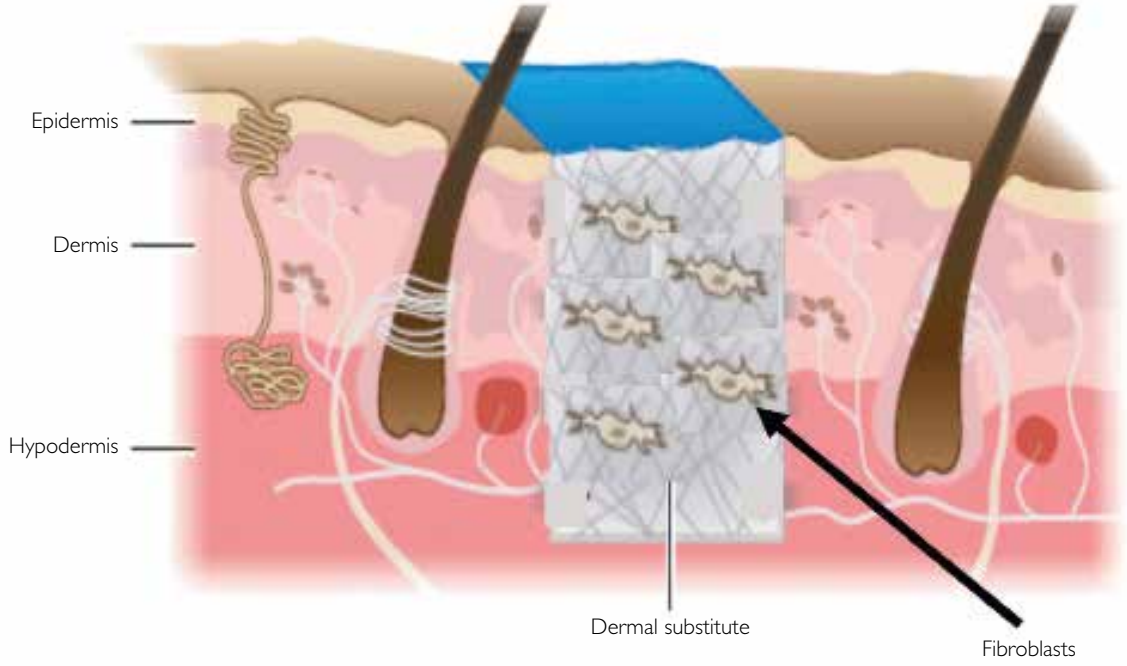


Fig 10: Autologous Keratinocytes on a membrane of hyaluronic acid, perforated with laser

reduction of all types of amputation (below the knee, foot, toe) and the necessity of bone resections as compared to standard care.[259]

Furthermore, a clinical trial using this dermal substitute for venous leg ulcers was described as well.[260] The allogenic substitute plus four-layer compression therapy (n=186) was compared with compression therapy (n=180) alone. The VLU



Source: Berthiaume F, Maguire TJ, Yarmush ML. Tissue engineering and regenerative medicine: history, progress, and challenges. Annu Rev Chem Biomol Eng 2011; 2:403–30

Fig 11: Application of a dermal substitute[228]

Table 13: Commercially available dermal skin substitutes

Product (company)	Description	Indications	Approval
Dermagraft	Cellular dermal substitute, bioabsorbable polyglactin mesh scaffold seeded with cultured allogeneic neonatal fibroblasts from neonatal foreskins	Diabetic ulcers	FDA – PMA approved

was present between the knee and ankle for at least two months and a maximum five years for the included patients. The trial described that the dermal substitute is comparable to standard therapy in regard to safety. Furthermore, the cellular substitute did not reveal a statistically significant improvement compared to control compression therapy for overall healing by week twelve. By week 24, 96 (52%) of the 186 patients from the dermal substitute group and 88 (49%) of the 180 patients from the compression therapy group achieved complete healing. In general, a better healing effect of the dermal skin substitute was observed in venous leg ulcers of 12 months' duration or less, but not in those of with over 12 months' duration.

Nevertheless, although the results for DFU patients were encouraging, other reports pointed out the

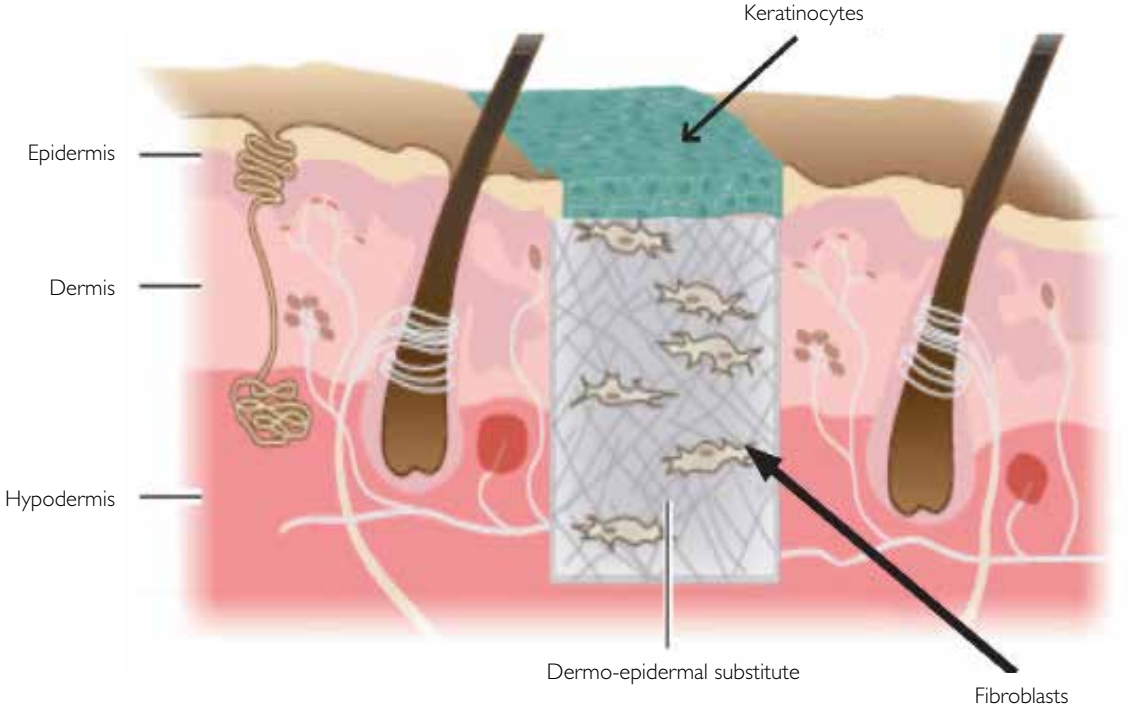
high costs of this dermal cellular substitute when compared with the use of non-cellular dermal substitutes, which result in similar outcomes.[261]

Dermo-epidermal Substitutes

These are characterised by the presence of keratinocytes and fibroblasts. Today, almost only allogenic dermo-epidermal skin substitutes are applied clinically on a regular basis.[262] These allogenic skin grafts are produced from cells collected from skin biopsies or from neonatal foreskin by healthy donors, which can be stored for a certain period until their delivery and use. This procedure reduces the costs for the production of such sophisticated skin substitutes (Figure 12, Table 14). Also, keratinocytes and fibroblasts can be seeded in a spongy matrix containing collagen. A skin substitute can fulfil an additional role in cases of large leakage of the skin, such as significant traumas or burns.[246,247]

The manufacturing of autologous dermo-epidermal skin grafts containing patients' own fibroblasts and keratinocytes is more expensive as they are produced and used only for one person. Today, several autologous skin substitutes are applied in clinical trials (phases I – IV), but they are not commercially available on a regular basis yet.[263,264]

The use of dermo-epidermal skin substitutes, particularly allogenic substitutes, and their effects on the healing of chronic wounds has been documented in a large number of peer-reviewed studies.[265,266] Two pivotal trials led to FDA



Source: Berthiaume F, Maguire TJ, Yarmush ML. Tissue engineering and regenerative medicine: history, progress, and challenges. Annu Rev Chem Biomol Eng 2011; 2:403–30

Fig 12: Application of a dermo-epidermal substitute[228]

approval in 1998 of a bilayered dermo-epidermal skin substitute produced from cultures of neonatal foreskin on a bovine collagen matrix for the stimulation of wound healing in venous leg ulcers and diabetic foot ulcers. In 2008, the product was approved as a transplantation product and used in clinical practice in Switzerland. A RCT involving 240 patients with venous leg ulcers resistant to previous conservative treatment for at least three months showed ulcer healing at 24 weeks in 57% of patients versus 40% in the control group (p=0.022). Of note, the effects were greater in a subgroup analysis of recalcitrant ulcers showing no healing progress for over one year (47% healing after 24 weeks versus 19% in the control group, p>0.005).[267] In a study with 208 patients with diabetic foot ulcers, weekly application of the bilayered skin substitute led to wound closure in 53% of patients after 12 weeks, compared to 38% of patients treated with moist gauze.[266]

Since then, more than 250 peer-reviewed studies have been published that show successful stimulation of wound healing. The mode of action of this allogenic skin substitute has not been fully elucidated. Per Stone et al., the application of a bilayered skin substitute changes the wound dynamics from chronic to acute.[250] A range of cytokines and growth factors that are present in the wound after its application have been demonstrated, but the product does not remain on the wound for the long-term. It has been shown in vitro that the wound healing microenvironment of chronic wounds after the application of this skin substitute resembles more closely the wound milieu of acute wounds.[250] Although the absolute reduction of time for healing with this skin substitute in the published studies was limited, it is one of the few therapeutic interventions for chronic wounds that has been shown to improve wound healing in a large number of prospective RCTs. Therefore, it is an interesting therapeutic option for hard-to-heal wounds. Data comparing its efficacy with autologous split thickness skin graft would strengthen the basis for its clinical use.

Future outlook

Melanocytes, vessels, genetic manipulation

The major drawback of all the above described cellular tissue-engineered skin substitutes is the lack of other main skin components or main cell types besides keratinocytes and fibroblasts. All commercially available products are free of other cellular components, for instance pigment producing melanocytes, or immunoregulatory Langerhans cells, and structures such as hair follicles, sebaceous and sweat glands, nerves, lymphatic and blood capillaries/vessels, and lack a hypodermis, the fat, as well.[268–271]

Research is currently ongoing in this field, regarding the integration of melanocytes, fat, and hair follicles, especially for large wounds (e.g. burn wounds). Regarding small and chronic

Table 14: Commercially available dermo-epidermal skin substitutes.[228]

Product (company)	Description	Indications	Approval
Apligraf (Organogenesis Inc.)	Cultured allogeneic dermo-epidermal substitute. A bovine collagen type I matrix seeded with allogeneic keratinocytes and fibroblasts cultured from neonatal foreskins.	Venous ulcers Diabetic ulcers	FDA/PMA approved for VLUs and DFUs
Abbreviation: FDA, Food and Drug Administration			
Source: Berthiaume F, Maguire TJ, Yarmush ML. Tissue engineering and regenerative medicine: history, progress, and challenges. Annu Rev Chem Biomol Eng 2011; 2:403–30			

Fig 13: Application of a dermo-epidermal skin substitute on an extremely recalcitrant leg ulcer which showed no healing tendency with conventional therapies. Presentation at the treatment start (a) and healing progress after 2 (b) and 8 (c) weeks

wounds, research currently focuses on exaggerating vascularisation of the skin substitutes. A faster and better vascularisation supports ingrowth

Fig 14: Dermoepidermal substitute derived by a co-culture of keratinocytes and fibroblasts

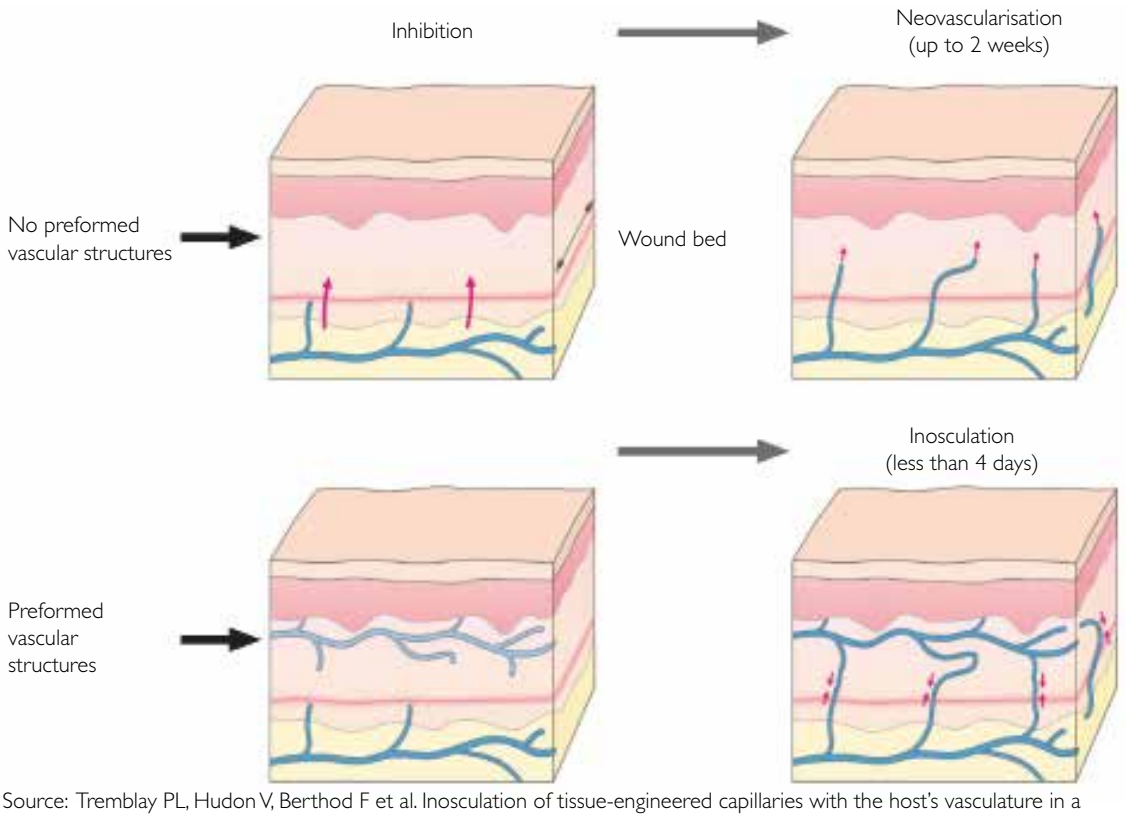
of the grafts and enhances wound healing, in general. Different strategies have been investigated. One approach is to tissue-engineer preformed (branched) capillaries in the skin substitutes *in vitro*. This is based on the concept of full-thickness skin transplantation, which contains vessels and capillaries rapidly connecting to the vascular structures already present in the wound bed. This process is known as inosculation, which results

in a faster blood supply and a greater likelihood of survival for the full-thickness skin transplant (Fig 15).

There is also interest in genetically manipulating cells, such as fibroblasts or keratinocytes. This idea is based on a diagnosis of bacterial contamination of the wound or molecular deficiency of the patients' tissue resulting in non-healing wounds. According to the results and needs, different strategies to manipulate cells *in vitro* could be employed. The cells could then overexpress factors increasing an inflammatory response, angiogenic factors, such as vascular endothelial growth factor (VEGF), or factors enhancing re-epithelialisation, such as platelet-derived growth factor (PDGF). The so-manipulated cells could be applied in the above-described manner, similar to the method use for CEAs or cellular dermal substitutes.

Automation

The tissue-engineered skin grafts mentioned before display several limitations. In particular, they are all still almost completely manually produced by well-trained and experienced persons. Therefore, the production of such skin substitutes is time-



Source: Tremblay PL, Hudon V, Berthod F et al. Inosculation of tissue-engineered capillaries with the host's vasculature in a reconstructed skin transplanted on mice. Am J Transplant 2005; 5(5):1002–10. Skin substitutes without vascular structures are nourished by diffusion until the vessels and capillaries slowly grow from the wound bed to provide blood supply. In full-thickness skin transplants or skin substitutes containing existing or preformed vascular structures, capillaries connect to vascular structures from the wound bed quickly, nourishing the transplant rapidly with sufficient blood supply.

Fig 15: Concept of inosculation[271]

consuming, labour intensive, bears a risk of contamination and are not perfectly reproducible, which influences the quality and are costly.

Automation of the production of skin grafts, or at least parts of the production, could allow for higher reproducibility, better safety, larger-scale production, and higher efficacy. A complete automation to produce three-dimensional (3D) skin, containing epidermis, dermis, and even hypodermis, requires a bio-reactor essential for gas concentrations, nutrient exchange, pH, and temperature in order to culture different cells types and a compartment in which to eventually generate the skin.

In general, the complete system needs to include sensors/surveillance to record and analyse the complete production process to fulfil the criteria created by authorities for clinical application in health care.

Today, the technology of 3D-printing offers, besides the well-known fields of automotive or aerospace, the possibility to print medical devices that can be used clinically. As an example, patients displaying craniofacial bone defects can benefit as medical scaffolds are custom-made printed that perfectly fit in place to reconstruct the bone defects. 3D-printing has emerged not only as a useful potential tool to fabricate acellular but also cellular structures. Hence, 3D bioprinting became a tremendous area of research in tissue-engineering and regenerative medicine.[272–275]

The material for the bioprinting process needs to be suitable for printing technology, biocompatible, support cellular viability, growth and function, and thereby provide structural and mechanical properties. So far, natural materials for bioprinting include substances such as collagen, fibrin, alginate, laminin, hyaluronic acid, gelatine, chitosan and fibronectin. On the other hand, synthetic materials, such as modified copolymers and acrylates, are also a potential option.

Three major bioprinting techniques based on different principles are used, namely inkjet, extrusion, and laser-assisted (Fig 16).

The inkjet printers are based on the known 2D printers that are used to print ink onto paper for a document. An electronically controlled elevator stage was introduced to control the third dimension, the z-axis. Thermal or acoustic forces are used to eject controlled volumes of liquid droplets out of the print head onto a certain substrate to a predefined location.

Extrusion bioprinters deposit, via an extrusion head, continuous beads of material onto a substrate. Directed robotically by CAD-CAM software, beads of material are deposited in two dimensions. The extrusion head is then moved along the z-axis, whereas the already deposited two dimensions layer serves as a foundation for the third-dimensional layer.

The less common laser-assisted bioprinting is based on principles of laser-induced forward transfer. Focused laser pulses are directed onto an absorbing layer of a ribbon to generate high-pressure bubbles that propel cell-containing materials towards a collector substrate.

Of course, each technique has advantages and disadvantages with respect to its automation, printing and resolution capabilities, precision in ejection and deposition, compactness and scalability.

A general challenge is to produce an architecture that at least resembles extracellular matrix (ECM) components so that skin cells can recapitulate their biological function. With the major techniques, several approaches were already performed

Box 2

Biofabrication can be defined as "the automated generation of biologically functional products with structural organisation from living cells, bioactive molecules, biomaterials, cell aggregates such as microtissues, or hybrid cell-material constructs through bioprinting or bioassembly and subsequent tissue maturation processes," according to the International Society for Biofabrication (ISBF).[276]

to bioprint skin for preclinical investigations, including keratinocytes, melanocytes, fibroblasts and endothelial cells.

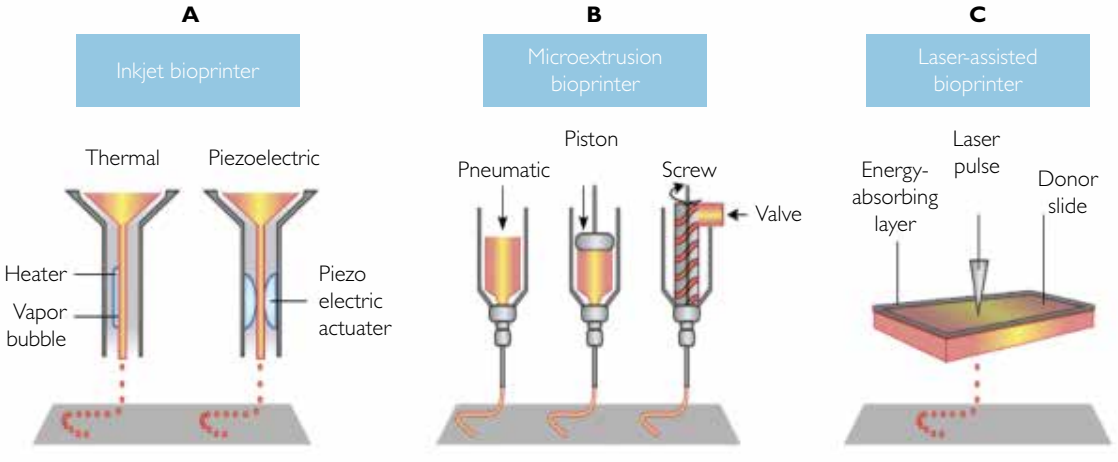
An alternative approach to the classical idea of bioprinting *in vitro*/*ex vivo* and subsequent transplantation onto a patient is the idea of bioprinting directly *in situ*. Cells and ink/materials are directly printed into the wound of a patient in this approach. This might be feasible, and preclinical research in this field is ongoing. It might become clinical reality to bioprint immediately after an injury or during surgery.

Taken together, although automation of skin substitutes for clinical applications is still not practicable, automation can result in fabricating large-scale effective and highly sophisticated therapeutic skin grafts for patients in the near future.

Algorithm for the use of cell therapies

As advanced dressings are usually associated with high costs, their use has to follow strict indication criteria. However, in the case of 'hard-to-heal' wounds, it can be argued that costs may be reduced with earlier use of advanced products.

It is therefore important to recognise early, when a wound is not proceeding through the regular wound healing phases and will eventually become a candidate for an advanced wound healing protocol. For this, it is useful to adopt surrogate markers: it was shown that healing of DFU after 12 weeks is unlikely, if the wound surface is not reduced by 50% after 4 weeks of appropriate moist wound treatment and proper off-loading.[278] Likewise, it was shown for Venous leg ulcers that healing after 24 weeks is unlikely if there is less than 40% wound area reduction after 4 weeks.[7–9]



Source: Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol 2014; 32(8):773–85. https://doi.org/10.1038/nbt.2958

Figure 16: Schematic components of inkjet, extrusion and laser-assisted bioprinters[277]

Table 15: Evaluation of evidence levels: cells and tissues

No.	Therapy	Indication for use	Level of evidence*	Comments
1	Mesenchymal stem cells	Acute wounds (such as burns)	1A	High-quality studies and good evidence of effectiveness and safety
2	Mesenchymal stem cells	Chronic wounds/ulcers	1A	High-quality studies and good evidence of effectiveness and safety
3	Platelet rich plasma	Acute wounds (such as burns)	1C	Few studies but good evidence of effectiveness and safety
4	Platelet rich plasma	Chronic wounds/ulcers	1C	Few studies but good evidence of effectiveness and safety
5	Platelet rich plasma	Aesthetic procedures	1C	Few studies but good evidence of effectiveness and safety
6	Monocytes	*In vitro* application	2C	Very few studies and low-quality evidence of effectiveness.Further research is requested
7	Epidermal skin substitutes	Acute wounds (such as burns)	1A	High-quality studies and good evidence of effectiveness and safety
8	Epidermal skin substitutes	Chronic wounds/ulcers	1A	High-quality studies and good evidence of effectiveness and safety
9	Dermal skin substitutes	Acute wounds (such as burns)	1A	High-quality studies and good evidence of effectiveness and safety
10	Dermal skin substitutes	Chronic wounds/ulcers	1A	High-quality studies and good evidence of effectiveness and safety
11	Dermo-epidermal skin substitutes	Acute wounds (such as burns)	1A	High-quality studies and good evidence of effectiveness and safety
12	Dermo-epidermal skin substitutes	Chronic wounds/ulcers	1A	High-quality studies and good evidence of effectiveness and safety
13	Acellular dermal matrix	Acute wounds (such as burns)	2C	Few studies with weak evidence
14	Acellular dermal matrix	Chronic wounds/ulcers	2C	Few studies with weak evidence
15	Placental-based allografts	Acute wounds (such as burns)	1C	Few studies but good evidence of effectiveness and safety
16	Placental-based allografts	DFU	1B	High-quality studies with good evidence of effectiveness and safety
		VLUs	1C	Few high-quality studies but good evidence of effectiveness and safety
17	Food-grade lactic acid bacteria	Chronic wounds/ulcers	1C	Few studies but good evidence of effectiveness and safety
18	Dressings based on autologous platelet-rich fibrin and leucocyte	Chronic wounds/ulcers	1C	Few studies but good evidence of effectiveness and safety

*for each indication

These surrogate markers should prompt clinicians to consider using an advanced wound healing protocol already early in the wound healing process.

