Universidade de Lisboa

Faculdade de Farmácia

Avanços tecnológicos na regeneração cutânea

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Mestrado Integrado em Ciências Farmacêuticas

2019

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Therapeutic Advances in Wound Healing

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Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

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2019

ACKNOWLEDGEMENTS

This work would not have been possible without the encouragement and collaboration of several people. I would like to express my gratitude to all those who contributed, either directly or indirectly, to making this work a reality.

To Professor Catarina Reis and Professor Andreia Ascenso for their constant availability and fundamental wisdom that allowed me a brilliant methodological orientation in order to conclude this work.

To my parents for their unconditional support. To my brother for his comprehension and helpful advice. To my grandmother for sharing valuable life experience and spiritual guidance at the most difficult times. To my niece for her presence and good disposition.

To all my close friends for their constant motivation and friendship.

A sincere thank you all.

ABSTRACT

Skin is the primary barrier against external agents be they chemical, physical or bacterial. A skin injury can compromise its integrity causing some complications if not properly healed. Therefore, wound healing is one of the most important but complex physiological processes that occur in the human body involving the sequential activation of multiple cell types and signaling pathways in a coordinated manner. Under some conditions, such as diabetes, proper wound healing might not be achieved leading to the development of non-healing chronic wounds. Depending on the possible etiologies, chronic wounds are classified as: pressure ulcers, diabetic ulcers, venous ulcers, and arterial insufficiency ulcers. Chronic wounds and burns decrease quality of life of the patients as well as for their families since it is associated with an increase in physical pain and socio-economical complications. Furthermore, incidence and prevalence of chronic wounds, unlike burns, have been increasing, mainly, due to population aging resulting in increased costs for national health systems and the trend is to further increase. Thus, the development of new, more cost-effective technologies/therapies is not only of big interest but also necessary to improve the long-term sustainability of national health systems. This systematic review presents an overview of the current knowledge on recent technologies/therapies developed (associated benefits and risks, clinical uses and availability) in the area of skin regeneration that have occurred in last two decades, including: (a) Skin Substitutes; (b) External Tissue Expanders; (c) Negative Pressure Wound Therapy; (d) Oxygen Therapy; (e) Wound Dressings; (f) Exogenous Growth Factor Based Therapy; (g) Systemic Therapy. Moreover, it explores future trends in wound care which includes the development of formulations using metallic (silver, gold and zinc oxide) nanoparticles and topical insulin. These novel formulations have been extensively studied and results have shown to be promising therapeutic options in the near future that may change the wound care paradigm.

Keywords: wound healing, skin regeneration, wound care, chronic wounds, burn injuries

RESUMO

A pele é a primeira barreira contra agentes externos, sejam eles químicos, físicos ou bacterianos. Uma lesão na pele pode comprometer a sua integridade, causando algumas complicações se não for curada de forma adequada. Portanto, a cicatrização de feridas é um dos processos fisiológicos mais importantes. Trata-se de um processo complexo que ocorre no corpo humano, envolvendo a ativação sequencial de vários tipos de células e cascatas de sinalização de forma coordenada. Sob determinadas condições, como acontece na presença de diabetes, a cicatrização adequada de feridas pode não ser facilmente conseguida, levando ao desenvolvimento de feridas crónicas não cicatrizantes. Dependendo das etiologias possíveis, as feridas crónicas são classificadas como: úlceras de pressão, úlceras diabéticas, úlceras venosas e úlceras por insuficiência arterial. As feridas crónicas e as queimaduras diminuem a qualidade de vida dos doentes e das suas famílias já que estão associadas a um aumento da dor física do doente e de complicações socioeconómicas. Além disso, a incidência e a prevalência das feridas crónicas, ao contrário das queimaduras, têm aumentado, principalmente, devido ao envelhecimento da população, resultando num aumento de custos para os sistemas nacionais de saúde. A tendência é de aumentar ainda mais. Assim, o desenvolvimento de novas tecnologias/terapias mais económicas não é apenas de grande interesse, mas também necessário para melhorar a sustentabilidade dos sistemas nacionais de saúde a longo termo. Esta revisão sistemática apresenta uma visão geral do conhecimento atual sobre tecnologias/terapias recentes desenvolvidas (benefícios e riscos associados, usos clínicos e disponibilidade) na área da regeneração cutânea que ocorreram nas últimas duas décadas, incluindo: (a) Substitutos de Pele; (b) Expansores Externos de Tecidos; (c) Terapia de Feridas por Pressão Negativa; (d) Oxigenoterapia; (e) Pensos; (f) Terapia Exógena Baseada em Fatores de Crescimento; (g) Terapia Sistémica. Além disso, esta revisão explora tendências futuras no tratamento de feridas, que incluem o desenvolvimento de formulações usando nanopartículas metálicas (prata, ouro e óxido de zinco) e insulina por via tópica. Estas novas formulações foram extensivamente estudadas e os resultados têm demonstrado serem promissoras opções terapêuticas num futuro próximo que podem mudar o paradigma de tratamento de feridas. Como as diferentes terapias apresentam características diferentes, a seleção da terapia apropriada ou da combinação de terapias para uma ferida específica é um passo fundamental para melhorar os resultados.