Physical therapies

Introduction

The physical approach to wound healing was probably the first approach ever implemented since physical means, such as compression, lavage and closure, have been available to physicians since ancient times and were used primarily in the case of acute wounds or traumas.[279]

In modern times, biological discoveries have emphasised a "biochemical" approach to the management of wounds with the basic idea that the interaction between a substance/compound and the surface of the wound would affect its evolution positively due to the modifications that the sub-stance/compound would induce in the biology of the wound.[280]

A typical example of this concept is the use of local antiseptics to contrast infection /contamination or the application of enzymes to debride the wound.[281,282]

Very recently, physical therapies regained an important role in the management of wounds, and new technologies and devices have been developed with this indication. With the term 'physical therapy', we refer to the interaction between the wound and a physical system in which there is a transfer of energy to/from the wound, which in turn translates into observable and measurable modifications in the system as well as in the wound.

A paradigmatic example regards the application of pressure, both positive (PPWT), for compression and oedema control in the case of venous leg ulcers, and negative (NPWT), for the treatment of a number of different chronic wounds.[283]

Although we will not cover PPWT and NPWT in this section, since both of them have been extensively treated in two recent position documents released by EWMA, they are probably the most successful and widely applied physical therapies with such a diffusion and success that they are considered nowadays the standard of care for a number of chronic ulceration.[284]

Other aspects of physical therapies which will not be treated in this section are those related to systemic and topical oxygen therapy and to physical means for debridement (hydrosurgery, ultrasound debriders) since both of them have recently been addressed in other EWMA documents, as well.[285,286]

The physical field has been progressively populated in recent times by a number of new technologies, ranging from shock waves to electrical fields, from magnetic fields to nanotechnologies, from light to laser, all with indications for wound management and all with some level of evidence behind them, although to a variable extent, due to the novelty of the proposals. We will try to critically examine the most significant of these new technologies and to provide relevant information needed to decide if and when a specific technology could eventually be beneficial to include in clinical practice.

Shock waves

From its first clinical application for urolithiasis

in the eighties, extracorporeal whock waves therapy (ESWT)[287] progressively moved to other indications, such as the treatment of tendons and fascia calcification,[288,289] bone fracture malunion and malalignment,[290] until a casual observation of a possible effectiveness in promoting wound repair prompted their adoption for a wide range of chronic wounds, including DFU,[291] VLU[292] and PU.[293]

According to this shift in the clinical indication, the technology beyond ESWT underwent an evolution from large equipment that generated focused high energy shock waves which could transfer a high amount of energy deep into the tissues and fragment stones to radial equipment that can produce lower energy waves on a wider surface, such as the surface of a chronic superficial wound.[294]

In both cases, shock waves are generated by a high voltage spark in a water medium (electrohydraulic) or in a metallic membrane (electromagnetic), which cause the rapid increase (nanoseconds) of pressure, generating a spike which may reach an intensity that is 100 times higher than the normal barometric pressure in less than five milliseconds.

The shape of the probe makes it possible to concentrate the waves and focus them according to the intensity set in the generator. This makes it possible to transfer the energy to the tissues in a higher or lower intensity and in concentrated shapes.[295]

While the mechanisms of action of high energy focused ESWT is clearly related to a sudden transfer of energy, which is able to disrupt gallstones, the effect of low intensity ESWT on wound healing still needs to be clarified. There is, however, evidence available, which supports that the application of stress to the cytoskeleton of the cells in the lesion (mechano-biological interaction) is able to produce a number of effects, including the repression/depression of genes[296] and changes in protein synthesis[297] of a number of cells, including keratinocytes,[298] fibroblasts,[299] endothelial cells,[300] and bone marrow stromal cells.[301]

ESWT have been demonstrated to increase vascular endothelial growth factor (VEGF)[302] and nitric oxide (NO)[303] concentrations, which promote angiogenesis.[304] Other observations are related to the reduction in the production of pro-inflammatory cytokines[305] and to the increase of the proliferation of fibroblast induced by ESWT *in vitro* and *in vivo*.[299]

Safety was also explored in all of the studies performed on ESWT, and from this point of view, the results were unanimous and positive, confirming a high safety standard for the technology.

These promising observations are unfortunately not paralleled by an adequate level of evidence generated via clinical trial. Two recent reviews on the subject are concordant in stating that according to the Cochrane standards, ESWT is not adequately supported by evidence.[306,307]

This is not necessarily related to the results of the available studies, which were generally positive, but rather to the poor quality of the trials, which either targeted a mixed population of chronic wounds,[294] were not sufficiently dimensioned,[307] or omitted important information about the details of the treatment, such as the number of impulses, the frequency of impulses and energy flux density (in millijoules per square millimetre).

In Table 16, a synopsis of the clinical studies on ESWT for the management of chronic wounds is reported.

Since the first report by Shaden et al. in 2007,[314]

Table 16: Studies on extracorporeal shock waves therapy (ESWT)

Author	Year	Condition(s)	No. of wounds	ESWT Specifications	Healing Rate	Comments
Aschermann et al.[308]	2017	CLUs	75	EFD: 0.136mJ/mm^2 Amount of pulses: 100 pulses/cm^2 Frequency: 4 pulses/sec	41%	No control group
Jeppesen et al.[309]	2016	DFUs	11	EFD: not available Amount of pulses: 250/500 pulses/cm^2 Frequency: not available	35%	Significant ($p<0.01$) reduction in the area of ulcers compared to controls
Omar et al.[310]	2014	DFUs	24	EFD: 0.11mJ/mm^2 Amount of pulses: 100 pulses/cm^2. Frequency: not available	54%	Faster healing than in control group ($p<0.05$)
Arnò et al.[311]	2010	Burns	15	EFD: 0.15mJ/mm^2 Amount of pulses: 100 Frequency: not available	80%	No control group
Larking et al.[293]	2010	PUs	9	EFD: 0.1mJ/mm^2 Amount of pulses: 200 + 100 pulses/cm^2 Frequency: 5 pulses/sec	56%	Crossover study favouring ESWT
Ottoman et al.[298]	2010	Donor sites	28	EFD: 0.1mJ/mm^2 Amount of pulses: 100 pulses/cm^2 Frequency: not available	100%	Faster re-epithelisation than in control group ($p<0.0001$)
Moretti et al.[312]	2009	DFUs	30	EFD: 0.03mJ/mm^2 Amount of pulses: 100 pulses/cm^2 Frequency: not available	53%	Faster healing and higher healing rates than control group ($p<0.001$)
Wang et al.[291]	2009	Recurrent DFUs	36	EFD: 0.11mJ/mm^2 Amount of pulses: 100 pulses/cm^2. Frequency: not available	31%	HBOT control group healing rates 22% ($p<0.001$)
Saggini et al.[313]	2008	VLUs, DFUs, PTUs	32	EFD: 0.037mJ/mm^2 Amount of pulses: 100 pulses/cm^2 Frequency: 4 pulses/sec	50%	Only 10% of ulcers in the standard of care control group healed ($p<0.01$)
Shaden et al.[314]	2007	Mixed chronic ulcers but not DFUs	208	EFD: 0.1mJ/mm^2 Amount of pulses: 100 pulses/cm^2. Frequency: 5 pulses/sec	75%	No control group

ESWT: extracorporeal shock wave therapy; DFU: diabetic foot ulceration; EFD: energy flux density; VLU: venous leg ulceration; PTU: post-traumatic ulceration; HBOT: hyperbaric oxygen therapy; PU: pressure ulcers; CLU: chronic leg ulcers

440 chronic ulcers have been treated with ESWT in a study aimed to evaluate the efficacy of this approach, with a mean healing rate of 57% (range: 31–80%). These results should be taken into consideration with prudence, specifically in view of the high healing rates in the control groups, when present, when standard of care was followed for the different aetiologies of chronic ulcers. At this point, more trials, and especially trials with more di-mensions, are needed to confirm the indications of ESWT for chronic wound management. It is, however, unlikely that this information will be available in the near future due to the difficulty and costs of these trials.

On this basis, considering the high safety profile and the varying documentation of effectiveness, we can consider ESWT as an adjunct therapy in addition to good quality standards of care to hasten heal-ing rates of DFUs, VLUs and PUs. A possible limitation includes the high costs of the equipment need-ed to generate the shock waves, which may limit the use of this technology to hospital-based practices.[294,306,307]

Electromagnetic fields (EMF)

Since the experiments of Galvani on frogs' isolated limbs in 1794, the role that electricity and mag-netism exerts on human physiology continuously grew until the present day. A wide range of interactions have been demonstrated and described, in virtually all of the mechanisms of function within an organism.[315]

Our body is able to produce electricity and magnetic fields and use them for a range of functions, from nerve conduction of information to muscle contraction, but also for polarising cells, inducing biochemical reactions, separating body fluids and a number of other functions.[316–318]

The main source of self-produced electricity is the ion exchange through the cellular membrane, which is a natural dielectric structure, normally charged with negative potentials on the outside and positive potentials on the inside (Fig 17).[319]

This potential can be measured, and the changes are related to cells and tissues functions as well as dysfunctions. An interesting observation, from this point of view, is that the reduction or nullification of this electric potential is a sign of sufferance and death of cells.[320]

Our tissues are also able to react to the application of electricity and magnetic fields from the outside, sensing them and reacting according to a complex paradigm in relation to the intensity, polarity, time and point of application.[321]

The intensity and frequency of both electric fields (EFs) and magnetic fields (MFs) are crucial to deter-mine the interactions with the tissues. There are 'windows' for both intensity and frequencies of currents that allow the interaction to occur in a way so that the organism is modified by the applica-tion of EF and MF. Outside these 'windows', the interaction simply does not occur or is destructive.[322]

Among the many aspects of human physiology which are influenced or regulated by EF and MF, tissue repair and wound healing are probably the healthcare areas that have accumulated the most evidence in previous years. The diagnostic and therapeutic applications of EMF have taken strong roots in these areas.[323]

The discovery that any tissue lesion produces an interruption in the normal polarisation of tissues, and that this in turn generates an electric current, opened a new window for interpreting the biology of tissue repair and the mechanisms that regulates wound healing.[324]

As illustrated schematically in Figure 18, the

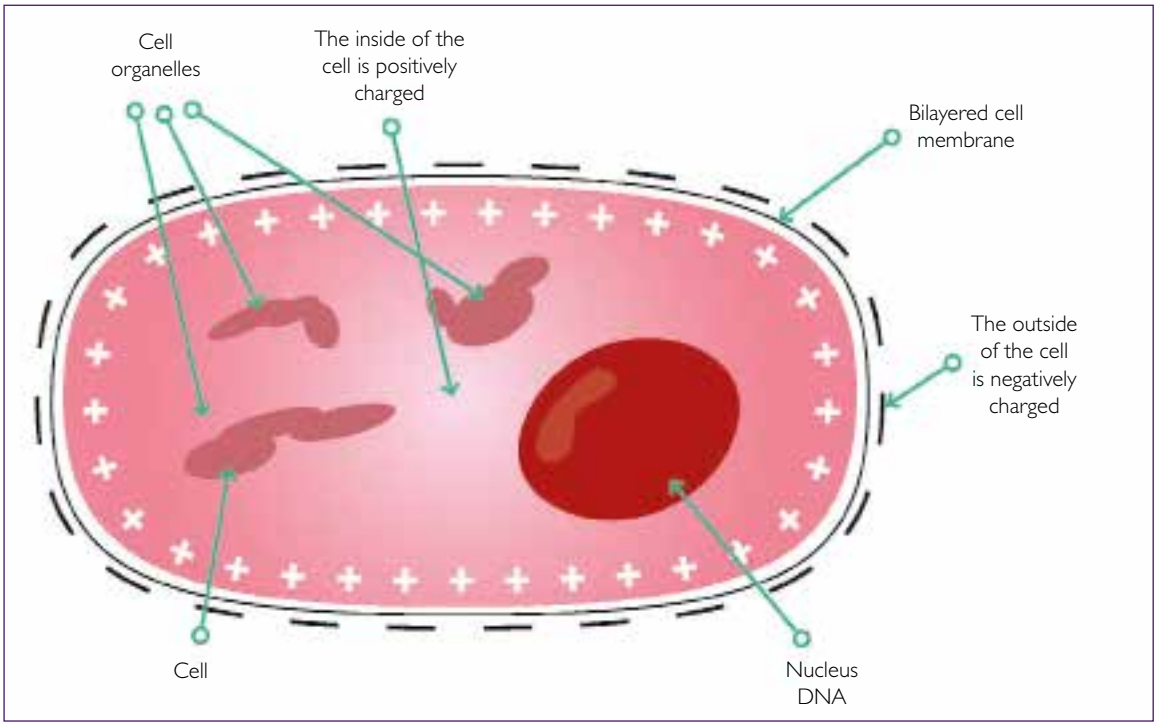


Fig 17: The cell as a dielectric structure and the compartmentalisation of electric charges

development of a difference in the polarity at the edge of a lesion is one of the mechanisms, which starts and sustain the movements of the edges of the ulcer towards the centre of the ulcers. The application of EF with an adequate intensity is able to stimulate or, vice-versa, stop and even invert the progression of such movement according to the polarity of EF in relation to the margin of the wound. Thus, it seems that EFs are the fast-responders to creation of a wound, overriding all of the other biochemical and hormonal mechanisms, at least in the initial moments.[317,322,323]

This has been demonstrated in different animal models and verified by use on human wounds of different aetiologies.[315,.317]

It has been demonstrated how the direct application of EFs may:

- Stimulate and orient the movements of different kinds of cells, including keratinocytes and fibroblasts
- Stimulate the production of cytokines and other proteins
- Guide the homing of bone-marrow-derived mesenchymal cells
- Activate/depress genes via intracellular second messengers: All oriented in promoting wound healing.[325–328]

A very elegant experiment in a cell culture demonstrated how by inverting the polarity of EF, fibroblasts not only inverted their active movements, but also shifted the polarity of their protein synthesis inside the Golgi's apparatus, orienting both movements and protein synthesis

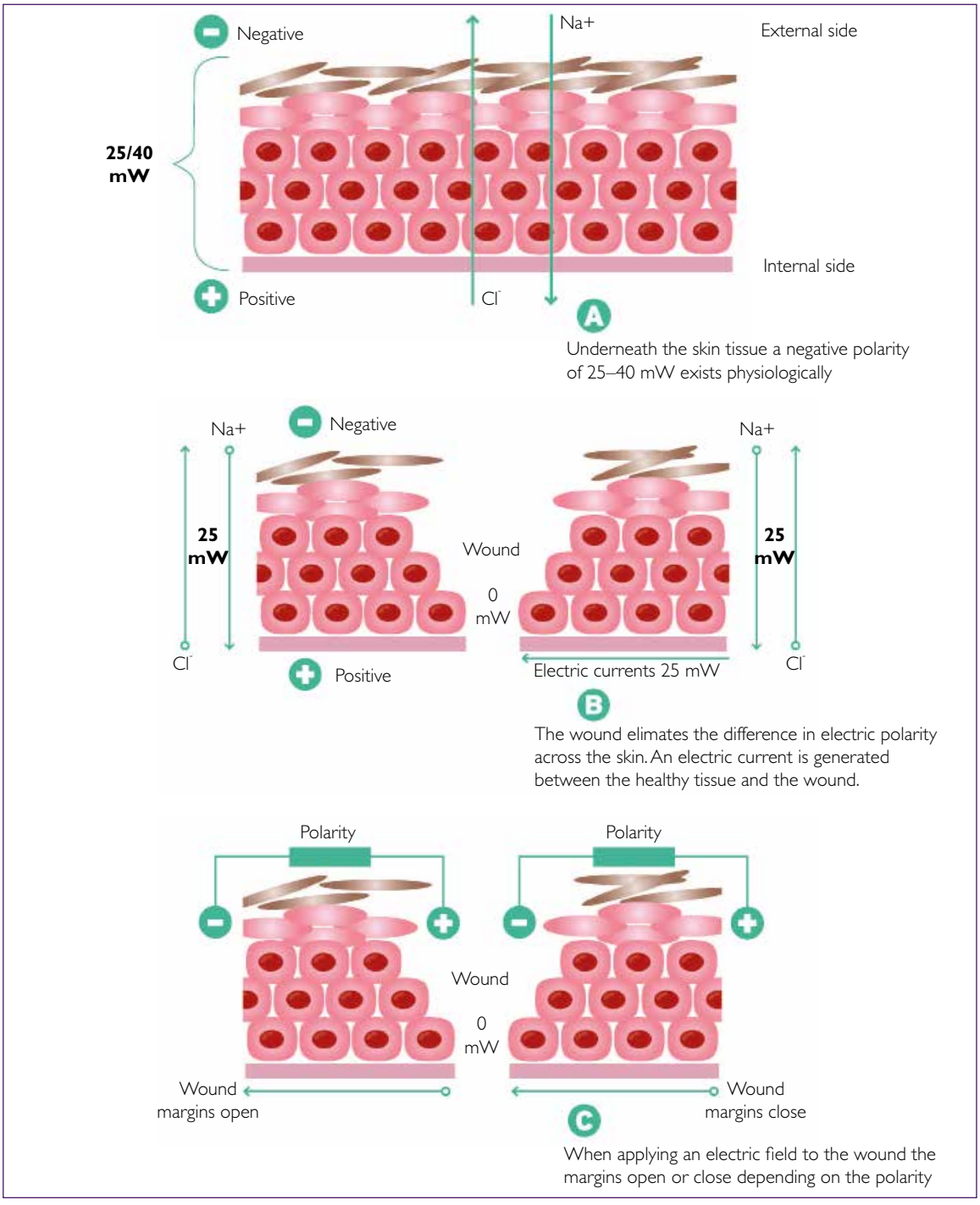


Fig 18: (A–C) The distribution of polarity in normal (A) and wounded (B) skin and the behaviour of lesions' edges to the application of an electric field to the wound (C)

according to polarity, towards the negative electrode (Fig 19).[329]

All the cells involved in wound repair are EF-sensitive, from those of the inflammatory phase (neutrophils and macrophages actively migrate towards the cathode as well as lymphocytes) to epithelial cells, fibroblasts and endothelial cells, which characterise the reparative phase. The reparative phase is not only actively moving when inserted in an EF, but also it increases the proliferative rate as well as the production of cytokines and growth factors, such as VEGF and EGF. This way it demonstrates an interplay between electrical and biochemical/hormonal regulation of wound healing.[331–334]

These observations have been translated into the production of devices and dressings that can apply EF directly to the wounds to stimulate wound repair.[335,336] Clinical trials have been carried out in different chronic ulcerative pathologies, such as PUs, VLUs and DFUs, over the last 30 years. Generally, positive results have been achieved (Table 17).

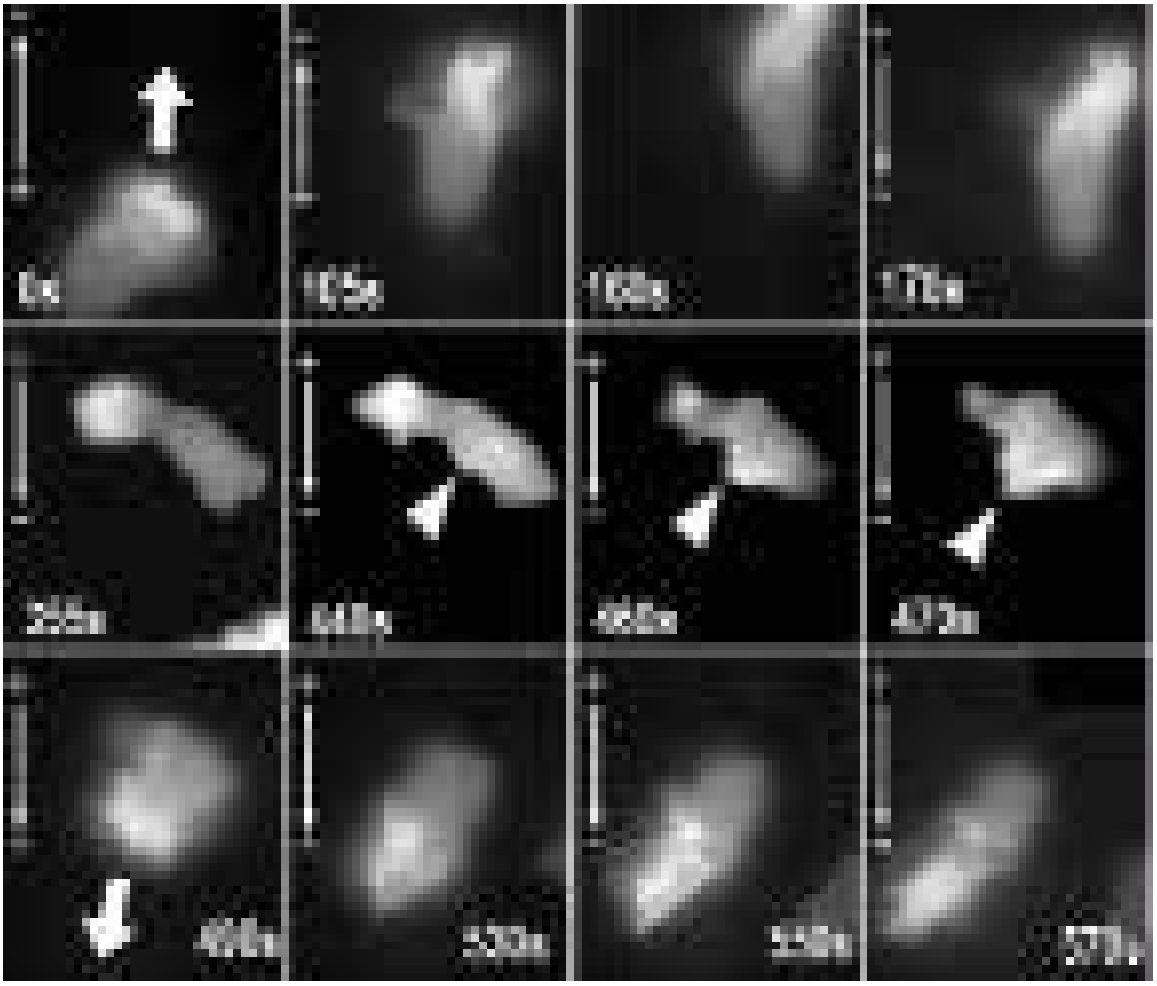


Fig 19: Electric-field-directed activation of PI3 kinases[330]

A meta-analysis of these trials, including only RCTs with control groups and adequate design, follow-up and reports, identified fifteen studies. In total, these included 876 patients (497 treated with EF and 379 controls) with an average duration of treatment of 6.53 weeks. The reduction of the area of the lesion at four weeks of treatment that was almost double in the EF-treated groups as compared to the controls (57.08% vs 29.34%).[352] The same authors calculated a positive OR of 26.77% for the use of EFs in a mixed chronic ulcer population. For the Pus, an OR of 42.70% was reached.

Unlike EFs, MFs are not produced by the cells and living tissue but can be generated by EFs when they change rhythmically. In the body, these changes are generally very short-lived. The generated MFs are therefore called pulsatile electromagnetic fields (PEMF).

Another difference between EFs and MFs is that MFs can penetrate the cells and tissue while EFs are stopped by the cell membranes. MFs can interact with a number of functions of the cells in a subtle way.[315] In addition, MFs are ubiquitous in our environment and can be divided into the following categories:

- Natural MFs, which have a very low intensity [5 x 10-5 Tesla (T); 1T = 100 Vs/cm2] but may

Table 17: Studies on Electric Fields (EF)

Author	Year	Condition	No. of wounds	Type of ES	ES-treated N. patients (PAR4)	Controls N. patients (PAR4)
Franek et al.[337]	2012	PU	50	Uni	26 (68.83%)	24 (23.24%)
Houghton et al.[338]	2010	PU	34	Uni	16 (37.02%)	18 (13.83%)
Petrofsky et al.[339]	2010	DFU	20	Bi	10 (68.40%)	10 (30.10%)
Ahmad et al.[340]	2008	PU	60	Uni	45 (62.35%)	15 (20.76%)
Jankovic et al.[341]	2008	VLU	43	Bi	24 (89.62%)	19 (56.42%)
Junger et al.[342]	2008	VLU	39	Uni	20 (15.11%)	19 (03.04%)
Franek et al.[343]	2006	VLU	55	Uni	28 (42.05%)	27 (28.27%)
Houghton et al.[344]	2003	VLU	42	Uni	22 (44.30%)	20 (16.00%)
Barczak et al. [345]	2001	PU	33	Uni	16 (69.21%)	17 (44.04%)
Peters et al.[346]	2001	DFU	40	Uni	20 (56.09%)	20 (34.17%)
Baker/ Chambers[347]	1997	PU	114	Bi	61 (64.77%)	53 (41.78%)
Baker/Rubayi[348]	1996	DFU	192	Bi	125 (38.49%)	67 (51.00%)
Wood et al.[349]	1993	PU	74	Uni	43 (60.37%)	31 (06.77%)
Feedar/Kloth[350]	1991	Mixed	50	Uni	26 (56.18%)	24 (32.82%)
Carley et al.[351]	1985	Mixed	30	Uni	15 (83.46%)	15 (37.92%)
Total	----	----	876	----	497 (57.08%)	379 (29.34%)

ES: electric stimulation; PAR4: percentage area reduction in 4 weeks; PU: pressure ulcers; DFU: diabetic foot ulceration; VLU: venous leg ulceration; Uni: unipolar stimulation; Bi: bipolar stimulation

af-fect many of our biological rhythms with its periodical variations

- Technical MFs, which are all the MFs that are artificially produced by technological means (any electric current generates a MF),which usually reach much higher intensities (up to 7 T in MRI machines, more than 10T in the particle accelerator).

MFs have a much lower intensity, but anyhow are more intense than the Earth's background MFs. These are generated by any electricity driven equipment from electric lamps to mobile phones. All of these, at least potentially, interact with our organisms, exerting a variety of effects of tissue and organ physiology. This happens either by directly interfering with the magnetic-sensitive molecules (all of the ones that contains charged ions or metallic components) or by directing and orienting the movements of molecules and organelles.[353]

PEMF are more frequently and constantly generated inside our body compared to static MFs. These are generated by endogenous and exogenous EFs, like those of neural action potential, or by a piezoelectric mechanism, generated by the movement of muscles, tendons, bones , joints and, in general, all of the moving proteic structure of our body.[354]

In the same way, any PEMF applied to a proteic structure is able to determine a movement and a change in its structure, and a typical example of this is the alignment that collagen fibers show when solicited by PEMFS.[355] This is one of the bases of the therapeutic application of PEMFs for tissue repair and wound healing.[356]

The first and more documented applications of PEMFs in therapeutic fields was the reparation of bone malunions. In this, the application of MFs was associated with faster and more stable stabilisation of the fractures with an increase in the speed and amount of callus formed and with a better alignment of the matrix fibers and an improved calcification.[357]

More recent evidence, including in vitro and in vivo studies, focused on the many positive effects that MFs can exert on virtually all of the phases of tissue repair. This was mediated both by the interaction with cells and their behaviour and by the modulation of cytokines and growth factors production, which promoted the therapeutic use of MFs in wound healing.[323]

MFs have been associated with an intense anti-inflammatory action, mediated by the shift in the production of cytokines from a pro-inflammatory pattern to an anti-inflammatory pattern. This can speed up the movement from the chronic inflammatory phase, which is typical for chronic wounds, to a more pro-reparative phase of tissue repair. This has also prompted the use on MFs not only in chronic ulceration, but also in a variety of chronic inflammatory conditions of both skin and the osteo-muscular apparatus, such as tenosynovitis, arthrosis, and traumas.[358,359]

In addition, MFs promote the proliferation and activation of fibroblasts and increase the neo-angiogenesis alongside the aforementioned orientating effect on collagen fibers. This promoted their application in the reparative phases of wound healing as well as in other conditions characterised by poor regenerative activity, such as osteoporosis.[360,361]

In Table 18, a report of the studies on the application of MFs for wound healing is reported.

The application of MFs in humans is not performed without concerns. Indications have been presented that the chronic exposure to MFs, especially in the case of high frequency and high

Table 18: Studies on Electromagnetic Fields (EMF)

Author	Year	Condition	No. of ptt.	EMF Specifications	Follow-up (days)	Results
Piaggesi et al.[362]	2016	DFU	140	TMR 24 + 24 min/day Exposure 4 weeks	70	Significant (p<0.05%) increase in rate of granulation tissue and symptom score in treated patients vs controls
Abbruzzese et al.[363]	2015	DFU	20	TMR 20 + 20 min/day Exposure 2 weeks	180	Significant (p<0.05%) increase in healing rate in treated patients (90%) vs controls (30%)
Gupta et al.[364]	2009	PU	12	PEMF - 1Hz sine wave, 45 min 5 x week Exposure 24 weeks	170	No significant differences between treated group and controls
Canedo-Dorantes et al.[365]	2002	ALU + VLU	26	PEMF 3.63 mT, 2-3 h/day, 3 x week Exposure 16 weeks	120	69% wound closure in treated group, healing lasted at least 6 months and up to 2 years
Stiller et al.[366]	1992	VLU	31	PEMF - 2.2mT, 3h/day Exposure 8–12 weeks	90	50% healing in treated group vs 0 in control group, significant (p<0.04) reduction in depth and pain perception in treated patients
Todd et al.[367]	1991	VLU	17	PEMF - 5Hz, 15 min 2 x week Exposure 5 weeks	45	Not significant improvement of clinical parameters in treated group
Ieran et al.[368]	1990	VLU	37	PEMF - 75Hz, 2.8 mT, 3–4 h/day Exposure 13 weeks	90	Significant (p<0.02) increase in re-epithelisation rate in treated patients compared to controls

Abbreviations: EMF electro-magnetic fields; DFU diabetic foot ulceration; ALU aterial leg ulcers; VLU venous leg ulceration; PU pressure ulcers; PEMF pulsatile electro-magnetic fields

intensity, is associated with carcinogenesis. For this reason, standards for the exposure to MFs of humans have been developed by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) and applied in several countries. These guidelines set the standards and limitations for the intensity and length of exposure and describe the possible long-term effects, which are not yet fully documented but cannot be excluded.[369]

In addition, but not exclusively for these reasons, a new generation of PEMF have been implemented and tested in chronic wounds with positive results. Their way of interaction with the biologic systems

is different from the traditional versions as they do not act directly due to their extremely low intensity. Instead, they 'communicate' with the MFs present inside the cells by frequency modulation sequences that characterise these MFs. This is phenomenon is known as bio-resonance. It modifies the frequencies of the MFs inside the cells. For this reason, they have been identified with the generic term 'therapeutic magnetic resonance' (TMR).[370]

The average intensity of TMR used is similar to the terrestrial magnetic field (approximately 40 microtesla), and this does not fully exploit the energetic parameters but rather the frequency of the electromag-netic signal. The system emits 'wave trains', which are picked up in spite of the low intensity, for example via a 'stochastic resonance' mechanism, which has already amply observed in nature. These produce a therapeutic effect. The cells affected by the signal are those requiring a rebalancing of function. It is assumed that, in the targeted tissue, the sick cells are affected by the signal through a realignment in frequency, whereas the other cells remain in tune with the signal transmitted. They receive the signal but are not affected by it.[370,371]

Recent studies on DFUs have demonstrated how TMR is able to increase the granulation tissue for-mation on recalcitrant ulceration after four weeks of application and how this clinical result is paralleled by histological and biomolecular findings of pro-reparative shifting in biopsies taken from the lesion under treatment.[362]

Photobiomodulation (PBM)

The use of light for medical purposes dates back to ancient times when phototherapy was empirically prescribed for a number of clinical conditions ranging from skin pathologies to asthma, behavioural disorders and, eventually, wounds.

More recently, advancements in human physiology, which elucidated many of the mechanisms behind the interaction between the organism and light, added many new dimensions to this field.[372] These mechanisms include its role in the synthesis of vitamin D, the photomodulation of biorhythms and the antidepressive effects.

Wound healing was probably one of the first and most important areas in which the application of light as a therapeutic tool was applied. This cumulated a body of evidence in the different pathologies, ranging from pressure ulcers to diabetic foot ulcers and venous leg ulcers.[373]

Despite its wide application, especially in the last 20 years, phototherapy was only recently defined in its different components. This separated the applications relying on the thermal effects of light application from the ones that are non-thermal and imply an interaction of light with endogenous photoacceptors.[374]

The latter were comprehensively grouped under the term 'photobiomodulation therapy', which in November of 2015 were included in the MeSH index of the US National Library of Medicine.[375]

The definition of photobiomodulation is "a form of light therapy that utilises non-ionising forms of light sources, including lasers, LEDs and broadband light, in the visible and infrared spectrum. It is a nonthermal process which involves endogenous light absorbing molecules (chromophores) that elicit photophysical and photochemical events at various biological scales. This process results in beneficial therapeutic outcomes including, but not limited to, the alleviation of pain or inflammation, immunomodulation, and promotion of wound healing and tissue regeneration".[375]

The interaction between light and our organism is conditioned by a number of different factors:

1. The 'optical therapeutic window': there is currently a relatively narrow range of wavelengths that can actually interact with the photoacceptors to exert photobiomodulation, and they are comprised between 600 and 1300nm; wavelengths of <600nm are absorbed by melanin and oxyhaemoglobin while wavelengths >1300nm are absorbed by the body's water. This window is located near the infra-red portion of the spectrum of visible light and is denominated near-infrared light (NIR) (Fig 20).[376]

2. There is also a range of doses on energy transfer that has to be taken in account when one considers the biological effects induced by photobiomodulation, and they are within the Arndt-Shultz curve (Fig 21).[377]

3. The sources of light: light for medical purposes may be generated either by LED (light emitting diode) or by LASER (light amplification by stimulated emission of radiation). In both cases, the emitted light is monochromatic. In the case of LED generated light, the emission is not unidirectional while LASER generated light is unidirectional and coherent, reaching much higher in-tensity with the same amount of energy, concentrating the area of application.[378]

To exert its effect, light has to interact with the structures of our cells. Although there are still some controversies regarding the possible targets for this interaction, cytochrome C oxidase appears to be the best candidate as the principal photoacceptor. This is due to its conformation with four possible sites of photoacception, the two copper centres (CuA and CuB) and the two iron centres (HemeA and HemeB). These are all involved in the transfer of electrons in the respiratory chain on the mitochondrial membrane of the eukaryotic cells.[380]

Other candidates, possibly with complementary roles, are phlavoproteins and porphirins, which are implicated in the generation of reactive oxygen species (ROS) after an interaction with photons.[381]

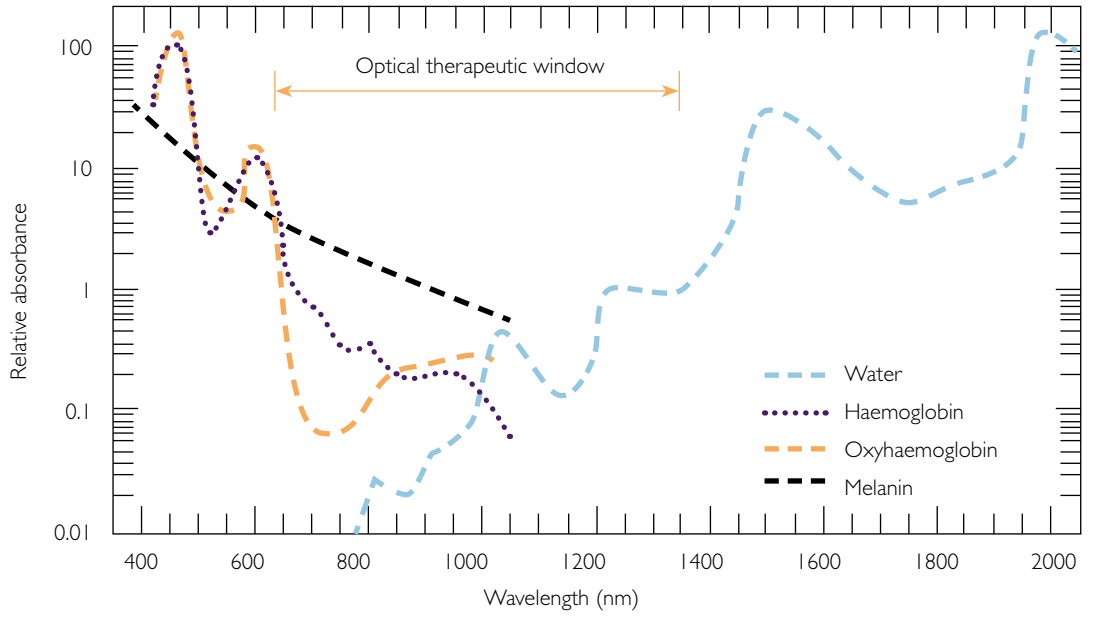
In both cases, and eventually in the other cases in which light may interact with biological structures, this has to be considered a so-called primary reaction. The secondary reactions include the effects that the first interaction induce within the metabolism of the cells and the tissue by transduction and amplification of the original signal, leading to a photoresponse.[382]

Secondary reactions include the production of NO, the intracellular increase of ROS, the increase in permeability of cell membrane, the increase of intracellular calcium levels, the increase in cell metabolism, the increase of RNA and DNA synthesis, fibroblast proliferation, activation of lymphocytes, macrophages and mast cells, and increased synthesis of interleukins and growth factors.[383]

An interesting emerging action of photobiomodulation on the wound healing process is the modulation of matrix-metalloproteinases (MMPs) and their inhibitors TIMP (1–4). Studies have demonstrated how irradiating chronic wounds with a laser (660nm 6.2 J/cm^2) results in a reduction of MMP-2/TIMP2 and MMP-9/TIMP2 as compared to non-irradiated controls.[384] A report confirmed by other studies in periodontitis models[385] opened a window on to what could be a next area of research in photobiomodulation and wound healing.[386]

Beyond the general concept that light exerts an important anti-inflammatory action, the new acquisitions of knowledge that are presented in the more recent studies, demonstrated many other effects, not only from a local, but also from a systemic, point of view.[387]

Photobiomodulation has now accumulated evidence of a positive action on all phases of



Source: Schindl A et al. Low-intensity laser therapy: a review. J Investig Med 2000; 48(5):312–26[376]

Fig 20: Therapeutic optical window.[376] The 'Optical Therapeutic Window' is the range of wavelengths at which light may interact with photoreceptors to exert a photobiomodulatory effect.

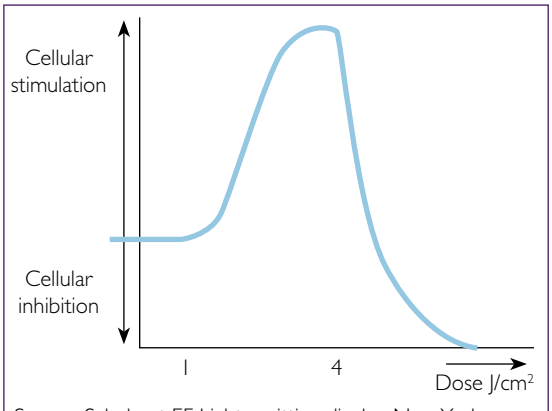
wound repair from the first inflammatory phases to the remodelling phase (Fig 22).

In Table 19, a selection of clinical studies on the application of photobiomodulation therapy to wound healing is reported.

While NIR is the main driver of photobiomodulation in wound healing, ultraviolet (UV) light has been proven to exert an important role in contrasting infections within chronic wounds.[396]

Depending on wavelength, UV light can be divided in four groups:

1. Vacuum UV (100–200nm)
2. UVC (200–280nm)
3. UVB (280–315nm)
4. UVA (315–400nm)



Source: Schubert EF. Light emitting diodes. New York: Cambridge University Press; 2003[379]

Fig 21: The Arndt-Shultz curve; with a peak around 5J/cm²; smaller doses of energy do not provoke effects, and higher doses may have negative effects.[377]

Vacuum UV and UVC are completely blocked by the ionosphere while UVB and UVA are in contact with our bodies with different grades of penetration, and UVA can penetrate deeper than UVB.[397]

Recent studies have demonstrated how it was possible to eradicate MRSA and *Pseudomonas aeruginosa* infections with short-interval (<1hour) applications[398] by irradiating chronically infected ulcers with UVC (254nm, 15.54mW/cm^2).

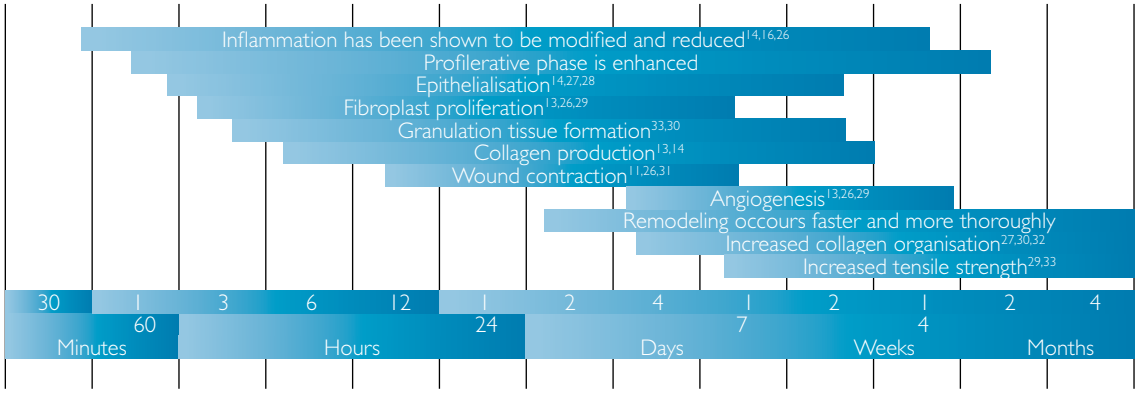
The antimicrobial effect of photobiomodulation has been confirmed for use on biofilm-producing bacteria, which include the majority of the cases of chronic wounds colonisation, and which are particularly resistant to systemic antibiotic therapy. Laser-generated light application can eradicate biofilm and infected wounds efficiently and safely.[399]

Other authors also observed pro-reparative effects of UV on various in vitro and in vivo models of chronic wounds, but the clinical experiences are so far too limited to result in any clear conclusions on these effects.

A novel and very promising approach to photobiomodulation in wound healing has been developed in recent years. This associates with the irradiation of a gel containing chromophores activated by LED generated visible light. When activated with a LED light (440 to 460 nm), the light absorbing molecules release large spectra of photons at different wavelengths in the visible range from 532nM to 615nM. The gel is applied on the wound surface and is not absorbed by the tissue, but it is activated by the light, which is applied for a duration of 5 minutes twice a week.[400]

This new way of realising photobiomodulation therapy has been named biophotonic treatment, and it has been successfully applied in pressure ulcers, diabetic foot ulcers, venous leg ulcers, and acne vulgaris with positive effects on pain perception, healing rates and the patients' quality of life.[389,394]

A non-secondary positive aspect of the biophotonic treatment is that it reduces the number of applications and the time required for effectively obtaining a therapeutic effect on wound



Source: Prindeze NJ, Moffatt LT, Shupp JW. Mechanisms of action for light therapy: a review of molecular interactions. Exp Biol Med (Maywood). 2012; 237(11):1241–8

Fig 22: Photobiomodulation can positively influence all phases of wound repair from the early inflammatory response to the late remodelling of scar tissue[380]

Table 19: Studies on photo biomodulation (PBM)

Author	Year	Condition	No. of patients	PBM specifications	Follow up (days)	Results
Romaneli et al.[388]	2017	DFU, VLU, PU	33	440–460nm (55–129J/cm^2)	224	QoL: 26.4% improvement of CWIS (Cardiff Wound Impact Score) post- vs pre-treatment (p=0.001) 52% achieved total wound closure with the study treatment
Nikolis et al.[389]	2016	FSR	32	400–470nm	84	Improvement of skin scores
Kajagar et al.[390]	2012	DFU	64	wavelength not speci-fied (2–4J/cm^2)	15	Significant (p<0.05) reduction in ulcer area in treated (1043 mm^2) and control (322 mm^2) group
Kaviani et al.[391]	2011	DFU	23	685nm (10J/cm^2)	140	Significantly (p<0.05) greater reduction in lesions' size in treatment group, no differences in healing rates and healing times between the groups
Landau et al.[392]	2011	DFU	16	400–800 nm (43.2 J/cm^2)	116	90% healing in treated group vs 33% in control group (p<0.05), significant (p<0.05) shortening of healing time in treated (7 weeks) vs control (11 weeks) group
Minatel et al.[393]	2009	DFU	28	890nm + 660nm (3 J/cm^2)	90	53% healed in treated group vs 7% in control group (p<0.05)
Shubert V.[394]	2001	PU	72	956 nm + 637 nm x 9 min pulsed (15.6 Hz - 8.58 kHz)	70	Healing rate 49% higher in treated group than in controls (p<0.05)
Papageorgiou et al.[395]	2000	AV	107	415nm (320J/cm^2) + 660nm (202J/cm^2)	84	76% improvement of inflammatory lesions at 12 weeks in treated group

PBM: photobiomodulation; AV: acne vulgaris; PU: pressure ulcers; DFU: diabetic foot ulceration; VLU: venous leg ulcers, FSR: facial skin rejuvenation

healing, which allows for a containment of the costs of management for these typically very costly chronic patholo-gies. This is both in terms of a reduced use of antibiotics and in terms of better resource use.[401]

Romanelli et al., in an interim analysis of prospective multicentred observational trials on 100 patients with DFU, VLU and PU from seven highly specialised centres in Italy, aimed to evaluate the safety and effectiveness of BPT on different models of chronic ulcers in a real-life setting, found a rate of closure of wounds of 53.8% for VLU, 52.9% for DFU and 33.3% for PU. The

percentage of full responders, which were defined by a decrease of the wound size area of more than 90% at study end and/or decrease of more than 50% of the size in 15 days or less, ranged from 33.3% in PU, to 61.5% in VLU and 70.6% in DFU. Moreover, the Cardiff Wound Index Score, an indicator of the quality of life in ulcerated patients, was found to be significantly (p=0.001) increased in all patients, irrespective of if they were full or partial responders probably due to the positive effects of BPT on pain.[388]

Despite the fact that the field of photobiomodulation is one of the most stimulating and rich among the physical therapies for wound healing with regard to clinical experiences, some controversies and uncertainties remain both from a methodological and clinical point of view:[374]

1. Optical parameters are extremely variable from study to study, and either frequencies, intensities and times of exposures change to an extent that it is difficult to compare the results between the different studies

2. There are many different photo-acceptors on the human cells, and the role of each of these are not yet fully understood. If more than one react to certain wavelengths, it may be difficult to determine the relationship between each stimulus and reaction to establish a causal pathway

3. The mechanisms of action of photobiomodulation are still not completely understood. We know that NIR and long visible wavelengths act via the cytochrome c oxidase while short visible wavelengths produce NO and ROS from nitrosated proteins and NADPH. However, these two mechanisms do not fully explain all of the observed effects of photobiomodulation in wound healing.

For all these reasons, there is still work to be done in this exciting field before we can promote it as a primary treatment option in wound management.

Nanotechnologies (NT)

On 29 December 1959, at a conference for the opening of the annual congress of the American Physics Society entitled 'There's Plenty of Room at the Bottom', the Nobel physicist Richard P. Feynman introduced for the first time the concept of a technology at the atomic dimensions, which can operate at the molecular level in a variety of environments. Although Feynman exposed the basic concepts, the term nanotechnology was actually coined by Kim E. Drexler in her book, entitled *Engines of Creation: The Coming Era of Nanotechnology* in 1986.[402]

With the term nanotechnology (NT), we refer to the research and application fields, which in the nanoscale dimension, range between one and 100 nanometres (nm: 1nm = 1 billionth of a metre); NT has potential within a number of different areas of development, ranging from electronic to engineering. Within medicine, many promising applications have been realised within a range of fields, from oncology to diagnostics and pharmacology and many others, including wound healing.[403]

The interest that NTs raises within wound healing relates to the physical characteristics of nanoparticles (NP) as well as their versatility and tunability, which make them suitable for use in the different phases of tissue repair.[404]

The high surface area/volume ratio makes it possible for NPs to have a high probability of interaction with the cellular elements and an enhanced penetration deep into the tissues. This also allows a higher bio-availability at lower concentration with a lower toxicity as a result.[405]

NTs have been explored in all phases of wound repair, from the acute inflammatory phase, in which they have primarily been tested as antibacterial agents, and for their modulatory effect on inflammation, to the reparative phases in which they have been applied due to their intrinsic properties and as vehicles of bioactive agents (Fig 23).[404]

In the acute inflammatory phase, the antibacterial properties of metallic and non-metallic nanomaterials have been tested in a number of preclinical studies *in vitro* and *in vivo* in animal models.[406]

Silver NPs and nanocrystals have been widely experimented with, also in clinical trials, for their ability to kill bacteria and to disrupt biofilms. The cytotoxicity of these heavy metal ions has been reduced, thereby decreasing the concentration due to the higher bio-availability in the NPs. The release of ions from NPs have been demonstrated to be more sustained over time, thus giving added value to this therapy in chronic wound infections.[407,408]

Zinc oxide (ZnO) NPs have demonstrated analogue effects at an even lower degree of toxicity, making them a very interesting alternative to silver, and also due to the possibility to insert them in different NM-dressings.[409,410]

Moreover, the efficacy of both Gram-and Gram+ strains and the activity against biofilm formation make both silver and ZnO NPs complementary or even alternative to the use of systemic antibiotic therapies. This may support the general efforts to reduce the risk of antibiotic resistance in chronically infected wounds.[411]

Non-metallic NMs have also been applied to the acute phase models with successful outcomes, mainly related to the anti-inflammatory effects that they exert on the wound biology. Carbon fullerenes has been demonstrated to significantly reduce inflammation and reduce the oxidative stress level in models of chronically inflammatory wounds.[412]

In the acute phase, NMs have also been tested as vehicles and carriers for bio-active molecules, such as nitric oxide (NO), antibiotic compounds and antioxidants.