Palavras-Chave: cicatrização de feridas, regeneração cutânea, tratamento de feridas, feridas crónicas, queimaduras

ABBREVIATIONS LIST

α-SMA	*Alpha-Smooth Muscle Actin*
AgNP	*Silver Nanoparticle*
AIU	*Arterial Insufficiency Ulcer*
AKT	*Serine-Threonine Kinases*
AuNP	*Gold Nanoparticle*
bFGF	*Basic Fibroblast Growth Factor*
CDOT	*Continuous Diffusion of Oxygen Therapy*
CEA	*Cultured Epithelial Autograft*
CETE	*Continuous External Tissue Expander*
DFU	*Diabetic Foot Ulcer*
DM	*Diabetes Mellitus*
DU	*Diabetic Ulcer*
ECM	*Extracellular Matrix*
EGF	*Epidermal Growth Factor*
EGFR	*Epidermal Growth Factor Receptors*
EMA	*European Medicines Agency*
eNOS	*Endothelial Nitric Oxide Synthase*
EO	*Essential Oils*
ERK	*Extracellular Signal-Regulated Kinases*
FDA	*Food and Drug Administration*
FGF	*Fibroblast Growth Factor*
GSH	*Cellular Total Glutathione*
HBOT	*Hyperbaric Oxygen Therapy*
HEKa	*Human Epidermal Keratinocyte*
hs-CRP	*High-Sensitivity C-Reactive Protein*
IAgNP	*Insulin-Coated Silver Nanoparticles*
IFN-γ	*Interferon γ*
IGF-1	*Insulin-Like Growth Factor*
IL	*Interleukin*
IR	*Insulin Receptor*
IRS	*Insulin Receptor Substrate*
KGF	*Keratinocyte Growth Factor*
LN332	*Laminin 332*
MMP	*Metalloproteinase*
NO	*Nitric Oxide*

NP	*Nanoparticle*
NPWT	*Negative Pressure Wound Therapy*
PDGF	*Platelet Derived Growth Factor*
PMN	*Polymorphonuclear Leukocytes*
PU	*Pressure Ulcer*
rh-bFGF	*Recombinant Human Basic Fibroblast Growth Factor*
rhEGF	*Recombinant Human Epidermal Growth Factor*
rhKGF-2	*Recombinant Human Keratinocyte Growth Factor 2*
ROS	*Reactive Oxygen Species*
SDF-1α	*Stromal Cell-Derived Factor 1 Alpha*
SPC	*Stem/Progenitor Cells*
TAC	*Total Antioxidant Capacity*
TBSA	*Total Body Surface Area*
TGF-α	*Transforming Growth Factor Alpha*
TGF-β	*Transforming Growth Factor Beta*
THOT	*Topical Hyperbaric Oxygen Therapy*
TIMP-1	*Tissue Inhibitor of Metalloproteinases 1*
TNF-α	*Tumor Necrosis Factor Alpha*
VAC	*Vacuum-Assisted Closure*
VEGF	*Vascular Endothelial Growth Factor*
VLU	*Venous Leg Ulcer*
VU	*Venous Ulcer*
ZnONP	*Zinc Oxide Nanoparticle*

INDEX

1. INTRODUCTION

Skin act as a physical, bacterial and chemical barrier and skin wound healing is an essential physiological process. Skin repair and regeneration is a complex and multifactorial process consisting on a highly sophisticated temporal sequence of molecular pathways and cellular events activated after injury and following an organized and harmonious way in order to restore the damaged tissue. However, under some conditions, the wound healing can be compromised resulting on prolonged wound healing phases or excessive responses of the organism to the injury (1). The result is generally the formation of an acute wound, which if not properly healed after a period, it can lead to the development of a non-healing chronic wound.