NO is a molecule that plays many different functions in wound repair, especially in the acute phases, when it has a vasodilatory effect, an antibacterial effect and acts as a scavenger for cellular and bacterial debris.[413]

The possibility to convey NO into the wound more efficiently and to extend the release of NO inside the lesion have been tested with positive results using nanocarriers [poly(lactic-CO-glycolic acid) (PLGA)-polyethyleneimine (PEI)] that satisfied these conditions.[414]

The same approach has been used for delivering antibiotics and antioxidants inside the wounds in a time/dose efficient method. In the first case, a nanoparticle, made by gold nanodots joined with the cyclic lipopeptide surfactin (SFT), showed much more intense antibacterial activity compared to SFT used alone.[415] This nanoparticle demonstrated antibacterial activity and included 1-dodecanethiol (DT). In the second case, curcumin, a molecule with antibacterial and antioxidant properties, was successfully encapsulated in a number of different nanocarriers.[416]

In the reparative phases, NM have been proposed as carriers for growth factors and cytokines and as a novel type of scaffold and matrices on which newly-formed tissue can grow in a more physiological way as compared with traditional methods.

The possibility to protect growth factors from

Source: Hamdan et al. Nanotechnology-driven therapeutic interventions in wound healing: potential uses and applications. American Chemical Society, 2016

Fig 23: Schematic representation of the nanotechnology-based therapies employed in wound healing.[404]

enzymatic degradation by the use of the proteases present in the chronic wound environment put nanoscale systems in the position of being taken into consideration as carriers for those proteins.[417] This method extended their release and bio-activity.

A recombinant epidermal growth factor (rhEGF) has been successfully encapsulated in PLGA nanoparticles and in solid lipid nanocarriers. In both cases, its release and activity on chronic wounds was extended, and the activity was prolonged. This was demonstrated on mouse models.[418–420]

In addition, a recombinant vascular endothelial growth factor (rhVEGF) has been successfully inserted in PGLA nanoparticles together with platelet derived growth factor (PDGF). This was done in a combined way to support the integration of the activities of these growth factors (VEGF pro-angiogenetic and PDGF pro-regenerative) in a chronic wound model.[421,422]

The possibility to realise three-dimensional structures within the nanoscale dimension has been exploited for realising scaffolds that mimic the characteristics of extracellular matrix (ECM). PLGA/silk fibroin hybrid nanofibers have been used to promote attachment and proliferation of fibroblasts in a diabetic ulcer model.[423] Highly-branched nanopolymers (dendrimers) with anti-inflammatory properties like gelatine-dendrimer with polyethilenglyciole and silver ions have been released and tested for antibacterial properties.[424]

The possibility to orientate the nanofibers at the nanoscale dimension has also been tested.[425,426] This aims to promote a faster migration of the cellular elements that form the granulation tissue during the reparatory phase and to promote the use of materials, such as silicon wafers. Silicon wafers minimise the scar formation while maintaining pro-reparative properties.

Newer and even more interesting applications of NM in wound healing are the applications related to the possibility of using them to carry gene fragments into the wounds. This application aims to 're-condition' the deranged biology of the chronic environments, such as by reducing the production of matrix metalloproteinases or mesenchymal stem cells, which may speed up the healing process.[427–429]

Despite the signs of a very promising and bright future of nanotechnology research, only a few clinical studies have so far been carried out on real patients with chronic wound pathologies. In Table 20 (NM), the available clinical studies on nanotechnologies are summarised.

In a prospective observational trial on silver nanocrystalline (SN), including 103 patients with chronic wounds of mixed aetiologies, which were followed for a median of 42.5 days, Verdù Soriano et al. found a significant ($p<0.05$) positive difference in the healing curves as compared to the controls.[430]

Miller et al. compared SN and cadexomer iodine in a study including 291 chronic ulcers outpatients with a prospective, randomised design. Despite a superimposable overall healing rate in the group, they found a faster healing rate in the group treated with silver.[431]

Tsang et al. performed an RCT with three arms, comparing SN to manuka honey (MH) and conventional dressings (CD). In this trial, they found a higher, but not significant, healing rate of 12 weeks for SN (81.8%) as compared to MH (50%) and CD (40%), respectively.[432]

Banchellini et al. prospectively compared nanoliposome carriers, charged with phosphatidylcholine (NLPP), with conventional treatment in a RCT involving neuropathic patients with anhidrosis in the feet. At six weeks, they found

Table 20: Studies on nanotechnologies (NT)

Author	Year	Condition	No. of Ptt.	Nanotechnology specifications	Follow up (days)	Results
Tsang et al.[432]	2017	DFU	31	SN vs manuka honey (MH) and CT	84	Higher but not significant healing rate at 12 weeks for SN (81.8%) as compared with MH (50%) and CD (40%), respectively
Miller et al.[431]	2010	VLU	281	SN vs cadexomer iodine (CI)	84	No differences in healing rates, faster healing time in SN
Verdù So-riano et al.[430]	2010	Mixed chronic ulceration	103	Silver nanocrystal-line (SN) vs CT	42.5	SN showed a signifi-cant ($p<0.05$) positive difference in the healing curves as compared to CT
Banchellini et al.[433]	2008	DF pre-ulcerative condition	30	Nano-liposomes charged with phosphatidilcoline vs conventional treatment (CT)	42	Significant ($p<0.05$) improvement in skin hardness, moisture and TEWL
NT nanotechnology; DF diabetic foot; DFU diabetic foot ulceration; VLU venous leg ulceration; TEWL transepidermal water loss						

Table 21: Table of evidence - physical therapies

Therapy	Indication	Level of evidence	Comments
ESWT	DFU, PU, VLU	1C	Few studies, very good risk/benefit ratio, can be considered an adjuvant therapy in a wide range of clinical conditions
EF	DFU, PU, VLU	1C	Good evidence of effectiveness in experimental models, but few studies with poor-quality in clinical fields, useful in stimulating wound edges' progression
MF	DFU, PU, VLU	1C	Relatively recent evidence, still few studies, few good-quality clinical trials, solid evidence in bone fracture repair, some indication of anti-inflammatory effects, evidence in stimulating collagen synthesis and granulation tissue formation
PBM	DFU, PU, VLU	2B	Still controversial mechanisms of action, not clear the full range of effects on wound repair, few studies of low- or very low-quality, some evidence of antibacterial activity and pain reduction
NT	DFU, MU, VLU	1C	Promising results, but a sufficient evidence base is not yet available, good results in prevention of DFU and antibacterial activity, few RCTs
ESWT extracorporeal shock wave therapy; EF electric fields; MF magnetic fields; PBM photobiomodula-tion; NT nanotechnologies; DFU diabetic foot ulceration; MU mixed ulceration; PU pressure ulcers; VLU venous leg ulceration			

a significant ($p<0.05$) improvement in skin moisture, skin hardness and trans-epidermal water loss (TEWL) in NLPP-treated patients compared to the control patients.[433]

In Table 20, a synopsis of the studies on NT in relation to wound management and repair is provided.

Smart technologies in wound management

Introduction

The prevalent and long neglected diabetic foot ulcer (DFU) and its related complications rank among the most debilitating and costly sequelae of diabetes. Currently, every six seconds somebody is diagnosed with diabetes, and every 20 seconds a limb is lost because of it.

Diabetes foot care costs represent the single largest category of excess medical costs associated with diabetes. It is estimated that one-third of all diabetes-related costs are spent on diabetic foot care in the United States, with two thirds of these costs incurred in the inpatient settings, constituting a substantial cost to society.[434,435] The lifetime incidence of diabetic foot ulcers (DFU) has been estimated to be between 19% and 34% among people with diabetes.[436] One in every 11 adults has diabetes (425 million worldwide), according to the latest report by the International Diabetes Federation (IDF) in 2017.[437] Ulcers requiring acute care can result in treatment costs of up to US$28,000 per event, varying with the severity of the wound.[438] Unfortunately, even after the resolution of a foot ulcer, recurrence is common and is estimated to be 40% within one year after ulcer healing, almost 60% within three years, and 65% within five years, according to the recent study by Armstrong et al.[436] Perhaps no complication of DFU is more significant than its associated 10–20% rate of lower extremity amputation (LEA); approximately 70% of such amputations are potentially preventable.[439] The consequences of DFU are not limited to amputation. In particular, DFU may put patients at risk for other adverse events, such as falls, fractures, reduced mobility, frailty and mortality.[440–442] For example, mortality after amputation because of diabetes is estimated to be 70% at five years, which exceeds many common cancers, such as breast cancer and prostate cancer.[443]

Fortunately, we live in a world where technology is increasingly being integrated into every aspect of our lives, representing an opportunity for creative solutions to prevent this devastating condition. In particular, thanks to the new 'smart' sensors and communication technologies available today, new opportunities have opened to smartly manage DFUs with personalised screenings and timely interventions. More importantly, with the given advances in wearable technologies and telecommunication, patients and their caregivers can be more engaged in enabling an optimised healthcare ecosystem. This chapter aims to provide an overview of the recent technological advances from wearables to mobile health, telemedicine and 'internet of things' with a great potential to revolutionise the smart management and/or effective prevention of DFU and its consequences, including lower extremity amputation. While the major focus of this chapter is on managing DFU, other types of chronic wounds including venous leg ulcer (VLU), pressure ulcers (PU), and some types of acute wounds, such as severe burns, are also discussed.

Even, if a DFU is successfully treated, patients may often suffer from significant lower extremity muscle atrophy, in particular if irremovable

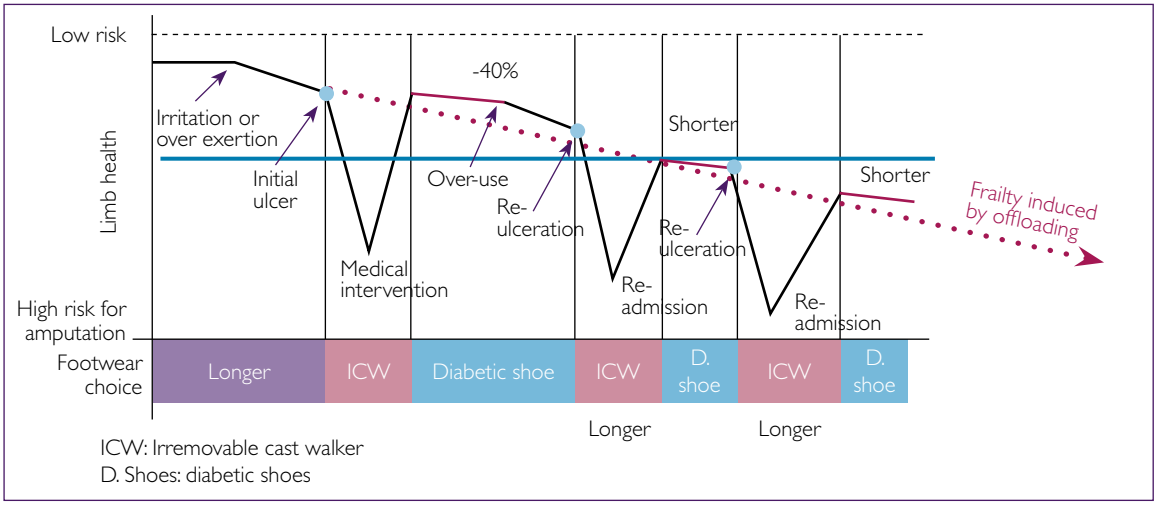


Fig 24: Pathway toward amputation because of frailty induced by offloading[444]

offloading was used for the duration of more than four weeks.[441] This may lead to premature frailty and reduced mobility. Figure 24 illustrates a general limitation of current DFU management inspired by a study conducted by Roser et al.[444] This illustration is intended to highlight the high frequency of re-ulceration (40% within 12 months of treatment[436] for people in diabetic foot 'remission'[445]), which places them at higher risk of future amputation. In particular, a recent study, in which daily physical activities of people with DFU was monitored every week, it was found that the activity level in those who were treated with irremovable offloading will be reduced on average by 49%. This may lead to muscle wasting in the lower extremities.[441] The amount of activity observed in the patient population after four weeks with treatment by offloading was less than 3000 steps per day, which is almost the same level of activity observed in a frail population.[446] This suggests potential frailty induced by offloading, which may have serious long-term consequences, including higher frequency of recurrence of ulcers, falls, higher risk of adverse events, disability, hospitalisation and mortality.[447] These data suggest an important gap in effectively managing and preventing DFUs, particularly in community hospitals and clinics.

In light of the impending diabetes epidemic and the high prevalence of DFU and its associated complications, the need for enhancing prevention of DFUs is clear. Thanks to the new 'smart' sensors and communication technology that is available today, new opportunities have opened to smartly manage DFUs with personalised screenings and timely interventions. With the help of automation, patients can even be prompted to check their feet, glucose level or weight and can enter the results

into mobile patient portals. Even better, they can transmit the results to their doctors in real-time. These fast-growing, low cost, and widely available resources can help predict the patient's risk of developing foot ulcers, infections, peripheral arterial disease, frailty and other diabetes associated complications, which can ultimately save limbs and lives. In the rest of this chapter, some of the emerging technologies, which could revolutionise smart management of DFU, are presented. This includes wearables used to screen mobility, management of activity, dosage, and 'internet of things' infrastructures that support the empowerment of patients and/or their care givers to effectively co-manage these chronic conditions and enable an optimised healthcare ecosystem.

The same considerations can be applied to other chronic ulcerations, like VLUs and PUs, which are the other major players in the field of chronic lesions, and which together account for the majority of ulcerated patients in the world. For all of these, the possibility of being adequately diagnosed, monitored and treated would increase significantly due to the introduction of sensors and IT. In light of this, DFUs can be used as a primer and a paradigm, which can be extended eventually to other types of ulcerations.

Wearables and applications to smartly manage chronic ulcers

Smart watches, smart pendants, other smart wearables or mobile-based applications already marketed to the young and healthy population will take on an ever-growing presence in the patient-care marketplace, including the management of the diabetic foot. Wearable devices can track nearly everything, from early stroke detection, to monitoring physiological parameters, quantifying physical activity, monitoring sleep quality, determining gait structures and standing plantar pressures and shear.[448–452] Their versatility and portability appeal to consumers and make them a consideration for insurance providers, who want to cut down on in-person visits by allowing physicians to remotely check in on patients, track patients' adherence to therapy and detect the early stages of serious medical conditions to triage those, who need an immediate supervised care. In addition, it enables the patient to receive personalised and targeted therapy and empowers them to take care of their chronic conditions themselves by engaging them in routine care and facilitates communication with their care-provider. Besides wearable inertial sensors, which are used for monitoring physical activities, gait assessment, and as a detection of falls, a variety of sensors have been designed, which facilitate monitoring of key risk factors associated with wound healing. These include pH, skin temperature, physiological stress response, moisture, oxygen, microfluidic analysis, and many more. Wearable technologies are not limited to monitoring. These technologies also enable daily interventions outside of the clinic via advanced wound dressings and nanotechnology-based therapy.

Different wearables have been designed to stimulate wound healing. These wearables include the use of electrical or mechanical stimulation, which may improve skin perfusion, smart wound dressing devices, which enable effective delivery of oxygen to the wound bed to hasten wound healing, and vacuum-assisted technology to support the closure of the wound to reduce the risk of infection. This section discusses whether or not and how such technology may assist in effective prevention and/or management of DFU or other types of wounds, including venous leg ulcers, pressure ulcers, or other chronic wounds like severe burns. Table 22 summarises the identified studies in which the benefits of wearables and advanced technologies have been compared to conventional therapy and were reported by *in vivo* testing in human subjects. Where a systematic review of RCT studies were available, only the results of the systematic reviews were summarised in this table.

Table 22: Selected studies related to wearable devices designed to stimulate wound healing and/or reduce risk of DFU/chronic wounds

Author	Year	Condition(s)	N. of Patients	Type of intervention	Primary outcome (s)	Duration of treatment (weeks)	Results
Edmonds et al.[453]	2018	DFU	240	Nano-oligosaccha-ride (sucrose-octasulfate) and SOC vs best practice (SOC)		20 weeks	18 points increase in healing rates (48% vs 30 %) (p=0.002) and 60 days shortening of healing time (p=0.029) vs control
Najafi et al.[454]	2017	Diabetes neuropathy	28	Electrical stimulation RCT design	Balance Gait Neuropathy severity Vascular health	6 weeks	Significant improvement in balance, gait, neuropathy severity, vascular health was only improved in sub-sample with peripheral arterial disease
Najafi et al.[450]	2017	Patients with history of foot ulcers	17	Smart insoles + real-time notification (case series)	Change in rate of adherence to prescribed footwear; reduction of recurrence of ulcers	Up to 12 weeks	Significant improvement in adherence for those who are receiving at least one alert every two hours; no recurrence of ulcers was reported during the follow-up period
Najafi et al.[441]	2017	Diabetic foot ulcers	49	Activity dosage (RCT design)	Success of wound healing at 12 weeks, weekly speed of wound healing	Up to 12 weeks	Significant correlation between number of daily steps with speed of wound healing irrespective of type of offloading (removable vs irremovable), significant association between duration of daily standing and success of wound healing at 12 weeks

Niederauer et al.[455]	2017	Chronic and non-chronic diabetic foot ulcers	100	Modern dressing – continuous diffusion of oxygen (RCT design)	Rate of successful wound healing at 12 weeks; time to heal	Up to 12 weeks	Significant higher rate of wound healing (46% vs 22%) with higher rate of success for chronic wounds (43% vs 14%), significant shorter time for healing
Kadry et al. 2016[456]	2016	Chronic lower limb ulcers	40	Pulsed radio frequency energy (RCT design)	Wound area reduction	6 weeks	Significant reduction in wound area compared to controls
Driver et al.[150]	2015	Neuro-pathic non-ischaemic chronic ulcers	307	Nano-technology-based therapy-integra dermal regeneration template (RCT study)	Success of wound healing at 12 weeks; weekly wound size reduction; time to health; adverse events	Up to 16 weeks (follow up for primary outcomes up to 12 weeks)	Significant higher rate of healing at 12 weeks compared to controls (51% vs 32%), significantly higher rate of weekly wound size reduction (7.2% vs 4.8%), less time for healing and less adverse events
Lewin et al.[457]	2015	Chronic venous ulcer	25	20 kHz ultrasound assisted treatment (case-control)	Success of wound healing; Rate of wound healing	Minimum 3 sessions	Significant improvement in average rate of wound healing (20.6%/week) compared to the control group (5.3%/week)
Thakral et al.[458]	2013	DFU PU VLU Leprosy ulcers Mixed Ulcers	21 RCT studies Total: 544 subjects	Electrical stimulation - RCT design	Wound area reduction (speed of wound healing)	Range from 3 weeks to 23 weeks	Significant wound area reduction compared to the controls
Rawe et al.[459]	2012	Diabetic and venous stasis ulcers	4	Pulsed radio frequency energy (case series)	Pain Reduction in wound size	6 weeks	Reduction in pain and wound size have been reported, but the sample is too small for any statistical power
Marston et al.[460]	2003	Chronic diabetic foot ulcers	314	Nano-technology-based therapy-fibroblast-derived dermal substitute (RCT study)	Success of wound healing at 12 weeks; adverse events	Up to 12 weeks	Significant improvement in rate of successful wound healing at 12 weeks compared to conventional therapy (30% vs 18%) with similar adverse events between groups

Veves et al.[266]	2001	Nonischaemic chronic plantar diabetic foot ulcers	208	Nano-technology-based therapy-bioengineered human skin equivalent (RCT study)	Success of wound healing at 12 weeks; adverse events	4 weeks	Significant improvement in success of wound healing at 12 weeks compared to control, adverse events rate was the same between groups
Smiell et al.[461]	1999	Chronic ulcers diabetic foot ulcers	4 RCT studies Total: 922 subjects	Nano-technology-based therapy-becaplermin gel (systematic review)	Rate of successful complete healing, time to complete healing	Up to 20 weeks	Significant healing rate compared to controls (50% vs 36%), reduction in the time to complete healing by 30% (14.1 vs 20.1 weeks)

DFU: diabetic foot ulcer; PU: pressure ulcer; VLU: venous leg ulcer; significant level was defined as $p<0.050$. The data with '- 'means' data is missing or not clearly reported.

Wearable device designed to stimulate wound healing and/or reduce risk of DFU

A recent systematic review by Thakral and colleagues,[458] which included 21 randomised clinical trials that used electrical stimulation for healing wounds such as DFU, VLU, PU, and mixed ulcers. This review suggested that electrical stimulation may offer a unique treatment option to hasten the heal-ing of complicated and recalcitrant wounds, improve flap and graft survival, and even improve surgical results. This systematic review concluded that electrical stimulation is effective to accelerate wound healing and increase cutaneous perfusion in human subjects. There are however, very few studies that have examined the effectiveness of electrical stimulation to prevent DFU or reduce the risk factors associated with DFU. A recent study, by Najafi et al.,[454] using a double-blind, randomised, control trial, demonstrated that daily home use of plantar electrical stimulation for people who are suffering from diabetes and peripheral neuropathy (DPN) is effective to improve plantar sensation, which is one of the key risk factors associated with DFU, as quantified by vibratory plantar threshold (VPT). In addition, this study also suggested that vascular health could be improved in the subgroup with peripheral arterial disease. Other observed significant improvements as compared to those of the control group were gait, balance and overall pain. In this study, off-the-shelf wearable technology (SENSUS, Neurometrix Inc, Waltham, MA, US) was used. This is a transcutaneous electrical nerve stimulator (TENS) system. However, the system was modified to provide electrical stimulation (~30 milliamps) to the plantar area via two electrodes placed on the hind and forefoot area rather than the leg. Considering the lack of plantar sensation in people with DPN, this configuration seems to be more acceptable and less inconvenient. This was supported by a near to 100% compliance in daily use as a therapy, according to the survey. The study consisted of a six-week treatment phase of daily-use of plantar electrical stimulations. The outcomes were assessed every two weeks.

Electrical stimulation is not the only modality that has been suggested to be effective for wound healing or reducing DFU risk factors. Other technologies, such as low frequency ultrasound,

mechanical stimulation, and pulsed radio frequency energy, have also been demonstrated to be effective in accelerating wound healing. In 2015, Lewin et al.[457] demonstrated that low frequency (tens of KHz frequency) ultrasound could improve wound healing outcomes in those with chronic venous ulcer if it was used in at least three sessions. However, this study was too underpowered to be clinically conclusive. In 2012, Rawe et al.[459] developed a lightweight battery-powered wearable device, which provides pulsed radio frequency energy for a duration of 6–8 hours. They suggested that daily use of this device for a period of six weeks could be effective to hasten wound healing. However, only four patients were tested using the device, and no control group was used as a comparator. Later on, in 2016, Kadry et al.[456] investigated the efficacy of pulsed radio frequency energy as a physical therapy modality in the treatment of chronic lower limb ulcers. Forty patients with chronic unhealed lower limb ulcers (diabetic foot ulcers) for over three months participated in this study. They randomly assigned patients to two groups. The intervention group received pulsed radio frequency with a pulse width of 400 msec, 70 pulses per second with an average power of 23w for 30 minutes, three sessions per week for six weeks, and medical care. The control group received medical care only. Their results suggested that the magnitude of the wound area reduction in the intervention group was significantly higher compared to the control group.

Very recently, the results of a prospective multicentric RCT on neuro-ischaemic DFU management using nano-oligosaccharides-impregnated dressing (sucrose-octasulfate dressing; TLC-NOSF) on top of the best practice treatment in highly specialised centres in five western European countries were published, bringing new information and evidence for the use of this component in the clinical management of chronic ulcerative pathologies in diabetic patients.[453]

In the study, which was implemented for 20 weeks, 240 DFU patients were randomised into two groups: one treated with the best standard care and the other with TLC-NOSF dressing in association with the same standard of care.

The results of this paramount study (EXPLORER), the first adequately dimensioned RCT in the field of wound dressings were extremely positive. The patients treated with TLC-NOSF showed a healing rate of 48% at 20 weeks as compared with 30% observed in the control group ($p = 0.002$). These very significant results were strengthened by those of four sensitivity analyses including a blind review done by external physicians.

Moreover, the healing time in the TLC-NOSF group showed a mean time of closure at 120 days vs 180 days in the control group ($p=0.029$). No significant differences were observed between the two groups when considering the adverse events.