A chronic wound can be defined as one that has failed to proceed through an orderly and timely reparative process to produce anatomic and functional integrity within a period of 4 weeks to 3 months or that has proceeded through the repair process without establishing a sustained, anatomic and functional result (2). Underlying conditions/pathologies such as malnutrition, stress, metabolic syndrome, diabetes and obesity predispose patients to chronic and non-healing wounds (3). Chronic wounds have a significant impact on the health and quality of life of patients and their families causing numerous physical as well as socio-economical complications. Physical complications may include infections, hemorrhage and lower-extremity amputations worsening wound outcomes and, in the worst cases, disability. On the other side, socio-economical complications comprise depression, distress, anxiety, embarrassment and social isolation and, consequently, financial burden. Ultimately, chronic wounds can lead to death (2). According to possible etiologies, The Wound Healing Society classifies chronic wounds into four categories: pressure ulcers (PU), diabetic ulcers (DU), venous ulcers (VU) and arterial insufficiency ulcers (AIU) (4).

In developed countries, it has been estimated that about 1 to 2% of the population will experience a chronic wound during their lifetime (2,5). Economic burden is estimated to be nearly 2-4% of the health budgets mainly associated with hospitalization, dressing changes, nursing time and related bacterial infections (6). Chronic wounds affect around 6.5 million patients in the United States and treating them cost about US$25 billion annually. Also, chronic wound care in the United Kingdom costs about US$3.4–4.6 billion per year (data in 2005) representing around 3 % of the total estimated national expenditure on health service for the same period (7). The burden is still growing since health care cost is increasing, population is aging and the incidence of highly risk of chronic wound development factors, such as diabetes and obesity, has also been rising (5).

Other common wound injuries are skin burns. Burn injuries are among the most devastating of all injuries since they are the fourth most common type of trauma worldwide, following traffic accidents, falls, and interpersonal violence. Burn incidents have been more prevalent among low socioeconomic populations and in less developed regions that generally lack the necessary infrastructure to reduce the incidence and severity of burns. Burns can often result in morbidity, prejudice of emotional well-being, and decreased quality of life (8). Actually, burn wounds are one of the major causes of disability-adjusted life years lost in middle and low income countries (9). Moreover, these injuries often require long-term treatment with numerous outpatient visits and multiple reconstructive surgical procedures and concomitant hospital stays constituting a socioeconomic burden for burn victims and their families. Ultimately, burns may result in death even though the majority is non-fatal (9). Fortunately, due to advances in burn care and social development, there has been a worldwide downwards trend of burn incidence and severity, length of hospital stay, and mortality rate (8).

Burn care is considered an expensive care since it requires long-term treatment and multiple surgeries in order to restore the skin, as previously mentioned (9). Despite detailed information about it, the cost of burn care is still scarce, and the most important costs are related to length of hospital stay, operative costs, dressings and staff. Also, the cost is directly proportional with percentage of total body surface area (TBSA) burned, being the primary determinant of costs (10,11). A systematic review of burn care costs reveals that the mean total healthcare cost per burn patient in high-income countries was US$88,218 ranging from US$704-US$717,306 (10). Another study from Australia reached a similar conclusion, being the cost US$73,532 for an average adult burn patient (11).

Taking into account the personal, social and economic impact of wounds for both patients and their families as well as for countries' national health systems, the search and development of novel wound healing cost-effective therapies/technologies have been of particular interest in the last decades. Some examples of these therapies are presented in this master thesis, namely skin substitutes; external tissue expanders; negative pressure wound therapy; oxygen therapies; wound dressings and growth factor based therapies.

2. AIMS

This master thesis aims to present an overview of the current knowledge and recent developments regarding wound healing technologies/therapies during the last two decades (late 1990s and early 2000s) as well as to explore potentially therapeutic options for the near future.

3. METHODS

The research for this literature overview was based entirely on electronic resources, mostly using the *Pubmed* database. Most of the research was performed between mid-April and early August and, occasionally, it was conducted outside these dates. From the analysis of literature search, the most representative articles were identified. Regarding article selection, there was no language restriction although most articles cited in this review are written in English. Also, most reported articles were published after 2000. Throughout the research and selection of information, there was always the objective of using the most reliable information and published in indexed scientific journals. At the end, 199 references were included in this research.

4. SKIN ANATOMY

The skin, a complex multi-layered structure, is the largest and the outermost organ of the human body, and it accounts for about 15% of the total body weight of an adult. It plays a critical role in protecting the underlying muscles, bones, ligaments and internal organs against external physical, chemical or biological agents (12,13). Furthermore, the skin is also important in thermoregulation, and provides sense of touch, prevents dehydration and synthesizes D_3 vitamin (14). Its structure is composed by three main layers: epidermis, dermis and hypodermis.