This is the first time that a dressing proved its efficacy in improving healing rates and healing times in neuro-ischemic DFU, and sucrose-octasulfate dressing has been indicated as a paradigm shift in the local management of neuro-ischemic DFU

Wearable wound therapy using nanotechnology

Nanotechnology-based therapy is another emerging technology, which has been demonstrated as a promising next generation therapy to advance wound healing and cure chronic wounds. In a recent review, Hamdan and colleagues[404] highlighted the most recently developed nanotechnology-based therapeutic agents and assessed the viability and efficacy of each treatment with an emphasis on chronic cutaneous wounds. They identified four FDA-

approved therapies used for chronic cutaneous wounds, including a bioengineered human skin equivalent,[266] two dermal substitutes[460,462] and recombinant human platelet derived growth factor (rhPDGF).[461] They concluded that nanotechnology-based diagnostics and treatment approaches offer an excellent opportunity to target the complexity of the normal wound-healing process, cell type specificity, and the plethora of regulating molecules as well as pathophysiology of chronic wounds.[404] The major advantage of nanomaterials over their bulk counterparts is the versatility and tunability of the nanomaterial's physicochemical properties, such as hydrophobicity, charge and size. This allows a higher probability of interaction with the biological target and an enhanced penetration into the wound site that thus accelerates the healing process.[403]

Modern wound dressing

Modern dressings are another emerging, wearable technology that could revolutionise wound management in people with diabetes. These active dressings enable a suitable microenvironment for successful healing by controlling the level of wound moisture and absorbing excess exudate.[463] Hydroconductive dressings and biologic dressings have also proven efficacious in advancing the wound-healing process through a variety of mechanisms.[41] One of the recent developments in the area is enhancing the tissue oxygenation using dressings with continuous diffusion of oxygen. Recently, Niederauer et al.[455] demonstrated in a randomised, controlled trial model that dressings with a continuous diffusion of oxygen are effective to improve the chance of successful wound healing at twelve weeks, in particular in those with chronic wounds. In this study, 100 subjects with DFUs were randomised to receive either active continuous diffusion of oxygen (CDO) therapy using an active CDO device or an otherwise fully operational sham device that provided moist wound therapy (MWT) with-out the delivering oxygen. The results suggested that continuously diffused oxygen over wounds leads to significantly higher rates of closure and reduced closure time as compared to similarly treated patients receiving standard therapy coupled with a sham device.

Wearables to monitor risks factors associated with poor wound healing or infection

The developed technologies were not limited to measuring those risks associated with DFU. Some recent efforts have also shown the benefits of technologies in monitoring the risks associated with a delay in wound healing and/or potential adverse events, such as infection. Aligned with these efforts, Farrow et al.[464] designed a real-time sensor system to monitor bacteria levels in the wound dressings. Their device is based on impedance sensors that could be placed at the wound-dressing interface that would potentially monitor bacterial growth in real time. Impedance was measured using disposable silver-silver chloride electrodes. The bacteria *Staphylococcus aureus* was chosen for the study as a species commonly isolated from wounds. Their results suggested that the impedance profiles obtained by silver-silver chloride sensors in bacterial suspensions could detect the presence of high cell densities, which may suggest that there is a potential to create a real-time infection monitoring system for wounds based upon impedance sensing. In 2015, Mehmood et al.[465] proposed a flexible and low-power telemetric sensing and monitoring system that would enable the measuring of wound-site temperature, sub-bandage pressure and moisture levels within the wound dressing. The clinical usefulness and the impact of the device for effective management of wounds still need to be confirmed. Other studies suggest new technologies for monitoring parameters of interest associated with wound healing, including Sharp's study in 2013,[466] which suggested printed composite electrodes that enable the interference-

free pH measurement even in the presence of high ascorbic acid concentrations across a wide analytical range (pH 4–10) in simulated wound fluid. A few other studies suggest technologies for measuring physiological and climate parameters that may contribute to delayed wound healing. In 2010, Sharp et al.[467] suggested a carbon fibre sensor for electrochemical pyocyanin detection, which could be used for intelligent infection diagnosis. In another study published in 2008, Sharp et al.[468] suggested carbon fibre composites to monitor uric acid in wound fluid. However, no study has, to date, been identified to demonstrate the clinical effectiveness of these technologies for management of wounds in people with diabetes.

Wearable technologies have also been used to monitor parameters, which may indirectly impact wound healing outcomes. In 2014 Parvaneh et al.[469] suggested the use of a chest-worn sensor to monitor the physiological stress response in patients with active DFU. In this study, physiological stress was continuously monitored in twenty patients with DFU for duration of approximately 45 minutes, including waiting, dressing change and the post-dressing period. Stress was quantified using a custom algorithm based on standard deviation of R-R intervals named heart rate variability (HRV). To identify the change in the level of stress, change in HRV was compared to the baseline HRV. Medium and high-stress periods were defined when HRV was in the range of 60–85% and below 60% of baseline HRV, respectively. Their results revealed that patients with DFUs experience moderate to high stress while visiting a wound clinic. This may impact wound healing outcomes negatively. In a follow up study, Razjouyan et al.[450] used a similar wearable sensor to examine whether stress could slow down wound healing. They recruited 25 patients with DFUs and monitored HRV during pre-wound dressing, using a wearable sensor attached to participants' chest. HRVs were quantified in both time and frequency domains to assess the patients' physiological stress response and vagal tone (relaxation). Change in the wound size between two consecutive visits was used to estimate healing speed. Their results confirmed an association between stress/vagal tone and wound healing in patients with DFUs. In particular, it highlighted the importance of vagal tone (relaxation) in expediting wound healing. It also demonstrated the feasibility of assessing physiological stress responses using wearable technology in an outpatient clinic during routine clinic visits.

Wearables to personalise wound care management

Recently, some efforts have been made to personalise wound care. These efforts were mainly based on measuring parameters such as moisture, pressure, temperature and pH inside the dressings, which have been shown to be indicative of the healing rate, infection, and wound healing phase.[470] In 2014, Mehmood et al.[465] proposed a low-power, portable telemetric system for wound condition sensing and monitoring, which enables the measurement and transmission of real-time information about the wound-site temperature, sub-bandage pressure and moisture level within the wound dressing. The proof of concept of the system was assessed on a mannequin leg using commercial compression bandages and dressings. A number of trials on a healthy human volunteer were performed where treatment conditions were emulated using various compression bandage configurations. They have also evaluated the level of comfort for the participants. Their results suggested that this non-invasive and flexible sensing device enables wireless reporting of instantaneous changes in bandage pressure, moisture level and local temperature at a wound site with average measurement resolutions of 0.5mmHg, 3.0% RH, and 0.2ºC, respectively. Effective range of data transmission was 4–5 metres in an open environment. However, the results

need to be confirmed in a patient population, and its validity for assessing wound healing should be determined. In 2016, Milne and al.[471] proposed a wearable sensor to measure wound moisture status without disturbing or removing the dressing. The technology was designed to determine when the dressings needed to be changed. In an observational study with no alteration of the usual care, it was demonstrated that of the 588 dressing changes recorded, 44.9% were performed when the moisture reading was in the optimum moisture zone. Of the 30 patients recruited for this study, eleven patients had an optimal moisture reading for at least 50% of the measurements before the dressing change. They concluded that a large number of unnecessary dressing changes are being made. Thus, this technology may reduce the likelihood of unnecessary dressing changes and, thus, limit the disturbance of the healing process. Other measurements, which could enable personalised wound care, are wound fluid pH and wound matrix metalloproteinases enzyme activity.[472] As described above, a few studies have suggested wearable sensors to measure these metrics. However, to date, we could not identify any published papers demonstrating their clin-ical validity.

Some other emerging technologies to improve management of wound healing are based on capturing wounds image and analysing healthy wound healing processes. In 2015, Aldaz et al.[473] presented the development and assessment of a hands-free image capture system named SnapCap. By leveraging the sensor capabilities of Google Glasses, SnapCap enables a hands-free digital image to be captured, tagged and transferred to a patients' electronic medical record (EMR). To evaluate of the perceived benefit of their system, they interviewed sixteen wound care nurses. They report that the features preferred by the wound care nurses are hands-free navigation features, such as barcode scanning for patient identification, double-blinking to take a photograph, and the ability of the system to allow sterile images to be captured.

Mobile health (m-health) to manage non-healing wounds

Cell phones and other consumer digital technologies have emerged as potentially powerful tools to empower patients to take care of their own chronic condition from accurate diagnosis to patient education, engaging them in their own care, monitoring the risk of DFU, and determining any complications associated with wound healing. However, many of these technologies are still in the early stages. To improve the classification of wounds in community health clinics, Ge et al.[474] developed a wound information management system that was created using an acquisition terminal, wound descriptions, a data bank, and related software. In this system, a 3G mobile phone was applied as acquisi-tion terminal, which could be used to access the data bank and determine wound classification. However, no clinical study was conducted to demonstrate its clinical value. In 2015, Parmanto et al.[475] proposed a mobile app to support self-skincare tasks, skin condition monitoring, adherence to self-care regimens, skincare consultations, and secure two-way communications between patients and clinicians. The system may help in supporting self-care and adherence to care management while facilitating communication between patients and clinicians. Wang et al.[476] developed an app for analysing wound images. The developed app enables capturing wound images with the assistance of an image capture box. The software allows for the detection of the wound boundaries and determination of healing status. Mammas et al.[477] proposed a smart phone as a mobile-telemedicine platform. They evaluated the feasibility and reliability of a platform based on simulating experimentation by ten specialists, who remotely examined a diabetic foot using the

proposed mobile platform. They demonstrated that this platform allowed for the remote classification of a wound as well as an evaluation of the risk of amputation with an accuracy of 89% on average. In addition, the acceptability of the platform was in range of 89–100% among specialists. A similar concept was proposed by Foltynski et al.[478] in which an app was designed to measure the wound area, send the data to a clinical database, and create a graph of the wound area changes over time. The team also suggested an elliptical method[479] to improve wound size estimation from 16 different wound shapes. Sanger et al.[480] proposed a mobile app to engage patients in wound tracking, which in turn could assist in identifying signs of wound infection. However, their study was limited to a design concept with no clinical study. An interesting application of mHealth was proposed by Quinn et al.[481] to improve the patient referral strategy from tertiary centres. Specifically, they proposed using mobile phone technology to decentralise care from tertiary centres into the community, improving efficiency and patient satisfaction, while maintaining the patients' safety. Their designed app enables the remote collection of patient wound images, prospectively, as well as the transmission of the image attached to clinical queries between the primary healthcare team to the tertiary centre. They tested this platform with five public health nurses in geographically remote areas of the region. They demonstrated that images could be transmitted securely and that the app is safe and reliable and could be used for remote wound bed assessment and to determine skin integrity and colour. They concluded that with minor adjustments, this application could be used across the community to reduce the necessity of patient visits at vascular outpatient clinics while still maintaining active tertiary specialist input to the patients' care.

Telemedicine/tele-monitoring in wound management

Telemedicine, also referred to as telehealth, telecare, remote care, or virtual care, has been defined as 'medicine practiced at a distance' and is mainly used for remote management of chronic disease.[482] The telemedicine interactions between the patient and the healthcare provider have so far been of two types, either taking place synchronously, in real-time through videoconferencing or the telephone or asynchronously, such as store-and-forward transmission of data using email. Monitoring applications have been either automatic (e.g. passive monitoring of activity using room sensors) or have required the patient to do something (e.g. transmit plantar wound pictures using buttons on a tablet or smartphone). Educational applications have employed specially designed home devices or depended on web access from PCs or smart phones.[482]

In recent years, thanks to the advances in telecommunication systems, telemedicine has emerged as one of the potentially most economic and patient-friendly methods for delivering follow-up care to patients with wounds.[483] In addition, considering that some wounds may take months to heal and can also lead to osteomyelitis and amputation, another way to track wound healing rather than traditional clinic visits is desperately needed. In particular, due to the shortage of wound care specialists (it is estimated that less than 0.2% of all nurses in the USA are wound care specialists), it is necessary to lessen the need for consultation with wound care specialists, which has promoted the application of telemedicine particularly in remote/rural areas. In addition, in the current organisation of wound management, it is often reported that the collaboration between primary healthcare personnel and wound specialists is not sufficient. This may cause problems with regard to ensuring timely referral practices between primary and wound specialist and healthcare services. A severe consequence of this is an increased risk of emergency and hospital admission.[435,484] Furthermore, telemedicine may assist in improving

communication with wound care specialists, improving access to care, optimising patient referral, reducing the need for transportation to outpatient clinics, and potentially reducing the cost of care while improving patient satisfaction and quality of care.

Increased connectivity among people via use of smartphones, tablets and the internet has made it possible to develop and implement telecare programmes for people with diabetes and foot problems, varying from the monitoring of wound healing to consultations concerning the prevention of DFU. The use of telemedicine to manage chronic conditions is increasing worldwide due to its promise of cost-effectiveness, decreased resource consumption, as well as timely and patient-centred care. While the use of telemedicine for managing chronic conditions, such as asthma, heart failure, COPD, diabetes, and hypertension, has been well established, high-quality studies on the effectiveness of telemedicine to manage diabetic foot and wound management are scarce, which make the generalisability of most findings limited. However, in this section, an overview of telemedicine applications available for the management of DFU, which were identified via our systematic search, is provided.

Telemedicine for wound care: patient acceptability and providers' perceptions of benefits

There are very few studies that examined how the incorporation of telemedicine impacts the experiences of the patients, who are receiving wound care. In-depth knowledge of patients' experiences as well as perceptions of the care providers regarding the implementation of telemedicine intervention can help evaluate whether the use of telemedicine is an appropriate method to improve wound care. In 2016, Strom et al. used individual semi-structured interviews to study patients' experiences with telemedicine during their follow-up wound care as compared to traditional care. A total of 24 patients were recruited and randomised in the intervention group (use of telemedicine, n=13) and the control group (use of traditional care, n=11). The results demonstrated clearly that competence in the wound management by the healthcare professionals was of great importance to patients' experience of security during their wound care, irrespective of the type of follow-up care. Specifically, patients lost confidence in the wound-care process if the doubted the competence of the healthcare professionals and if the continuity of care was absent. They concluded that telemedicine can be an important supple-ment in the wound care process, but its efficacy will depend on whether it is used as intended and whether continuity of care is present. They also recommended that education and practical training in the use of telemedicine should be provided to all healthcare professionals in primary healthcare and not simply to a few. In 2015, Rasmussen et al.[485] explored the key organisational factors in the suc-cessful implementation of telemedicine in wound care. They conducted eight semi-structured interviews, including individual interviews with leaders, and an IT specialist, as well as focus group inter-views with clinical staff. A qualitative data analysis of the interviews was performed in order to analyse the healthcare professionals' and leaders' perceptions of the organisational changes caused by the implementation of the intervention. They reported that the telemedical setup enhanced confidence among collaborators and improved the wound care skills of the visiting nurses in the municipality. The need for a focus on the training of the visiting nurses was highlighted as a key factor in the success of implementation. Several concerns have also been identified, such as lack of multidisciplinary wound care teams, patient responsibility and a lack of effective patient interactions with the physician. Finally,

this study concluded that telemedicine may provide an additional option to offer patients after an individual assessment of their healthy condition. In 2017, Kolltveit et al.[486] conducted a qualitative study in ten focus groups to identify the perceptions of healthcare professionals in different work set-tings concerning the facilitators support of engagement and participation in the application of telemedicine. They identified four key conditions for successful implementation of telemedicine for wound care, including user-friendly technology and training, a telemedicine champion located in the work setting, support of committed and responsible leaders, and effective communication channels at the organisational level. They concluded that attention to the distinct needs of each staff group is an essential condition for effective implementation of telemedicine in wound care.

Does telemedicine improve wound care and wound outcomes?

A few studies have examined the effectiveness of telemedicine to improve wound outcomes and wound care. However, convincing evidence to support the clinical efficacy of telemedicine in wound management as compared to traditional care is still lacking. In 2015, Zarchi et al.,[487] using a pro-spective cluster, controlled study, examined whether advice on wound management provided by a team of wound-care specialists via telemedicine would significantly improve the likelihood of wound healing compared with the best available conventional practice. A total of 90 chronic wound patients in home care, of which 50 received telemedicine care and 40 received the conventional care, were recruited. During the one-year follow-up, complete wound healing was achieved in 35 patients (70%) in the telemedicine group compared with 18 patients (45%) in the conventional group. After adjusting for several covariates, between-group differences were statistically significant with an adjusted hazard ratio of 2.19. They concluded that telemedicine is effective to connect homecare nurses to a team of wound experts in order to improve the management of their chronic wounds. In 2013, Vowden et al.,[488] proposed the use of digital pen-and-paper technology and a modified smartphone to remotely monitor and support the effectiveness of wound management in nursing home residents. To demonstrate the effectiveness of this programme, they conducted a randomised, controlled pilot study conducted in 16 selected nursing homes. In these, 39 patients with a wound were identified. They reported that the proposed telemedicine care delivery system provided improved patient outcomes and that it may offer cost savings by improving dressing product selection, decreasing inappropriate onward referral and decreased healing time. They have also reported that, despite initial anxiety related to the technology, most nursing-home staff found the system of value, and many were keen to see the trial continue to form part of the routine patient management. In 2009, Terry et al. compared wound outcomes in subjects randomly assigned into three groups: Group A (n=40) received weekly visits via telemedicine consulting with a wound care specialist, group B (n=28) had weekly visits with in-person consulting with a wound specialist, and group C (n=35) received the usual and customary care. Their results suggested that group A had increased time for healing, increased length of stay, increased costs, and more visits as compared with groups B and C despite a similar wound status in all groups. They did, however, conclude that telemedicine is a useful communication tool in wound management, but its efficacy depends on the wound size and type. They also recognised several limitations in their study, including insufficient power and a large distribution in the wound severity with-in their recruited subjects. In 2015, using a RCT study design, Ramussen et al.[489] compared telemedical

and standard outpatient monitoring in the care of patients with diabetic foot ulcers. A total of 401 cases with DFUs met the study inclusion and exclusion criteria and were randomised to telemedicine (n=119) or standard outpatient monitoring (n=181). Telemedical monitoring protocol consisted of two consultations in the patient's own home and one consultation at the outpatient clin-ic. Standard practice consisted of three outpatient clinic visits. The three-visit cycle was repeated un-til the study's endpoints. The study's endpoints were defined as complete ulcer healing, amputation or death. While a trend in increasing wound healing ratio (hazard ratio=1.11) and reducing foot am-putation (hazard ratio=0.87) were found in telemedicine monitoring, these trends were not statistical-ly significant ($p>0.40$). However, a mortality incident was observed in the telemedicine group (hazard ratio=8.68, $p<0.001$). They recommended further study to better identify these patient subgroups that may have a poorer outcome through telemedicine monitoring. In a critique of the Ramussen et al. study, Muller et al.[490] shared their experience implementing telemedicine with home nurses in France. They claimed that they stopped their trial prematurely because they realised that the homecare nurses and private nurses involved in their study were not adequately trained to deal with chronic wounds, and such training is essential for a successful implementation of telemedicine in wound care. They also claimed that this challenge was not addressed in the Ramussen et al. study. Furthermore, they claimed the quality of data and wound pictures, which are needed for an effective judgment, were not controlled in the study done by Ramussen et al. These factors may partly explain the poor outcomes observed from the telemedicine implementation in the Rasmussen et al. study. They further concluded that a successful implementation of telemedicine in wound care would re-quire initial training and ongoing support.

Does telemedicine optimise wound care delivery and the quality of care?

Inefficiencies and communication gaps continue to hamper effective delivery of care and progress towards improving the quality of healthcare and improving the population's health outcomes at a lower cost.[491] With the rapid evolution in the healthcare industry, healthcare delivery organisations are leveraging innovative solutions to meet these challenges. Several studies have suggested that telemedicine is an effective tool to improve care access for patients with a need for wound care and a facilitation of the communication between wound care specialists and patients. In 2017, Turnin et al.[492] examined whether telemedicine could improve healthcare access in rural areas for the management of DFUs. A vehicle was equipped with a satellite dish and medical equipment for screening ophthalmological, renal, vascular, and neuropathic damage and assessing the level of risk of diabetic foot ulceration. Onboard, a nurse performed some or all of the tests on patients, who have received no diabetes care review for over a year. The data was entered into a computer and transmitted via satellite for interpretation by designated specialists. The results were sent to patients, general practi-tioners (GPs), and diabetologists. Over approximately three years, 228 screening days were performed in six rural departments, in which 1545 patients were screened in whom 93.4% were diagnosed with type 2 diabetes. Pathologies were detected in 17–32% of the tests including 18.7% diabetic retinopathy, 31.9% microalbuminuria, 17.2% lower limb arteriopathy, 28.3% peripheral neuropathy, and 28.2% high risk of foot ulceration (grade 2: 20.6% and grade 3: 7.6%). They concluded that telemonitoring created an opportunity to screen a larger number of patients who are in need of urgent care and thus helped improve healthcare access through its innovative

organisation and the use of satellite technology. In 2016, Kolltveit et al.[493] explored healthcare professionals' experiences in the initial phases of introducing telemedicine technology in 10 different wound care groups, which included home-based care, primary care and outpatient hospital clinics. The participants reported experiencing meaningful changes to their practice arising from telemedicine, especially associated with increased wound assessment knowledge and skills and improved quality of documentation. They concluded that using a telemedicine intervention enabled the participating healthcare professionals to approach their patients with diabetic foot ulcers with more knowledge, better wound assessment skills and increased confidence.

Does telemedicine reduce the cost of wound care?

The main purpose of telemedicine is to facilitate a productive interaction between the patient and the healthcare provider in order to achieve improved treatment results and lower treatment costs. While, as described above, several studies have examined the benefit of telemedicine to facilitate interaction between patients and specialists and the potential benefits with regard to improved out-comes and timely care, very few studies have examined whether telemedicine could also reduce the cost of care as compared to conventional face-to-face patient consultation. In 2013, Sparsa et al.[494] proposed the use of telemedicine to manage chronic wounds (leg ulcers, pressure ulcers, and diabetic ulcers) in older adults living in retirement homes. Specifically, they explored whether telemedicine intervention for wound care could reduce the number of ambulance transportations. Of the 40 establishments invited to take part, 22 agreed to do so, but only the first 10 respondents were accepted for participation in their study. Each participating establishment was provided with a digital camera and its own secure e-mail address in order to allow photographs to be sent anonymously. To demonstrate the effectiveness of their telemedicine programme, they documented the number of tele-expertise consultations provided, the chronic wound type, the number of hospitalisations or medical consultations, and the number of ambulance trips avoided over the two years of follow-up. During this period, photographs of 34 patients presenting 26 chronic wounds, including 10 pressure ulcers, two diabetic feet and 14 leg ulcers, were sent by the recruited establishments to receive telemedicine consultations. They concluded that this programme helped avoid 20 trips for patients over a two year period, and enabled rapid hospitalisation of nine patients in the university hospital, which in turn helped to provide timely and optimised chronic wound management for patients residing in establishments for the elderly. In 2008, Dobke et al.[495] evaluated the impact of the telemedicine consultations on patients with chronic wounds by recruiting 30 patients from long-term care skilled nursing facilities, referred to the ambulatory wound care programme for wound assessment and preparation of management plans. To facilitate communication with a surgical wound care specialist, telemedicine feedback was provided prior to the face-to-face consultation for 15 randomly selected patients out of 30 recruited patients. The telemedicine consult included a virtual consultation with a field wound nurse, who provided remote wound assessment, described the rationale for the suggested wound management with an emphasis on wound risk projections, and explained the prevention and benefits of surgical intervention. The telemedicine impact was measured by assessing the duration of the subsequent face-to-face consultation and patient satisfaction with further care decisions as well as by a validation of a decisional conflict scale. Their results suggested a significant reduction in the duration of the face-to-face consultation time on average by 70% and an increase in the patient satisfaction rate by 46% on average.

They concluded that telemedicine consultations preceding face-to-face evaluations improved patients' satisfaction and understanding of their care as well as an in-crease in the perception of a shared decision-making process regarding the wound care. In 2016, Fasterholdt et al.[496] examined the cost-effectiveness of telemedicine of DFU patients using a RCT study design. A total of 374 patients were randomised to either telemonitoring or standard monitoring groups. Telemonitoring consisted of two teleconsultations in the patient's own home and one consultation at the outpatient clinic. Standard monitoring consisted of three outpatient clinic consultations. Total healthcare costs were estimated over a six-month period at the individual patient level from a healthcare sector perspective. Amputation rates were similar in the two groups; however, a reduction of costs—on average by €2039 per patient—was observed, thanks to telemonitoring care. However, the observed reduction in cost was not statistically significant, and it was therefore concluded that a telemonitoring service in this form had similar costs and effects as standard monitoring. In 2007, Litzinger et al.[497] examined the potential benefits of telemedicine with regard to reducing the need for wound ostomy continence (WOC) nurses' visits over a two-year prospective study design. In their study, home health aides, specifically trained in telehealth technology, assisted with the evaluations of severe wounds using video teleconferencing (VTC) equipment and advanced camera technology that enabled the WOC nurse to evaluate wounds from a remote location. This decreased the travel time for the WOC nurse, increased the frequency of specialised wound consultations, and facilitated the development of comprehensive treatment plans for multiple patients. To estimate the cost benefits from telemedicine, they recruited 35 patients receiving multiple wound care evaluation, averaging seven visits in the first year to 11.3 visits in the second year, with a total number of virtual visits of 470. They reported that nursing visits saved by the video programme totalled 421.2 hours, reducing the healthcare costs by $9,449. Miles-not-travelled totalled 30,500, which reduced the costs by an additional $11,875.87 (mileage reimbursement), and the travel time saved totalled 916.8 hours, which reduced the costs still further by $20,850. After deducting the administrative cost, they claimed that the net saving of this programme was $25,208. However, the costs of the equipment were not factored into the savings.

Is telemedicine as reliable as the in-person visit for purpose of wound care?

Very few studies have compared telemedicine care and in-person care head-to-head. Telemedicine for wound care is mainly dependent on the quality of the wound images. Even with high-quality pictures, some valuable information needed for care decisions may be limited in order to accurately determine the need for debridement or to detect signs of infection. In 2011, Bowling et al.[498] examined the ability of wound inspections using wound images in comparison with in-person wound inspections. They requested two clinicians to document some primary, clinically relevant features by reviewing 12 different wound images captured using a novel wound imaging system, which provides three-dimensional wound images, including wound area and depth. As a validation, the wounds were also inspected in a face-to-face consultation, and the results were compared via the written notes. They reported an overall agreement between the remote and in-person assessments. However, a lower degree of agreement was identified with regard to the subjective clinical assessments, such as the value of debridement to improve healing, which was linked with the limitation of imaging techniques to capture certain characteristics, such as moisture or exudation. It was, however, reported that clinicians gave positive feedback on visual fidelity and concluded that the

three-dimensional wound images could accurately measure and assess a diabetic foot wound remotely. In 2007, Binder et al.[499] conducted a case series study including 16 patients with 45 leg ulcers of different origins. After an initial outpatient visit where the leg ulcers were assessed and classified, teledermatological follow-ups were performed via home care nurses. Relevant clinical information, and one to four digital images of the wound and surrounding skin, were transmitted weekly via a secure website to an expert in the wound care centre. The expert assessed the wound and made therapeutic recommendations. They claimed that 89% of transmitted images (644 out of 707) had excellent or sufficient quality for providing confident therapeutic recommendations. They concluded that the acceptance of telemedicine in wound care for recommendation of treatment by wound experts is very high, and that telemedicine offers great potential for long-term wound care.

'Internet of things' and remote management of wounds

One of the fastest developing infrastructures, promising to revolutionise the wound care industry, is the 'internet of things' (IoT).[500] It is expected that 50% of healthcare over the next few years will be delivered through virtual platforms. This has accelerated the development of a new market named 'digital wellness', which combines digital technology and healthcare.[500] Digital technology-based healthcare is regarded as a natural and ultimate choice for remote, home-based, and long-term care for patients with chronic conditions due to its low cost, high accuracy and continuous monitoring and tracking capabilities. The IoT involves a system of devices, machines, or anything with the ability to transfer data without the need for a human to implement the communication.[501] Fuelled by the recent adaptation of a variety of enabling wireless technologies, such as radio-frequency identification (RFID) tags and wearable sensor and actuator nodes, the IoT has stepped out of its infancy and is the next revolutionary technology in transforming the internet into a fully integrated 'Future Internet'.[501] As we move from www (static pages web) to web2 (social networking web) to web3 (ubiquitous computing web), the need for data-on-demand using sophisticated intuitive queries increases significantly. What has made IoT the next big thing is not just its machine-to-machine component but the potential of sensor-to-machine interactions. With the increasing development of health sensors, there is a growing opportunity to utilise the IoT for medical data collection and analysis. It is expected that an integration of these tools into the healthcare model has the potential of lowering annual costs for chronic disease management by close to one-third.[502] The use of the IoT for medical applications is, however, still in infancy. In particular, our systematic search did not identify any studies related to the application of IoT for management of DFUs. However, significant business decisions have been undertaken recently by major information and communication technology (ICT) players, like Google, Apple, Cisco, and Amazon, to position themselves in the IoT landscape. For example, in 2014, Novartis was working with Google on sensor-technologies, such as the smart lens and a wearable device to measure blood glucose levels.[503] In 2017, Amazon teamed up with Merck and Luminary Labs on an effort called the Alexa Diabetes Challenge, with the goal of finding the ultimate way to monitor diabetes using voice-enabled solutions.[504] As the IoT continues to develop, further potential is estimated to be developed to facilitate the management of chronic conditions at home including effective and timely management of diabetic feet at risk as well as facilitating the delivery of care for accelerated wound healing.

Conclusions

We live in a world where technology is increasingly

Table 23: Table of evidence-smart technologies

No.	Therapy	Indication for use	Level of evidence (for each indication)	Comments
1	Electrical stimulation	Wound healing DFU PU VLU Mixed ulcers	1B	There is a clear effectiveness evidence, including a systematic review of 21 RCT studies that confirmed benefit and safety of TENS to accelerate wound healing irrespective of the type of ulcers. The major hurdles seem to be poor adherence to regular therapy and difficulty of stimulation parameters adjustment by none-tech savvy patients. Thus, successful implementation at large remains unclear
2	Electrical stimulation	Improving postural control and gait	2A	Recent RCT studies confirmed acceptability, safety, and efficacy of TENS for use to improve balance, gait, and skin perfusion. It seems delivering electrical stimulation via plantar region could improve acceptability and adherence particularly among people with a loss of plantar sensation, who may not feel uncomfortable tingling caused by the electrical stimulation
3	Nanotechnology-based therapy	Wound healing —chronic DFU, deep wounds, ischaemic wounds	1A	Several level one evidence studies, including few systematic reviews, are supportive of the benefit of dermal substitutes and its low risks. However, there are very few comparative studies to demonstrate which dermal substitute product is superior to the others. While in low complicated wounds, there is no noticeable difference between products, it seems the difference is more pronounced for complicated wounds, such as ischemic wounds. However, most studies excluded those with ischaemic wounds, which makes a fair comparative comparison difficult
4	Ultrasonic assisted treatment	Chronic VLU	2A	There is level two evidence (case-control) indicating the effectiveness and the low risk in its ability to accelerate wound healing
5	Pulsed radio frequency energy	VLU	1C	There few studies including a recent level one study (RCT trial) supporting the safety and effectiveness of this therapy to speed up wound healing
6	Active dressing with continuous diffusion of oxygen	Chronic and non-chronic DFU	1C	A recent RCT study and multicentre study is supportive for benefit of active dressing with continuous diffusion oxygen to speed up wound healing. However, more independent studies are needed to confirm the effectiveness of such therapy
7	Physical activity dosage management	DFU	2B	There is a recent RCT study supporting the importance of managing the dosage of physical activity including the total number of daily steps and standing bouts to hasten wound healing. However, more studies are required to confirm the ease of implementation for this guideline to hasten wound healing
8	Stress man-agement	DFU	2C	Few recent studies suggest that stress management could speed up wound healing. However, there is no level one study to confirm the effectiveness of implementing stress management strategies to speed up wound healing

9	Mobile health	DFU	2C	Few recent studies have suggested that mobile health and smart phones could assist in improving adherence to therapy, which in turn could assist in speeding up wound healing, reducing costs, improving patients' satisfaction, monitoring outcomes, and prevention of ulcers. However, most of the studies are underpowered or poorly designed. There is no level one study to date to confirm the benefits and disadvantages
10	Telemedicine	DFU, VLU, PU, and mixed ulcers	2B	Several recent studies suggested that telemedicine could reduce costs, improve outcomes and improve patients' satisfaction. However, results are still controversial. It seems the key challenges are having access to high-quality images, which are essential for effective decision-making as well as adequate training for the staff, which is essential for successful implementation
11	Sensorised dressing		2C	Few recent studies have suggested that sensorised dressing could assist in reducing the cost of wound healing by reducing the number of dressing changes, and it could assist in reducing adverse events, such as infection, which potentially would accelerate wound healing. However, to date, no level one study exists to confirm the benefit versus the disadvantages and cost
12	'Internet of things'	DFU, VLU, PU, Mixed Ulcers	2C	Recent developments in the area of 'Internet of Things,' including voice-enabled technologies, have opened up new op-portunities to effectively manage wound, assist care givers, improve communi-cations between care pro-viders and pa-tients/caregivers and po-tentially reduce costs. However, to date, there is no quality study to confirm the benefit vs disad-vantages/cost.

being integrated into almost every aspect of our lives. With the miniaturisation of processors, advancements in sensing technologies, consistent availa-bility of electrical power, ubiquity of access to the internet, and significant strides in machine learning and artificial intelligence, new emerging solutions have been developed to improve healthcare delivery, patient satisfaction, and the population's health across different disciplines while simultaneously reducing the cost of care. Recent studies have suggested that technologies are effective to promote patient involvement, care coordination, and effective communication between patients and caregivers. Technologies, such as telemedicine and wearables, enable a reduction of in-person visits and allow physicians to remotely check in on patients, track patients' adherence to therapy, and detect early stages of serious medical conditions and triage those who are in need of immediate supervised care. Technology can be used to supplement healthcare provided wound care by offering both educa-tional and motivational support. The advances in sensing technologies enable physicians to collect valuable objective data from wounds, such as moisture levels, pH, temperature, and many more, to track healthy wound healing, reducing unnecessary wound dressing change, providing timely intervention to prevent infection and reducing the likelihood of amputation. While, the application of such technology for effectiveness of wound care is still in its infancy, and its cost effectiveness is still debated, by the exponential speed of technology development and the exponential increase in technology investment for healthcare applications, it is anticipated that healthcare and care delivery

for chronic conditions, such as the diabetic foot, will be dramatically changed in the near future.

The economic scenario

Wound healing is a complex cascade of events that have a significant impact on patients, society and the economy. Across Europe, 2–4% of healthcare expenditure is spent on wounds; in the US, wound care affects 5.7 million people (~2% of the population) at an annual cost of US$20 billion.[505] The mean cost of treating wounds in Europe ranges from €6,000 to €10,000 per year.[506] A recent study performed in Wales showed that the cost of managing patients with chronic wounds is 5.5% of the total healthcare expenditure. Most of the costs are accumulated by hospital stays and nursing time dedicated to treat patients in the hospital or at home while the materials, such as dressings, represent a smaller portion of the total costs.[507] The costs of wound management are different with respect to wound type, complexity and site of care. In the US, the average cost of VLUs amounts to $4,000 per month per patient; the mean cost of DFUs in 2012 ranged from $9,650 to $19,431.[508] In the US, the cost of treating pressure ulcers (PUs) is estimated to be $11 billion/year.[509] In Europe, the cost of managing DFUs is €4–6 billion/year.[506] Furthermore, an important issue is represented by the incidence of complications with a significant impact on patients and the healthcare system; these constitute a third of the cost drivers after hospitalisation and nursing time. These complications, such as infections, may lead to hospital admission, surgical intervention, and extended or increased use of resources.

Cost items are direct costs, such as dressings and devices, diagnostic equipment, clinician time, hospital/clinical overheads (e.g. administration services, building costs, etc.) and transport of the patient to the healthcare services. Indirect costs include the loss of income by the patients and/or their caregivers due to reduced time or ability to work, and costs due to a reduced ability to undertake domestic responsibilities[510] (Table 24). An important driver of cost is represented by the necessity of changing dressings several times during the week. The wound care also creates a human cost, such as a decrease in the physical, mental and social wellbeing that can affect families and caregivers, as well as the patients. Wound management is complex, prolonged and expensive. In this scenario, it is necessary to reflect on the role and contribution of advanced wound dressings. If the advanced treatments are often more expensive than traditional ones, it might make sense to use these products when the traditional therapy is not efficient and effective and has not reached the defined clinical and economic outcomes.

Since the turn of the 20th century, medical innovation has produced extraordinary improvements both in the diagnostic fields and in the therapeutic fields, contributing to an improvement in the quantity and quality of patients' lives. On the economic side, the growing number of procedures, the aging of the population and, most importantly, the chronic nature of many diseases, which were previously fatal up until now, are driving up healthcare costs and raising serious concerns over the economic sustainability of the healthcare systems. In this context, the role of the economic evaluations in health care as

Table 24: Cost of items related to hospitalisation[510]

Initial patient and wound assessment	• Clinician time • Facility cost • Diagnostic tests • Laboratory test • Dressing, drugs and other disposable • Patient and carer travel time • Patient out of pocket payments • Patient/carer lost work time
Wound treatments	• Clinician time for dressing changes • Facility cost • Clinician travel time • Dressings, drugs and other disposables • Antibiotics • Diagnostics and laboratory tests • Special equipment • Patient and carer travel time • Patient out of pocket payments • Patient/carer lost work time
Inpatient costs	• Inpatient bed days • Dressings, drugs and other disposables • Antibiotics • Diagnostic and laboratory tests • Surgical procedures • Rehabilitation costs • Outpatient follow-up visits • Special equipment • Patient out of pocket payments • Patient/carer lost work time

'the comparative analysis of alternative courses of action in terms of both their cost and consequence' is becoming more and more important in supporting the decision-makers at the European, country and the local level with respect to the technologies to invest in and reimburse with available resources.[511,512] There is an increasing need for scientifically robust cost and resource-use studies. Currently, there are few studies with regard to wound management, and there is confusion as to how these studies should be performed, especially with regard to endpoints and resource use. Furthermore, there is a limited number of health economic studies on advanced therapies that conduct a cost-effectiveness analysis. The selected economic studies on advanced therapies are presented below.

Health economics of advanced technologies

For the purpose of this document, a literature

search was performed in major clinical and economic databases, such as Pubmed, Embase and Cochrane. Of the 14 economic articles retrieved, two focused on cell/tissue therapy, seven on materials and dressing, four on physical therapy and one on smart technology.

The paucity of economic studies on cell/tissue therapy, physical therapies and smart technology underlines how the economic evaluation of these fields is still under/unexplored. In the area of advanced physical therapies, no studies on electromagnetic fields, show waves and photobiomodulation have been yet performed. Only one economic study has been performed on nanotechnology.

In February 2018, research into the economic aspects of the use of wearable technologies and telecommunication to manage patients with diabetic foot ulcers and wounds in people with diabetes was conducted, and that one article was included.

Economic impact of cell/tissue therapy

In cell/tissue therapy, four cost-effectiveness analyses were retrieved.[513,514] The cost-effectiveness and the comparative analysis were focused on DFUs and VLUs treatments using cellular/tissue skin substitutes: Apligraf, Dermagraft and OASIS (Table 25).

The cost-effectiveness study conducted by Carter et al.[513] compared Apligraf (HSE), Dermagraft (LSE) and OASIS (ECM) used as adjunct therapies to standard of care (SC) with standard of care alone (compression therapy) for VLUs over a period of one year. A Markov model derived from the four RTCs and wound care specialists' interviews were developed. The final model outputs included cumulative costs, clinical outcomes as ulcer-free weeks and the incremental cost-effectiveness ratio (ICER). Regarding the clinical outcome, ulcer-free weeks were 31 for OASIS, 24 for standard of care, 29 Apligraf and 27 Dermagraft. With respect to the costs, Dermagraft showed a higher expected cost of $11,237, followed by Apligraf $10,638, OASIS at $6,732 and standard of care at $6,132.

Although wound closure time was similar among the three skin products, costs for the application of the product were substantially higher for Apligraf ($1,578) and Dermagraft ($1,518) than for OASIS ($152).

The direct costs include initial and established clinic visit costs, cellular and/or tissue-derived products costs, prescription drugs costs, hospitalisation costs, home healthcare costs, and compression stockings costs. Indirect costs were not evaluated. The data cost is based on Medicare's national average reim-bursement rates. However, the probabilistic sensitivity analysis showed that OASIS is economically dominant with lower total costs and better clinical outcomes compared with the other two products. The ICER for OASIS relative to standard care was approximately $86 per ulcer-free week. This indicates that if a patient is willing to pay an additional $86 (approximately $12/d), he/she will gain one additional ulcer-free week. This work is one of the first to investigate the cost-effectiveness of three different cell/tissue substitutes in the management of VLUs.

A second cost-effectiveness study compared two cellular/tissue-derived products presented in the previous article. The number of the medical devices, type of chronic wounds, the length of the study and the country were different when compared with the previous study.

Gilligan et al.[514] determined the cost-effectiveness of OASIS and Dermagraft on DFU wound closure. A Markov model was developed to compare the costs and outcomes of OASIS versus Dermagraft

using data from a 12-week, randomised clinical trial. The clinical outcome was an average wound closure time of 36 days for OASIS and 41 days for Dermagraft. There was no significant difference between these results. The average cost is $2,522 for OASIS and $3,889 for Dermagraft. In this study, direct costs have been considered as costs for low and high-cost substitutes, cost of hospital established clinic visit, physician rate skin substitute application, cost for application of a skin substitute, physician rate evaluation and management visit level. The perspective of the analysis is the third person payer perspective, specifically the centres for Medicare and Medicaid services. The total treatment cost using Dermagraft is approximately 54% higher than using OASIS. Although this study is different in duration, number of medical devices, and pathology from the previous one, it also demonstrated that OASIS yields similar clinical outcomes relative to Dermagraft at a lower cost.

Martson et al.[515] used wound care specific electronic medical records (NetHealth) from 158 wound centres to compare the effectiveness of a bilayered living cellular construct (BLCC) and an acellular porcine small intestine submucosa collagen dressing (SIS) for the treatment of venous leg ulcer. Data from 1489 patients with 1801 refractory VLUs (as defined by failure to have >40% reduction in size in the four weeks prior to treatment) with surface areas between one and 150cm^2 in size, who were treated between July 2009 and July 2012 at 158 wound care facilities across the US, were analysed. Patients' baseline demographics and wound characteristics were comparable between the groups. Kaplan-Meier–derived estimates of wound closure for BLCC (1451 wounds) was significantly greater (p=0.01, log-rank test) by weeks 12 (31% versus 26%), 24 (50% versus 41%), and 36 (61% versus 46%), respectively, compared with SIS (350 wounds). BLCC treatment reduced the median time for wound closure by 44%, achieving healing 19 weeks sooner (24 versus 43 weeks, p=0.01, log-rank test). Treatment with BLCC increased the probability of healing by 29% compared with porcine SIS dressing (hazard ratio=1.29 [95% confidence interval 1.06, 1.56], p=0.01). The authors concluded that the 19-week difference in median healing time (week 24 versus 43) in the current analysis should result in substantial cost savings (between $7,000 and $10,000 additional savings), considering that each additional week for non-healed ulcers may cost more than US$377 per week.

Rice et al.[516] analysed DFU patients, who were selected from Medicare deidentified administrative claims using ICD-9-CM codes. The analysis followed an 'intent-to-treat' design, with cohorts assigned based on the use of (1) BLCC, (2) HFDS, or (3) CC (i.e., ≥1 claim for a DFU-related treatment procedure or podiatrist visit and no evidence of skin substitute use) for treatment of DFU in 2006–2012. Propensity score models were used to separately match BLCC and HFDS patients to CC patients with similar baseline demographics, wound severity, and physician experience measures. Medical resource use, lower-limb amputation rates, and total healthcare costs (2012 USD; from payer perspective) during the 18 months following treatment initiation were compared among the resulting matched samples. Data for 502 matched BLCC-CC patient pairs and 222 matched HFDS-CC patient pairs were analysed. Increased costs associated with outpatient service utilisation relative to their matched CC patients were offset by lower amputation rates (–27.6% BLCC, –22.2% HFDS), statistically significantly (p<0.05) fewer days hospitalised (–33.3% BLCC, –42.4% HFDS), and emergency department visits (–32.3% BLCC, –25.7% HFDS) among the BLCC/HFDS patients. Consequently, BLCC and HFDS patients had per-patient average healthcare costs during the 18-month follow-up period that were lower than their respective matched CC

Table 25: Cells and tissues

Author	Year	Country	Condition	Treatment	Objective	Methods
Gilligan et al.[514]	2015	USA	DFUs	OASIS ECM Dermagraft LSE	Determine cost-effectiveness of OASIS relative to Dermagraft for the treatment of DFU	A Markov model was developed to compare the costs and outcomes of OASIS vs Dermagraft using data from a randomised clinical trial. Time horizon: 12 weeks Perspective: third-party payer
Carter et al.[513]	2014	UK	VLUs	Three cellular tissue derived products (CTPs): -OASIS (ECM), -Apligraf (HSE), -Dermagraft (LSE) vs. standard of care (compression therapy)	Develop a cost-effectiveness model derived from a systematic literature review to compare three CTPs used as adjunct therapies to SC to SC alone	A three-state Markov mod-el derived from the medical literature was developed. 10 studies: • 5 used to populate the clinical outcomes • 5 used to supply information on health economic, resource utilisation, and ulcer recurrence. Time horizon: One year Perspective: payer
Marston et al.[515]	2014	USA	VLU	Two cellular/ tissue derived products (CTPs): -OASIS (ECM), -Apligraf (BLCC)	Compare the effectiveness of BLCC and SIS for the treatment of VLUs	Using de-identified EMRs from wound care facilities across the US for a three-year period
Rice et al.[516]	2015	USA	DFU	Two cellular/ tissue derived products (CTPs): -Apligraf (BLCC), -Dermagraft (HFDS) vs standard of care	To assess the real-world medical services use and associated costs of Medicare patients with DFU treated with BLCC or HFDS compared with those receiving conventional care (CC)	DFU patients were selected from Medicare de-identified administrative claims using ICD-9-CM codes. The analysis followed an 'intent-to-treat' design, with cohorts assigned based on use of BLCC, HFDS, or CC from 2006–2012. Propensity score models were used to separately match BLCC and HFDS patients to CC patients with similar baseline demographics, wound severity, and physician experience measures (matched pair analysis). Medical resources used during the 18 months following treatment initiation were compared among the resulting matched samples

No. patients	Costs/outcome	Results
40 patients screened. 26 of the 31 patients who met the inclusion criteria completed the study 12 in OASIS group 12 in Dermagraft group	Only direct medical costs of care: • costs for low and high-cost substitutes, • cost of hospital established clinic visit • physician rate skin substitute application • cost for application of a skin substitute, • physician rate evaluation and management visit level two. Clinical outcomes: number of ulcer-free weeks. Total cost: • 2,522$ ECM • 3,889$ LSE Outcome: average days wound closure time • 36 ECM • 41 LSE No significant difference	ECM yielded similar clinical outcomes to LSE, but a lower cost, with an additional cost savings of more than 1,360$.
No patients	Cost • initial clinic visit • established clinic visit • cellular and/or tissue-derived products • prescription drugs • hospitalisation costs • home healthcare costs • compression stockings. Total cost: SC= 6,133$ ECM+SC= 6,732$ HSE+SC= 10,638$ LSE+SC=11,237$ Clinical outcomes: number of ulcer-free weeks	OASIS is the most cost-effective CTP when used in the management of VLUs as an adjunct to standard care
ECM Group n=302 BLCC Group n= 1187	19-week difference in median healing time (week 24 vs 43) in the current analysis should result in substantial cost savings (between $7,000 and $10,000 additional savings) Difference in healing time was significant p=0.01	More BLCC patients healed faster in a shorter period of time, using significant fewer treatment applications as compared to ECM
BLCC & CC, n=502 HFDS & CC, n=222	Increased costs associated with outpatient service use relative to matched CC patients were offset by lower amputation rates (–27.6% BLCC, –22.2% HFDS), statistically significantly ($p<0.05$) fewer days hospitalised (–33.3% BLCC, –42.4% HFDS), and emergency department visits (–32.3% BLCC, –25.7% HFDS) among BLCC/HFDS patients	Consequently, BLCC and HFDS patients had per-patient average healthcare costs during the 18-month follow-up period that were lower than their respective matched CC counterparts (–$5,253 BLCC, –$6,991 HFDS)

counterparts (–$5,253 BLCC, –$6,991 HFDS).

Economic impact of materials

Selected articles related to the materials have presented differences in terms of condition, treatment and methodology: six articles covered studies performed in Europe (UK, Germany, France, Italy and Spain) and two were conducted in the US; five papers covered VLUs, one covered DFUs, one examined chronic wounds with exposed bones and/or tendons due to trauma, and one reviewed postoperative wounds (Table 26).

In the field of dressings which stimulate wound healing, a German study performed by Augustin et al. evaluated the cost-effectiveness of two neutral foam dressings (UgoCell versus UgoStart) used in the hydroactive treatment of exuding chronic wounds in venous and mixed leg ulcers.[517] The innovative foam dressing UgoStart is based on the same matrix and carrier of the standard of care (SC) including lipo-colloid technology (TLC) plus a nano-oligosaccharide factor (NOSF technology). This technology is able to inhibit supernatant matrix metalloproteinases, which are responsible for the lack of extracellular matrix compound synthesis and the persistence of an inappropriate local inflammatory process. Cost-effectiveness analysis was carried out from a German statutory health insurances per-spective using a decision tree model for a period of eight weeks. Clinical outcomes and resulting costs obtained by the clinical trial have been combined.[518] The study included 187 patients (93 on UrgoStart and 94 on SC) with venous and mixed leg ulcers. After eight weeks of treatment, the trial showed an average reduction of wound size of 6.9cm^2 in the top of care versus 2.6cm^2 in the comparator. The primary endpoint of the study was the reduction of wound size within eight weeks: 65.6% for UrgoStart and 39.4% for the standard of care. The economic model included only direct medical costs, such as cost of nursing, wound care products, medical devices, hospital treatment, outpatient care and pharmacotherapy costs. In the model, the total treatment costs for eight weeks were €557.51 in the UrgoStart group as compared with €526.17 in the SC group, resulting in a mean difference of €31.32. Effect-adjusted costs advantage generated were €485.64 in the advanced therapy, UgoStart, coming from an effect-adjusted costs of €849.86 in UrgoStart and €1335.51 in the SC.