The epidermis is the most superficial and biologically active of these layers as the basal layer of the epithelium (*stratum basale*) is constantly renewing (15). The epidermis is mainly constituted by keratinocytes, apart from Langerhans cells and melanocytes, and its outermost layer, the *stratum corneum*, has a key role in protecting the underlying tissues against infections, dehydration and chemical or mechanical stress. In the basal layer, the lowest layer of the epidermis, stem cells differentiate into keratinocytes. This differentiation and maturation occur while these cells migrate towards the skin surface, but alongside this process, their ability to undergo mitosis is lost converting them to unviable cells (12,16). Langerhans cells participate in the skin immune system while melanocytes produce melanin, a pigment that colors the skin and filters out the ultraviolet radiation from the sunlight (12).

The dermis is the thickest of the three layers and it is located underneath the epidermis. It is a connective tissue made of extracellular matrix (ECM), fibroblasts, vascular endothelial cells, in addition to hair follicles, sweat and sebaceous glands, blood vessels and nerve endings (17).

Fibroblasts constitute the majority of the dermis population and have the ability to secrete collagen and elastin contributing for cutaneous elasticity and mechanical strength (12).

Below the dermis is the hypodermis layer (subcutaneous layer). Its primary function remains on isolating and dampening between the skin and skeletal structures, such as bones and muscles, besides storing energy in adipose tissue (17).

5. WOUND HEALING

Regeneration of injured skin tissue is an important biological process due to its vital function of maintaining the body in haemostatic conditions, but a very complex one since it depends on multiple types of cells and biological pathways. Typically, in mammals, the normal response to injury occurs in four overlapping but distinct stages: haemostasis, inflammation, proliferation and remodeling phases (18). Excessive wound healing (e.g. hypertrophic scars and keloids) or chronic wound healing (e.g. ulcers) can compromise the normal physical function (19).

5.1. Haemostatic Phase

In order to prevent blood and fluid losses, remove necrotic tissue and avoid infection, the coagulation cascade is quickly activated as well as inflammatory pathways and the immune system (19). Platelet aggregation is achieved through some activators, such as collagen and tissue factor, resulting in the release of growth factors and chemotactic factors to form the blood clot (20).

5.2. Inflammatory Phase

The inflammatory stage starts a few minutes after tissue damage. This is followed by increased blood flow and vascular permeability resulting in edema and redness due to the rise of local inflammatory agents' levels (histamine, activated complement, inflammatory cytokines, fibronectin, etc.) which promotes the infiltration of neutrophils, monocytes and lymphocytes to the injury site. These cells are necessary to remove necrotic tissue and control infection by killing bacteria through phagocytosis and free radical production (17,21,22). In addition, macrophages remove apoptotic neutrophils and other dead cells, acting as antigen presenting cells and releasing cytokines and growth factors that stimulate angiogenesis (23,24). Lymphocytes exert specific response against microbes usually for 72h (13,22).

5.3. Proliferation Phase

The proliferative stage usually starts 2 days after the injury overlapping with the inflammatory phase (25). It begins with the degradation of the fibrin-platelet matrix and is characterized by migration and proliferation of fibroblasts (fibroplasia) and endothelial cells to the wound site resulting in the secretion of the cytokines and growth factors mentioned above, ECM deposition, angiogenesis and re-epithelialization (12,25). Fibroblasts have a critical role in this stage since they produce matrix proteins (fibronectin, collagen, hyaluronic acid and proteoglycans) which are essential to the development of new ECM. These cells also help in angiogenesis and migration of keratinocytes which are essential to the process of re-epithelialization (26,27). This allows the previous fibrin matrix to be gradually replaced by granulation tissue composed of fibroblasts, blood vessels, macrophages, and a loose matrix rich in type I collagen, fibronectin, glycoprotein and hyaluronic acid (1). In the later part of this stage, some fibroblasts, which are attracted from the bone marrow or from the edge of the wound, are stimulated by macrophages, and can be differentiated into myofibroblasts (28). These last cells are quite important in the process of wound contraction since they secrete alpha-smooth muscle actins (α-SMA) and elastic fibers, being only found in the adult wound (1,29,30). Typically, this stage might last for about 3 weeks (25).

5.4. Remodeling Phase

The remodeling or maturation phase is the last of the four phases occurring during a long period of time up to 1 year or more. During this stage, initial processes engaged after the injury become less intense and cease (18). Apoptosis of macrophages and myofibroblasts is noticeable as well as the decline of the neovasculature (31). The maturation stage is marked by both wound contraction and collagen remodeling (degradation of type III collagen and formation of type I collagen) (32). While remodeling, wounds' tensile strength gradually increases as a consequence of myofibroblasts' action even though the tissue never regains the properties of uninjured skin (33). Collagen remodeling is achieved by maintenance of its balance determined by the regulation of matrix metalloproteinases´ (MMP) activity (34).