The clinical trial designed by Meaume et al.[518] illustrated that the advance foam dressing with Nano-oligosaccharide factor accelerates wound healing two times faster as compared with the non-NOSF foam dressing. The effect-adjusted costs demonstrated that UrgoStart is superior in cost-effectiveness to the SC. Furthermore, the quality of life for the patients was also explored showing significant improvement in the advanced therapy group for two of the five dimensions, pain-discomfort and anxiety-depression.[66]

Guest et al.[525] used a decision model to estimate the clinical outcome and the cost-effectiveness of using a skin protectant compared with not using a skin protectant in the management of VLUs. Patients' data was derived from The Health Improvement Network (THIN) database. Patients had their first diagnosis between January 2008 and December 2009. The number of patients included was 510: 255 patients received a Cavillon formulation (166 Cavillon no sting barrier film (NSBF), 89 received a Cavillon durable barrier cream (DBC)), and 255 received no skin protectant. The model showed a significant difference among groups in terms of the reduction of the wound size (NSBF: 31%, DBC: 23% and control: 9%, $p<0.001$). Mean six-monthly NHS cost of resource use per patient did not present significant differences since the cost was about £2200 in all groups. There were no significant differences in clinical outcomes. The therapy with NSBF was the preferred treatment as it leads to a significant reduction in the wound

size. Like the Guest study, the study performed by Panca et al.[524] was based on patients' data collected from the THIN database. It evaluated the clinical outcome and the cost-effectiveness of using sodium carboxymethylcellulose dressing (CMC) and four superabsorbent dressings (Dry Max Extra (DM), Filvasorb (F), Kerramax (K) and sachet (S)) in the treatment of highly exuding VLUs. The study showed that the cost-effective therapy was the S: the six-month NHS cost of managing VLUs was £3700 per patient, which was 15–28% lower with respect to the other treatments and more QALYs. The Italian study by Romanelli et al.[521] aimed to assess the cost-effectiveness of single-layer ECM in addition to SC (petrolatum-impregnated gauze) compared with SC alone for patients with VLUs and mixed arterial/venous ulcers. Clinical data were derived from an eight-week randomised clinical trial (RCT) of adults ≥18 years with VLUs or mixed A/V ulcer. Patients were randomised to a ECM group (n=25) or a standard of care group (n=23) and were followed monthly for 32 weeks to assess wound closure. Economic data originated from Markov models were developed to compare the clinical outcome and costs of ECM versus SC using wound closure rates and expected VLU and mixed A/V ulcer cost per patient. The study did not present significant differences in terms of costs, but ECM was more clinically effective with respect to SC as the number of open-wound weeks was lower (six weeks versus 10 weeks); and complete wound closure was significantly higher in patients with ECM ($p<0.05$). The study of Nherera et al.[520] used a Markov model to compare the expected cost and outcomes of managing patients with VLUs with a topical antimicrobial dressing (Cadexomer Iodine (CI)) plus compression bandages (standard of care) versus standard of care alone. Patients treated with CI-SC experienced 25 ulcer-free weeks and 0.86 QALYs versus 19 ulcer-free weeks and 0.82 in SC group. Total cost per patient in CI+SC treatment over 52 weeks was $7259 versus $7901 in the standard of care. The authors affirmed that prospective, controlled clinical studies were needed to confirm the results of their study.

A recent study performed by Guest et al.[519] assessed the cost-effectiveness of using adjunctive porcine small intestine submucosa tri-layer matrix (SIS), a three dimensional biomaterial consisting of a biocompatible, acellular, collagen-based extracellular matrix, as adjunct to SC versus SC alone (one of the following: silver dressing, hydrogel wet-to-dry dressing, alginate dressing, Manuka honey and triple antibiotic dressing) in patients with diabetic foot ulcers according to Medicare's perspective. This was a decision-modelling study populated with data derived from a clinical trial, information related to the patients obtained from the clinical authors, and published literature. The Markov model simulated the management of diabetic neuropathic lower extremity ulcers over a period of one year in USA. The effectiveness measures were the number of ulcer-free months, probability of having a healed ulcer at twelve months, probability of avoiding a complicated ulcer at 12 months and probability of avoiding an amputation over 12 months. At 12 months after start of treatment, the use of adjunctive SIS instead of SC alone led to a 42% increase of number of ulcer-free months, a 32% increase in the probability of healing, a 3% increase in the probability of avoiding a complicated ulcer and a 1% in-crease in the probability of avoiding an amputation.

Expected healthcare costs (2016 prices) over the 12 months after the start of therapy amount were at $13,857.61 in adjunctive SIS and $13,962.23 in SC alone. Debridement procedures represented 42% of the total cost in SC alone and SIS application 22% in the SIS group. SIS plus SC improved clinical outcomes for less cost.

A study performed by Guest et al.[523] assessed the cost-effectiveness of using Polyheal compared with

Table 26: Materials

Author	Year	Country	Condition	Treatment	Objective
Guest et al.[519]	2017	USA	DFUs	OASIS Ultra + SC (silver dressing, hydrogel, wet-to-dry dressing, alginate dressing, Manuka honey and triple antibiotic dressing) vs SC alone	Estimate the cost-effectiveness of using Oasis Ultra as an adjunct to SC compared with SC alone in managing DFUs in the US over 12 months after the start of treatment
Nherera et al.[520]	2016	USA	VLUs	Cadexomer Iodine (topical antimicrobial dressing) plus SC vs SC alone (compression bandages)	To estimate the clinical and cost difference between Cadexomer+SC vs SC alone according to payer's perspective
Romanelli et al.[521]	2016	Italy	Mixed arterial/venous (A/V) or VLUs	Single layer extracellular matrix (ECM) as an adjunct therapy to standard of care (SC) compared with standard care alone (compression therapy, debridement and maintenance of a moist wound environment)	To assess the cost effectiveness of single layer ECM plus SC vs SC

Methods	N. patients	Costs	Results
A Markov model was constructed based on patient-level data obtained from clinical trial: • information pertaining to patient management from the clinical authors • published literature Perspective: Medicare	Adult patients with diagnosis of type 1 or 2 diabetes mellitus	The model only analysed direct healthcare costs borne by Medicare and excluded direct costs incurred by patients and indirect costs incurred by society as a result of employed patients taking time off work. Total costs: 13,962.23$ SC vs 13,857.61$ OA-SIS+SC	The use of OASIS instead of standard care alone improves outcome for less cost and OASIS was found to be a dominant strategy when compared with starting treatment with SC alone
Markov model to simulate the expected cost and outcomes of manag-ing VLUs. Outcomes (wound healing, infection rate, HQOL and health resource use) over one year	No patients	Expected healing rates at 52 weeks: Cadexomer: 61% SC: 54% Ulcer-free weeks: Cadexomer: 25 SC: 19 Expected total cost at 52 weeks: Cadexomer: $7901 SC: $7259 QALYs at 52 weeks: Cadexomer: 0.82 SC: 0.86	Cadexomer iodine+SC is dominant treatment for chronic VLUs. It is needed to perform prospective, controlled clinical studies to confirm the results of the study
Data derived from an eight-week RCT of patients with VLU or mixed A/V ulcer: 50 patients (23 with A/V and 27 with VLU) visited in outpatient setting at University of Pisa Markov model to compare clinical outcomes and costs of ECM vs SC using wound closure rates to estimate n. closed wound weeks and A/V and VLU cost per patient Costs came from standard cost references and medical supply in USA Perspective: third payers Direct medical costs (2015 US dollars)	ECM group: 25 patients SC: 23 patients	Wounds healed after eight weeks: ECM: in 5.4 weeks SC: 8.3 weeks Complete wound closure: ECM: 80% (20 pts) SC: 65% (15 pts) Expected cost per ulcer at the end of 32 weeks: ECM: $2527 SC: $2540 ICER: $–3.75	ECM provides better clinical outcome at a slightly lower cost

Arroyo et al.[522]	2015	Spain	Post operative wounds.	Polyurethane film surgical dressing vs gauze surgical dressings	To evaluate the clinical and cost-effectiveness of polyurethane film with absorbent pad (OPQV) respect to the use of gauze and tape
Guest et al.[523]	2015	France Germany UK	Chronic wounds with exposed bones and/or tendons due to trauma	Polyheal (non-biodegradable, chemically inert, synthetic, nega-tively charged 5-micron polystyrene microsphere) vs surgery	To assess the cost-effectiveness of using Polyheal vs surgery
Augustin et al.[517]	2014	Germany	VLU	UrgoStart (U) vs UrgoCell (SC)	Evaluate cost-effectiveness of NOSF (nano-oligosaccharide factor) containing wound dressing (U) in vascular leg ulcers compared with SC (without NOSF) for eight weeks

Primary endpoint: rate of superficial site surgical infection Secondary endpoints: • rate of complications related to the surgical dressings used • number of dressings changes during patient's hospital stay Dressing performance: Likert scale from 0 to 4	416 patients (15 hospitals): • 199 gauze/tape group • 217 polyurethane film group	Economic analysis base on the outcome of the study. Infection rate refers to a hypothetical cohort of 1000 surgical patients treated with either polyurethane film and with gauze/tape. Direct costs of postoperative management of surgical site Gauze/tape: €22,350 OPOV: €12,740 The difference is due to the nursing time costs (€19,350 in gauze vs €7740 in OPOV) Cost of managing superficial SSI Gauze/tape: €59,400 OPOV: €22,400 Difference due to cost of hospitalisation (€46,200 in gauze vs €19,600 in OPOV) and antibiotic treatment (€13,200 vs €2,800)	The use of polyurethane film can significantly reduce the rate of surgical site infections and other type of wound complications respect to the use of gauze and tape. In gauze/tape group the incidence of surgical wound was 6.6% vs 1.4% in OPOV group. The perception of professionals and patients is significantly better respect to polyurethane film vs gauze/tape ($p<0.001$)
Three decision models based on published clinical outcomes related to surgery, predicted healing rates with Polyheal derived from clinical studies, and patient pathway and associated healthcare resources derived from interviews with clinicians	No patients	Initial management Total healthcare costs (€2010/2011) per patient Polyheal vs Surgery France: €7984/€12,300 Germany: €7571/€18,137 UK: €8860/€11,330 Polyheal group: Primary cost driver Nurse visits: 36% in France and 42% in UK Surgery and hospitalisation: 50% of total in Germany Surgery group: Primary cost driver Hospitalisation 72% in France 67% in Germany 69% in UK	Polyheal potentially provides a cost-effective treatment in France, Germany and UK respect to surgery but it is dependent on Polyheal's healing rates in the clinical practices in terms of when it will be commonly available
Decision analytic model based on clinical outcomes and costs by a two arm, randomised, multicentred phase III study conducted in France but with real-world conditions in Germany. Perspective: German statutory health insurance	187 patients: • 93 U • 94 SC	Direct medical costs: • costs for nursing • wound care products • medical devices • hospital treatment • ambulant care • pharmacotherapy	Wound size reduction: 65.6% U vs 39.4% SC Total treatment costs: 557.51€ U vs 526.19€ SC (difference: 31.32) Effect-adjusted costs: 849.86€ vs 1335.51€ Difference: 485.64 U is cost-effective

Panca et al.[524]	2013	UK	VLUs	Sodium carboxymethyl-cellulose dressing (CMC) and four superabsorbent dressings (DryMax Extra (DM), Flivasorb (F), Kerramax (K) and sachet (S))	To evaluate the clini-al and cost-effectiveness of using CMC and four dressings
Guest et al.[525]	2012	UK	VLUs	Skin protectant: Cavillon No Sting Barrier Film (NSBF) or Cavillon Durable Barrier Cream (DBC) vs not using a skin protectant.	To assess the clinical and cost-effectiveness of skin protectant vs not using skin protectant

surgery in chronic wounds with exposed bones and/or tendons due to trauma in France, Germany and UK from the payer's perspective. Total healthcare cost following initial use of Polyheal were €7984 in France, €7517 in Germany and €8860 in UK; total healthcare costs after surgery were €12,300, €18,137 and €11,330, respectively. Polyheal resulted in a dominant treatment in each country as compared with surgery. These results will be dependent on Polyheal's healing rate in clini-cal practice when the product becomes more accessible.

Arroyo et al.[522] compared the clinical outcomes and cost-effectiveness of using polyurehtane film surgical dressing (OPOV) versus gauze surgical dressings in postoperative care. The study involving 416 patients (OPOV group=217, gauze=199) in 15 Spanish hospitals had as a primary endpoint the rate of superficial surgical site infection (SSI) during initial hospitalisation and as a secondary endpoint the rate of complications related to the surgical dressing used and the number of dressing changes during the hospital stay. Data showed that the polyurethane film dressing had a significant reduction of SSI (1.4% versus 6.6% in gauze, p=0.006). The unit of cost of the polyurethane film dressing was higher in respect to gauze/tape, but the polyurethane film was associated with fewer dressing changes which implies a reduction of auxiliary dressings and nurse time.

Economic impact of physical therapies

Table 27 presents four papers related to the

Decision model based on patients' data from a database	CMC: 99 DM: 43 F: 99 K: 99 S:99	Mean 6-monthly NHS cost of resources per patient (2010/2011 prices): CMC: £2452.8 DM: 4346.7 F: £5127.6 K: 4768.1 S: £3647.3 Primary cost driver: prac-tice nurse visits CMC: 33% of total DM: 22% F: 14% K: 19% S:19% Community nurse visits: range from 42% in CMC and 64% in F.	The use of S is lower costly: over 6 months of the start of treatment with S, the NHS cost of venous leg ulcer management is -£3,800 (15-28% less than the cost of other super-absorbent).
Decision model based on case records of cohort of matched patients from a database First diagnosis: between January 2008 and De-cember 2009	Cavillon formulation: 255 NSBF: 166 DBC: 89 Control group: 255	Mean 6-monthly NHS cost of resources per patient (2009/2010): DBC: £2,152.88 NSBF: £2,245.02 Control: £2,234.18 Main driver cost: practice nurses visits (58% of total costs in 3 groups) Dressings costs are high-er in control group and accounted for <10% of the total cost.	NSBF leads to significantly greater wound size reduc-tion respect to other groups (31% vs 23% in DBC and 9% in control).

physical therapies for venous leg ulcer treatment. In the arena of electromagnetic fields, no economic evaluation studies were available. In literature concerning the externally applied electroceutical device in managing VLUs, two studies on electric fields were found.

The aim of the first study performed by Taylor et al.[527] was to evaluate the cost-effectiveness of treating patients with chronic, non-healing VLUs using electric stimulation (ES) (Accel Heal) therapy in addition to dressings and compression bandaging from an NHS perspective in the UK. A Markov model spanned a period of five months, which was the maximum period in which patients were followed. Clinical evaluation of ES therapy among 22 patients with chronic, non-healing VLUs was performed. Data originated from patient's case report forms completed during the clinical evaluation were evaluated to measure clinical outcomes and the use of healthcare resources for each wound. Furthermore, data over a period of six months before the start of ES treatment from the patients' medical records were considered. The model estimated the cost-effectiveness of ES therapy based on 2008–2009 prices.

Patients receive three units of ES therapy in addition to dressings and compression bandaging; during the clinical evaluation, patients continued to use the same bandages and dressings utilised before the start of ES therapy.

The model generated two measures of cost-

Table 27: Physical therapies

Author	Year	Country	Condition	Treatment	Objective
Guest et al. (526)	2015	UK	VLU	Externally applied electroceutical (EAE) device+dressing+compression vs combination dressing+ bandage (SC)	Estimate cost-effectiveness of treating patients with VLU with an EAE device plus dressings and compression bandaging VS SC
Zhou et al.[508]	2015	USA	• VLU, • traumatic/ surgical wounds (SW) • PU • DFU • Other type (OT)	Primary treatment: 45 minutes of high voltage pulsed current electric therapy. Occasionally: • whirlpool therapy • ultrasound • ultraviolet C therapy (UVC)	Calculate the healing rates, the costs and time required for closure wound care (CWC) to assess the cost difference between healing and non-healing wounds and to compare cost-effectiveness between VLU and non-VLU as DFU, PU, OT in a PT outpatient wound care clinic in US
Taylor et al.[527]	2011	UK	Non-healing VLS	Externally applied electroceutical (EAE) device+dressing+compression VS com-bination dressing+ bandage (SC) of >6 months duration	Estimate cost-effectiveness of treating patients with non-healing VLU with an EAE device plus dressings and compression bandaging vs SC

effectiveness as an expected probability of being healed and the expected number of QALYs at five months after ES therapy. The expected outcomes at five months after the start of electric stimulation therapy reported that 38% of all wounds were expected to heal in the ES plus dressings and compression with respect to 9% in the previous care plan. The use of ES therapy can lead to a 27% reduction of required nurses' visits (from 49.0 to 35.9 visits per patient) and a minor reduction in the number of bandages required from 7.9 to 3.5 (–56%). These improvements were expected to lead to a 6% of health gain of 0.0017 QUALYs over five months.

From an economic standpoint, the expected total healthcare costs at five months from the start of ES therapy were £748.94 in the ES plus dressings and bandaging versus £879.90 in the patients without ES (difference £131). The cost of electric stimulation amounted to £120 (£40 per unit) and represented 16% of the total healthcare costs.

Methods	No. of patients	Costs	Results
Decision model based on clinical outcome, resource use and costs from prospective, single-arm, non-blinded, clinical and economic evaluation of EAE therapy in the management of VLUs in 2013–2014 (over 12 months before-12 month after the start of treatment) Perspective: NHS	30	Direct medical costs • healthcare resource (community nurse visits, practice nurse visits, tissue viability nurse visits) • dressings and bandages (dressings, compression bandages, non-compression bandages) • electric stimulation	EAE therapy affords the NHS a cost-effective treatment although this was dependent on the duration of the wound Incremental cost per QALY gained was £2,522
Retrospective cohort study based on patient data extracted from the electronic medical database from September 2012 to January 2015	261: 159 included: • 72 VLU • 48 SW • 11 PU • 16 PDF • 12 OT	Costs included: • dressing cost • reimbursement rate from insurance companies • breakeven rate for the clinic	Respect to the com-parison between VLU (n=63) and non-VLU (n.56) in the healed group the only significant difference was in wound dressing costs (p=0.001) Incorporation of PT in wound care appeared to be cost effective
A five-month Markov model based on clinical, resource use and utility coming from patients' case report forms, medical case notes Perspective: NHS	22	Direct medical costs • electric stimulation, • nurse visits • bandages • dressings • creams, ointments and emollients	• EAE therapy is expected to reduce the NHS cost by 15% from £880 to £749 due to a reduction in the requirement for nurse visits over the first five months after the start of treatment • 6% health gain of 0.017 QALYs (from 0.299 to 0.316 QALY) over five months

The nurse visits amounted to 67% in the ES plus dressings and compression bandaging versus 77% in the dressings and compression bandaging alone. With respect to cost-effectiveness analysis, the ES therapy was a dominant treatment and potentially afforded the NHS a cost-effective treatment for patients with chronic venous ulcers of >6 months duration, depending on the number of ES therapy units, the unit cost of the device, and the number of required nurse visits.

A study performed by Guest et al.[526] evaluated the cost-effectiveness of treating patients with VLU using an applied electroceutical device (EAE) (Accel Heal) in addition to dressings and compression bandaging according to the NHS in the UK.

The aim of the prospective, single-arm, non-blinded study was to estimate clinical outcomes, cost impact and cost-effectiveness of EAE therapy in patients affected by VLUs in 2013–2014. Data associated with the wound over 12 months before

the start of EAE therapy were compared with the first twelve months after the start of the therapy.

Professionals involved were 13 nurses based at 11 centres of which six centres were com-munity-based clinics, and five were hospital outpatient clinics. Patients involved (n=28) were treated with six active units of EAE therapy (each unit for two days) plus dressings and compression bandaging over 12 days. Hereafter, the patients were treated with dressings and bandages. Data collected over a period of 12 months from the start of the therapy included age, gender, wound duration, wound size, pain, exudate levels (classified as low, medium or heavy), clinical visits and the use of bandages and topical treatments. This data was compared with the information collected from the patients' clinical records over the 12 months prior to the start of the EAE treatment. A computer-based decision model was performed to represent the treatment pathways and associated management of the wounds in the data set.

The patients' mean age was 66.0 years, 62% were female, 8.7cm^2 was the mean size of VLU, and 2.2 years was the mean duration of their wound before the start of EAE therapy.

At 12 months after the start of the treatment, 77% of all wounds had healed, and 23% had improved. The number of dressings was decreased by 26% (from 197.0 to 146.1) over 12 months after the start of the treatment. Total healthcare costs over the 12 months prior to the treatment amounted to £1908.99 versus £1753.87 after the therapy. Before the therapy, the costs related to the practice nurse visits represented 40% of the total cost, and after the therapy, they only represented 18% of the total. The cost absorbed by the electroceutical device was 14% of the NHS total cost. The difference in effectiveness between before and after EAE therapy yielded a 12% improvement in health gain of 0.09 QALYs ($p<0.01$). The EAE therapy results thus supported EAE therapy as a dominant treatment for VLUs, which could potentially provide the NHS with a cost-effective treat-ment for patients with VLUs.

The study does have some limitations since the nurses were self-selected, patients were not random-ised to a treatment, and the study had no comparator group. Within the model's limitations, the cost-effectiveness of treating patients with VLU with EAE therapy depends on healing rates, duration and size of the wound. It is necessary to collect and use more clinical data in the data set for an accurate final estimation of the cost-effectiveness of the device.

A retrospective study performed by Zhou et al.[508] aimed to calculate the healing rates, the costs and the time required for closure wound care (CWC) in patients with VLUs and non-venous leg ulcers like DFUs, PUs and other types of wounds in an outpatient wound care clinic in the US. The patients received 45 minutes of high voltage pulsed current electric therapy as primary treatment, and Whirlpool therapy, ultrasound and ultraviolet C therapy were occasionally used. The study aimed to assess the cost difference between healing and non-healing wounds and to compare the cost-effectiveness between VLU and non-VLUs, such as DFUs, PUs and other types of wounds with physical therapy (PT).

Data referred to patients treated from September 2010 to January 2015 in a single centre (n= 261). Included are 159 patients (75 males and 84 females), and 72 had venous ulcers, 48 had traumatic/surgical wounds, 11 had PUs, 16 had pressure DFUs, and 12 had other wound types. Of these patients, 151 received 45 minutes of high voltage pulsed current electric therapy as their primary treatment. Sometimes, the patients were also treated with Whirlpool therapy, ultrasound

and ultraviolet C. The mean age was 63.78 years. 74.84% of patients (n=119) represented the healing group and 25.16% the non-healing group (n=40). Treatment duration was 98.01±76.12 days in the healed group versus 144.50±133.84 in non-healing group ($p<0.001$). The number of visits was 27.10±22.64 in the healed group with respect to 37.48±32.23 in non-healing group.

Costs included reimbursement rates from insurance companies and breakeven costs for the clinic. Reimbursement rate included electric stimulation ranging from $18 to $40 per patient visit, plus $70 for initial evaluation, and $40 for re-evaluation every 30 days. Dressing costs were not reimbursed from insurances companies, so it also considered the total dressing cost per treatment episode. Breakeven cost for episode were $83 for the number of visits plus total dressing costs (operational costs were $83/hour and included the salaries for one full-time therapist and one full-time PT aid).

The reimbursement rate (USD) was 1327±1143.53 in the healed and 1751±1536.58 for the non-healed; the breakeven rate (USD) was 2492.58±2106.88 versus 3362.50±2914.03 ($p=0.002$), respectively.

With respect to the comparison between VLU (n=63) and non-VLU (n.56) in the healed group, the only significant difference was in wound dressing costs ($p=0.001$).

The study presented preliminary data on the cost-effectiveness of wound care when physical therapy is included, but further studies are necessary.

Economic impact of smart technologies

From an economic literature search, covering the period January 2007–January 2018, 263 articles were retrieved, but four papers were considered, and only one was included in the report. The Danish study performed by Fasterholdt et al.[496] compared the cost-effectiveness of telemonitoring (TM) versus standard monitoring (SM) in patients with DFUs. An economic evaluation was related to the clinical trial performed in seven departments and outpatient clinics of five hospitals in Southern Denmark. The patients enrolled in the TM group performed two teleconsultations in the patient's own home conducted by telephone or online written consultations and one consultation at the outpatient clinic. The SM group performed three visits at outpatient clinic. A total of 374 patients were enrolled (193 in TM group and 181 in SM group). Groups did not present significant differences in terms of demographic and clinical characteristics. Total healthcare costs per patient over a six months period were lower in telemonitoring as compared to standard monitoring, €12,356 versus €14,395 (cost difference: €2039), but the difference was not statistically significant. The difference was related to fewer hospital admissions and lower outpatient costs. A significant difference was related to the total staff time used on outpatient consultation, amounting to 156 minutes for the TM group versus 266 minutes in standard group. The amputation rate was similar in the two groups.

This was the first study that employed a strong methodology in terms of economic evaluation for telemonitoring of patients with DFUs in a field with limited previous research.

Conclusions

Due to the scarcity and limited robustness of the available economic studies on advanced therapies in wound management, further analyses on advanced therapies in chronic wound care are necessary to shed more light on the economic implications of alternative technologies, procedures and therapeutic approaches.

For these reasons, we would encourage public and private organisations, the scientific societies, and the professional associations to promote prospective, multicentred studies that could allow for the accurate assessment of direct and, no less important, indirect costs, such as loss of productivity, indi-vidual patient and his/her family's costs. Moreover, as patients suffer because of pain, lack of sleep, immobility and social isolation, with substantial impairment in their daily life, more detailed analyses should focus also on the assessment of the different therapeutic strategies in regard to the patients' quality of life.

In our opinion, all of these factors should be taken into account to perform future clinical and economic evaluations and to provide to different stakeholders—clinicians, patients, hospital administrators, payers, industry, and health policy makers—valuable information.

Regulatory issues: what needs to be considered for an integrated regulatory strategy

Development of advanced therapy medicinal products for wound management–a challenging field

The great potential of regenerative medicines in wound care was just recently demonstrated by a case study describing the regeneration of an entire human epidermis for a boy with Junctional epidermoly-sis bullosa (JEB) by a gene therapy product consisting of autologous transgenic keratinocyte cultures.[528] Cell and gene therapies and their use in regenerative medicine are one of the most innovative achievements in the medical field. They hold enormous promise to cure some of the most troubling and intractable diseases. In wound healing, technologies that have the potential to regenerate as opposed to repair tissue are also gaining ground as demonstrated by the JEB case above. Wound healing is a logical target for early development of regenerative strategies due to the regenerative nature of wound healing and the physical features of the skin since it is relatively avascular, flat and accessible.[529] Moreover, new and highly effective treatments are urgently needed for wound care as chronic wounds show a high prevalence, produce high treatment costs and are extremely debilitating for patients.

Despite regenerative medicines' game changing potential, the success rate of a marketing authorisation application (MAA) for regenerative medicines in the EU remains rather poor as they face substantial challenges regarding the transition from a research to a development stage. Within wound care, the success rates of new drugs in general also remain poor. Over the last eighteen years just two products, Regranex (beclapermin)[530] and Episalvan (birch bark extract)[531] were centrally approved for wound healing in the EU whereas the Marketing Authorisation Holder of Regranex has in the meantime withdrawn the marketing authorisation due to commercial reasons. The poor success rate of medicinal products for wound management medicinal products is attributed to the challenging indications, which especially lack well-designed, comparative clinical trials in well-defined patient cohorts.[529] Thus, companies, who are engaged in developing innovative regenerative medicines in the field of wound care, are facing both innovative products and a demanding indication. Therefore, it is of the outmost importance to have a well thought-out integrated regulatory strategy in place in order to successfully develop regenerative medicines for wound healing.

Overview of relevant legislation

Though most new regenerative medicines are classified by the European Medicines Agency as Ad-vanced Therapy Medicinal Products (ATMPs), the wound healing area comprises diverse products, such as medical devices (MD), combination products

and advanced therapy medicinal products (ATMPs). Different legislations for these products exist, which are partly overlapping. An overview of the different legal frameworks is provided in the following.

For MDs the most relevant piece of legislation is currently the Medical Device Directive (MDD 93/42/EC),[532] defines the CE certificate as a prerequisite for placing a MD on the market in Europe and in European Free Trade Association (EFTA) countries. CE certificates are issued by a Notified Body, who is designated to perform this task by the designating authority in their country, and since the classification of devices is based on risk, the scrutiny applied for conformity assessment depends mainly on the classification and risk of the device.[533] Currently, the European regulatory framework for medical devices is undergoing significant changes, and the MDD will soon be replaced by the Medical Device Regulation (MDR) 2017/745/EC (534), which will take effect beginning in mid-2020. Changes occurring under MDR concern, amongst others, are the introduction of a life-cycle approach to ongoing CE-marking compliance, more complex conformity assessment procedures, increased post-market surveillance, post-market clinical follow-up studies and delivery of periodic safety update reports (Class IIa devices and above).[535]

In case a product consists of a MD and a medicinal product (MP), the product is called a combination product. Here, it is critical to understand the primary mode of action of the product since this will determine whether it will be regulated as a MD or as a MP in the EU. For example, a wound dressing containing an antimicrobial agent will be regulated as a MD whereas a wound treatment product for the delivery of an antimicrobial agents will be considered as a MP.[536]

A third possible scenario for the regulation of a combination of a MP and a MD would be the classifica-tion as an ATMP, for example with autologous chondrocytes seeded onto a collagen membrane to repair cartilage. The autologous chondrocytes represent the integral part of the product, and thus, the whole product falls under the Advanced Therapy Medicinal Product Regulation (EC) No 1394/2007.[537] ATMPs comprise four distinct product categories, which are gene therapy medicinal products (GTMP), somatic cell therapy medicinal products (sCTMP), tissue-engineered products (TEP) as well as combined ATMPs. Table 28 provides an overview of the characteristics of each of these categories. Following the implementation of the ATMP Regulation, it became mandatory for ATMPs to follow a centralised procedure to obtain a marketing authorisation pursuant to Regulation (EC) No. 726/2004.[538] As a consequence, ATMPs have to fulfil the same high regulatory standards as other pharmaceuticals.

Where do we stand with ATMPS in wound management?

Over the last 18 years, only two medicinal products for wound healing were granted marketing authorisation in the EU. Both products do not fall under the ATMP classification, which shows that despite ATMPs' game changing potential, no ATMP with an indication in wound management has been approved yet. However, when looking at the Committee for Advanced Therapies (CAT) classification procedures since 2011, 27 procedures refer to products with a wound management related indication, in detail 24 TEPs, two sCTMPs and one GTMP.[539] The classified products comprise various TEPs based on human autologous keratinocytes, an sCTMP consisting of autologous adipose tissue-derived mesenchymal stem cells and the one GTMP composed of living, genetically modified Lactobacillus reuteri bacteria with a plasmid containing the gene for human CXCL2-1a indicated for chronic skin wounds in patients with diabetes.[539]

Table 28: Overview of GTMP, sCTMP, TEP and combined ATMP definitions

Category	Definition
GTMP	• Contains an active substance which contains or consists of a recom-binant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence • Its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the prod-uct of genetic expression of this sequence
sCTMP	• Contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physio-logical functions or structural properties relevant for the intended clinical use have been altered, or of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor • Is presented as having properties for or is used in or administered to human beings with a view to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues
TEP	• Contains or consists of engineered cells or tissues, and • Is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue
Combined ATMP	• It must incorporate, as an integral part of the product, one or more medical devices within the meaning of Article 1(2)(a) of Directive 93/42/EEC or one or more active implantable medical devices within the meaning of Article 1(2)(c) of Directive 90/385/EEC, and • Its cellular or tissue part must contain viable cells or tissues, or • Its cellular or tissue part containing non-viable cells or tissues must be liable to act upon the human body with action that can be con-sidered as primary to that of the devices referred to

In the US, StrataGraft Regenerative Skin Tissue (Mallinckrodt plc) indicated for the treatment of severe burns and other complex skin defects received, just recently, Regenerative Advanced Therapy (RMAT) designation. This designation aims in speeding up the timeframe for approval of innovative and prom-ising regenerative therapies and speaks to the strength of the clinical data generated with StrataGraft during phase I and II clinical trials.[540]

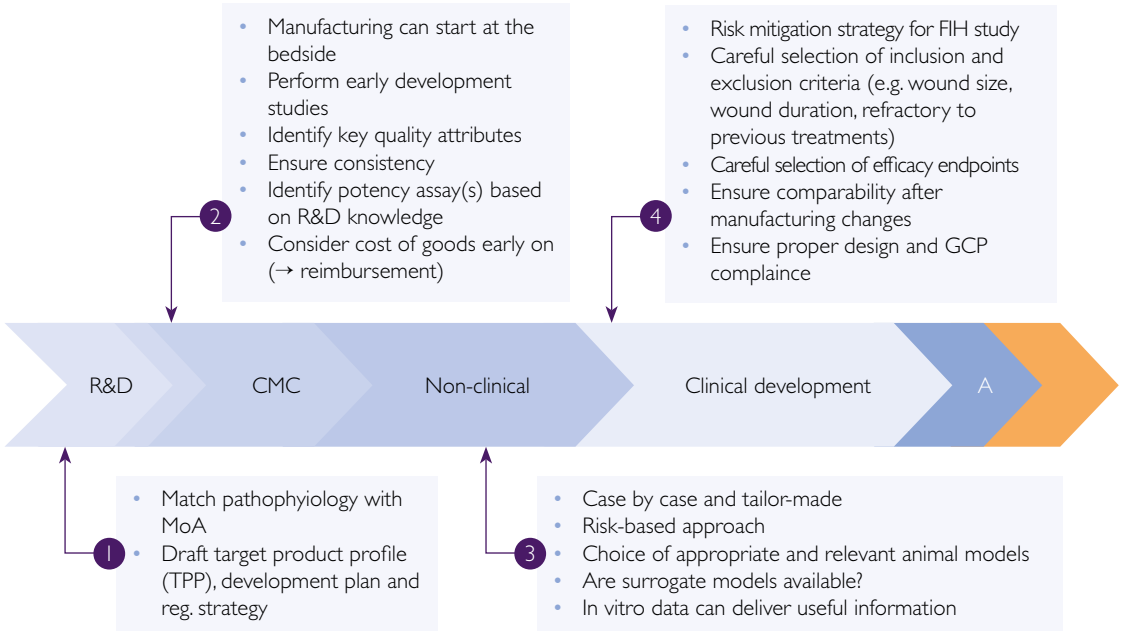
This demonstrates that diverse efforts are being made to take advantage of the great potential of regenerative medicines to transform wound management, and this gives reason to be optimistic that innovative products for wound healing can be expected to reach the Marketing Authorisation Application (MAA) status over the next few years.

How to best address challenges during atmp development for wound management?

Despite ATMPs being a heterogeneous group of products, developers of ATMPs face common devel-opment features. The awareness of this is important to ATMP developers in order to steer drug devel-opment effectively. Figure 25 summarises important points to be considered at key transition points in drug development of ATMPs for wound management.[541]

Points to consider at the R&D stage

In order to select the lead indication where a pathophysiology matches a mechanism of action (MoA), it is of the utmost importance to characterise the MoA thoroughly and to understand the pathophysiology of the target



Acronyms: R&D=Research & Development, FiH=First in Human, GCP=Good Clinical Practice, MoA=Mode of Action, TPP=Target Product Profile

Source: Seimetz D. ATMPs: How to successfully master Challenges and foster the Regulatory Success rate? Pharmazeutische Medizin. 2016;(18) 3.

Fig 25: Points to consider at key transition points in drug development[541]

disease. Already at this early stage, drafting a target product profile is helpful to guide lead candidate selection and to guide the development and regulatory strategy.

Points to consider for manufacturing

Unlike for other proprietary medicinal products, the manufacturing process of certain ATMPs starts already at the patient's bedside, which is not necessarily a qualified Good Manufacturing Practice (GMP) unit. In addition, the set-up of an ATMP manufacturing process including its qualification and validation is not a trivial task. The identification of key quality attributes of an ATMP is critically important and helps set up the process and ensure consistency. Likewise, the identification of a suitable potency assay is important as this represents the only direct link to the product's clinical efficacy. In addition, it is highly recommended to consider cost of goods already early on, such as when the initial GMP process is being designed, to lower the expected commercial challenges.

Non-clinical challenges

For the non-clinical development of ATMPs in wound care management, there are rarely off-the-shelf solutions available. Sufficiently sensitive and relevant models are frequently lacking to accurately assess safety and pharmacodynamic properties and to guide clinical development. Therefore, ATMPs require careful considerations and tailor-made solutions more than any other class of products. Currently, there

are no ideal animal models available for areas such as chronic wounds or extensive burns. Therefore, multiple animal models should be used to assess the activity of wound-treatment products.[542]

Based on our practical experience, two models complementing each other can be used for chronic inflammatory wounds, such as the diabetic mouse model and a common minipig model. Thereby, the diabetic mouse model reflects the inflammatory status, and the minipig model is more representative of the human skin architecture.

When no appropriate *in vivo* model can be identified or is known to be able to complement *in vivo* studies, *ex vivo* surrogate models or *in vitro* data can be used to provide valuable information.

The extent of pharmacodynamic, pharmacokinetic or shedding studies will depend on the particular nature of the ATMP. While detailed investigations will be feasible and needed for genetically modified cells or bacterial based products in wound care management, this might not be feasible nor necessary for a non-genetically modified skin graft. For the latter, engraftment and graft survival will be important pharmacokinetic and pharmacodynamic properties to examine.

Clinical challenges

Under consideration of the non-clinical limitations, which the majority of ATMPs face, it is important to design a well thought-through risk mitigation strategy for the First in Human (FIH) study. A proper risk mitigation strategy combined with an indepth knowledge of the MoA and the pathophysiology guides the selection of the most appropriate patient population. The critical selection of inclusion and exclusion criteria for enrolment and the choice of relevant efficacy endpoints are also important. Some authorities, particularly the FDA, accept only complete wound healing as an efficacy outcome for chronic wound treatment, which might be difficult to demonstrate since many patients' wounds may not heal over the course of the study (543). This aspect can be addressed by the addition of other endpoints, such as wound measurements or health-related measurements of quality of life. At the late stage of clinical development and specifically when changes have been introduced into the manufacturing process, it is important to carefully assess comparability to ensure that the clinical performance is not impaired by a changed quality profile of the ATMP.

What regulatory tools should be considered for setting up an integrated development and regulatory strategy?

Drug development times have increased enormously over the past decades, and the cost of bringing a drug to market has more than doubled in the past 10 years. Today, it takes far more than a decade to bring a new drug from discovery to the market. A well-considered regulatory strategy is key to success at the time of approval and aims at aligning the regulatory activities involved in bringing a product to market with the drug development process and business strategy.[544]

Tools are available to support the development strategy and should be considered at dedicated time points during the development to increase the chances for a successful drug development (Table 29, Fig 26).

Outlook and conclusion

Regenerative medicines are on the rise and about to shift the focus of medicine from replacing and repairing tissue to regenerating it. Although regenerative medicine is not yet a reality in wound management, the ongoing development activities in the field of ATMPs hold the realistic promise of revolutionising the standard treatment. Gene therapies producing wound healing factors may

Table 29. Overview on regulatory tools

Tool	Description
Specific guidelines from agencies	• FDA guidance for industry- Chronic Cutaneous Ulcer and Burn Wounds- Developing Products for Treatment[545] • Specific ATMP guidelines[541]
Small- and medium-sized enterprises (SME) status[546]	• Administrative, regulatory and financial support provided by EMA • Annual headcount <250 and an annual turnover ≤50 million Euros • Substantial fee reductions for regulatory procedures
Classification of the ATMP[547]	• Confirmation if a medicine meets the scientific criteria for defining an ATMP and under which category it falls
Certification of CMC and nonclinical documentation[548]	• Pre-assessment of quality data and, when available, non-clinical data • Aims to identify any potential issues early on • CAT may recommend issuing a certification confirming the extent to which the available data comply with the standards
Support from the EMA innovation task force[549]	• EMA Innovation Task Force (ITF) is a multidisciplinary group that includes scientific, regulatory and legal competences • Establishes a discussion platform for early dialogue with applicants • For companies not yet experienced in the regulatory arena
PRIME scheme[550]	• Aim: to enhance support for the development of medicines that target an unmet medical need • Offers more frequent interaction and early dialogue with developers of promising medicines
Scientific advice procedures by National Competent Authorities or European Medicines Agency[551]	• Authorities give advice to developers on the appropriate tests and studies in the development of a medicine to avoid major objections regarding the design of the tests during evaluation of the MAA • Authorities give scientific advice by answering questions posed by medicine developers • Received advice is not legally binding

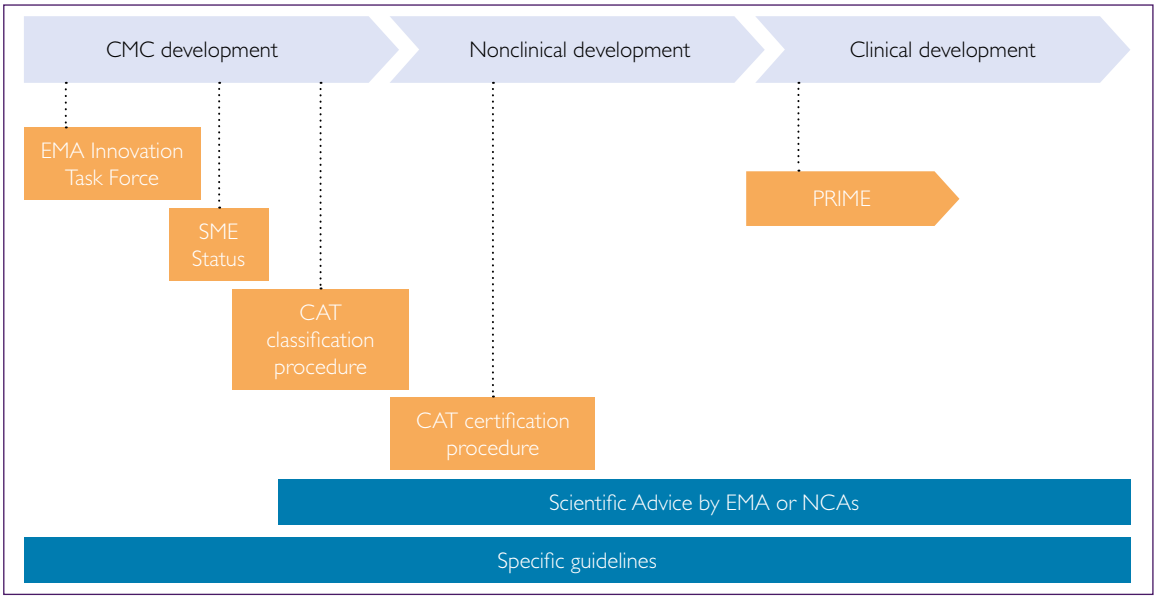


Figure 26: When to best use the regulatory tools

soon become reality and open new horizons for treatments. Gene editing might also find its way into the area of wound management. For example, Aushev et al. demonstrated the elimination of dominant-negative mutations in keratin genes in epidermal stem cells by transcription activator-like effector nucleases (TALENs).[552] This might be a promising approach for the treatment of keratopathies, like epidermolysis bullosa, in the future.

As was outlined in this article, most regenerative medicines are classed as ATMPs and are, thus, con-fronted with high product and development standards. Thus, their development can be very challenging for companies due to the inert complexity of the products. In addition, detailed EU guidance related to emerging gene editing technologies, in particular, but also to wound management related indications, is missing. Timely engagement with regulatory authorities can be key for a successful development process. Therefore, the integration of regulatory tools in the overall development strategy is crucial as it enhances early dialogue with regulatory bodies.

The wish list – for a better future

Based on an extensive review and critical reappraisal of the existing evidence and of the problems related to the implementation of new technologies in wound healing, the authors responsible for this EWMA position document agree on the following recommendations for future developments:

1. Development of new technologies: As the development of new technologies is a time- and resource-consuming process, often lasting several years, companies interested in developing and introducing both new technologies and medical devices for wound healing are advised to consult preliminarily with an interdisciplinary team of stakeholders, including basic scientists, bioengineers and clinicians with a specific expertise in wound healing, in order to test the originality and applicability of their ideas/projects.

2. Health technology assessments (HTAs): The limited financial resources in all healthcare sys-tems across Europe, which are typically financed via a taxpayer system, emphasise the need for an adequate allocation of resources based on updated evidence and principles of cost-effectiveness. HTAs have become the standard approach whenever new technologies are proposed for introduction into the field. The fact is that HTA procedures vary from country to country, or, in some cases, from region to region within a country. As part of a rationalisation process, which should be promoted and endorsed by the EU in the framework of legislative action, HTA procedures should be defined and standardised across the EU. This would simplify the process of bringing new technologies from the lab to the patients. It would also reduce the amount of resources that companies must invest in these procedures, eventually saving those funds for further research activities.

3. Implementation of new technologies in clinical practice: In order to bridge the gaps that almost unavoidably develop between the realisation of new technologies and their implementation in clinical practice, it is important to define minimum standard requirements for testing/implementation in clinical practice. These requirements must be related to Items 1 and 2 in this list, tested under controlled conditions and following the recommendations of good clinical research. RCTs are the preferred approach. However, due to the cost- and method-related difficulties linked with the organisation of an RCT, prospective observational trials may be considered if they are independent and relevant for wound management.

4. Translational science: Despite the increasing number of options in terms of the variety and quality of technologies available for clinical use in wound management, there is a diffuse underuse of new technologies when they initially become available to clinicians. Often, the implementation in clinical practice does not meet the expectations of the manufac-turers. One major component of this bias is related to

a poor understanding of the basic principles of the new technologies and their materials among healthcare professionals. Their level of knowledge may eventually be improved by translational science initiatives aimed at bridging this technological gap.

5. The need for investments in research: Important economic resources are needed to sustain the growth of research and the development of new technologies for wound management. Beyond the commercial interests of the industries in the field, institutions at the European level must also recognise the importance of investing in a field that will be of in-terest to one out of every four EU citizens over the next decades.

6. Access to new technologies in the EU: The possibility of accessing new technologies varies significantly across the different countries in the EU, not only for the reasons described below in Items 7 and 8 in this list. Another key factor in ensuring the accessibility of new technologies is that the companies must be willing to market the new technologies in all European countries despite the economic arguments for targeting certain countries before others. When new technologies are not available across the European healthcare systems, this creates idiosyncrasies in the actual possibility of patients being treated with new technologies. Therefore, companies are advised to extend their diffusion of new technologies across Europe to the extent that it is possible.

7. Regulatory controversies: Detailed EU guidance related to emerging gene editing technologies is available, but for wound management-related endeavours, it is so far missing. It would be advisable to engage with regulatory authorities in the future in order to make them aware of the challenges related to the development of medical products for wound management and this lack of guidance. This will hopefully lead to the development of specific guidelines from which product developers can benefit in the future.

8. Definition of outcomes, direct and indirect costs: Cost studies vary in approach and quality. The wide variety of outcome measures and costs hinder comparisons of interventions and progress. Thus, there is an increasing need to define outcomes, direct costs and indi-rect costs that should be included in the economic evaluations, clearly. Promoting re-search and clinical trials on advanced therapies and involving health economists and health statisticians in the planning, execution and analysis of the studies, is essential for ensuring the appropriate economic assessment of the impact of these interventions. Moreover, given the paucity of studies on the quality of life for patients, more analyses focused on this dimension should be performed.

9. The growth of a wound care centred research field within the telemedicine and wearables milieus: Technologies, such as telemedicine and wearables, enable the reduction of in-person visits and allow physicians to check on patients

remotely, track patient adherence to prescribed therapies, detect the early stages of serious medical conditions and triage those who are in need of immediate supervised care. While the application of such technology for effectiveness on diabetic foot care is still in its infancy, and its cost-effectiveness is still debated, it is anticipated that general healthcare and chronic wound care delivery will change due to this technology dramatically in the near future. Thus, more research is recommended in this field to translate these telehealth technologies into a better management system for chronic wounds and improved patient-centred outcomes, including the number of in-person visits required.

10. Evaluation of outcomes: A major challenge for a fair comparison between new technologies and conventional therapies is the lack of consensus and guidelines for the standardisation of reporting of outcomes. In addition, new outcomes that are more sensitive to new technologies should be defined and standardised, such as the number of in-person visits for telehealth applications and levels of restriction in mobility during the wound healing phase. Moreover, most research in the area of chronic wound management is currently focused on wound outcomes during the wound-healing phase without taking into consideration the high rate of recurrences. It is recommended that the time of recurrence for ulcers, as well as their frequency, should also be taken into consideration when examining the effectiveness of new technologies.

Contributions from EWMA

1. With regards to the development of new technologies (NTs), EWMA puts forward its 25-year long experience in the field and candidates for a pivotal position in an initiative to establish an interdisciplinary consultancy committee, including basic scientists, bioengineers, clinicians, and industry.

2. With regards to pushing for health technology assessments (HTAs) to be conducted on new technologies in wound management, EWMA offers to act as a consultant in the process of developing wound repair-related HTA procedures.

3. EWMA is available to initiate a programme, in collaboration with other stakeholders, offering endorsement for NTs, as well as dissemination and advertisement, via EWMA's communication and network platforms, such as the EWMA Journal and scientific meetings.

4. EWMA will promote actions targeting EU-level stakeholders and decision-makers to promote politics that facilitate the process of ensuring equal access to NTs across the European countries.

5. EWMA will work to influence the politics of the EU with an aim to increase the public investments in NT research.

6. EWMA will commit to initiatives supporting translational science that aims to bridge the technological gaps between research and clinical practice. This should take place in collaboration with all of the stakeholders involved in this fields, including clinicians, caregivers and industry leaders.

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