6. THERAPEUTIC ADVANCES IN SKIN HEALING

6.1. Skin Substitutes

When a major skin loss occurs, the probability of the risk infection, water loss and hypothermia increases leading to a higher morbidity, cost and period of hospitalization and, sometimes, it can be fatal (35). Although the most popular treatment options resort to the allogenic skin graft technique, the problem is that the donor area skin is too often limited. Thus, skin substitutes of synthetic or human origin present an efficient alternative for substituting donor skin grafts (1,25).

The preparation of skin substitutes involves cells and/or ECM (36). Ideally, synthesized skin substitutes should provide immediate replacement of both epidermis and dermis with permanent wound coverage (37). They should also be able to act as a barrier preventing the events mentioned above; not provide any toxicity; without antigenicity to avoid any immune response; be hypoxia tolerant; facilitate angiogenesis; be resistant to shear; have long shelf-live and be easy to store; finally, they should be easy to apply and agile enough in order to adjust to irregular wound surfaces (12,35,36,38,39).

There are different ways to classify skin substitutes, such as coverage duration; anatomy structure; cellular/acellular; biologic/synthetic, but the most accepted and commonly used classification is the Kumar's system (40). The Kumar's system consists of three categories: class I - Temporary impervious dressing materials; class II - Single layer durable skin substitutes; and, class III - Composite skin substitutes (41). This system is fully described in the following section (A1).

6.1.1. Class I - Temporary Impervious Dressing Materials

Class I skin substitutes function as an epidermal barrier and despite lacking cell components, they act as a mechanical barrier to bacterial invasion, reduce evaporative water loss and help to produce moist environment for improved healing (35,41).

6.1.1.1. Single Layer Materials

Natural Materials

Natural material include those from natural sources like human amniotic membranes (e.g. Biomembrane®), and biocompatible vegetal biomembranes, such as those derived from the *Hevea brasiliensis* rubber tree (42).

Biomembrane® (Matrix Company, Egypt) comprises of four sheets (epithelial sheet, basement membrane, connective tissue fibroblasts and a spongy sheet) and has been reported to have angiogenic properties (42). Furthermore, it decreases pain, protects from infection, controls the loss of electrolytes and albumin, hastens the re-epithelialization and prevents invasive bacterial infection (42–44). Additionally, amniotic membranes inhibit the α-SMA decreasing the formation of scar tissue compared to a moist wound dressing control (45). Biomembrane® is mostly used in the treatment of burn wounds.

The vegetal biomembrane that comes from the *Hevea brasiliensis* rubber tree seems to be useful in chronic VUs. Its activity has shown to be more intense in the inflammatory phase which seems to be directly related to intense angiogenesis followed by re-epithelialization (46).

Synthetic Materials

Synthetic material include membrane or synthetic polymer films (e.g. Tegaderm®; 3M, USA); biosynthetic cellulose layers (e.g. Nexfill®; MedLevensohn, Brasil); biocellulose layers (e.g. Veloderm®; Applied Pharma Research, Switzerland) and biocellulose layers derived from sugar cane, foam, or polymeric spray (e.g. Mepilex®; Mölnlycke Health Care, Sweden). (35)

6.1.1.2. Double Layer Materials

Transcyte® (Advanced Tissue Sciences, USA) is a human fibroblast-derived skin substitute often used for excised burns and consists of a polymer membrane and neonatal human foreskin fibroblast cells (allogenic) cultured under aseptic *in vitro* conditions on a nylon mesh. Within the nylon mesh, fibroblasts proliferate and secrete matrix proteins (fibronectin, type I collagen, tenascin, proteoglycans and glycosaminoglycans) and growth factors contributing to the promotion of angiogenesis and further fibroblasts´ regrowth (12,47). In addition, Transcyte® has been shown to significantly reduce the length of hospitalization of pediatric patients with partial thickness burns being quite safe and effective (48).

6.1.2. Class II - Single Layer Durable Skin Substitutes

Class II skin substitutes can be either epidermal or dermal.

6.1.2.1. Epidermal Substitutes

Epidermal skin substitutes are like the human epidermis in its form and function. However, products in this category tend to present poor quality scar since they are prone to breakdown

and susceptible to infection (41,49). The only product available in this category is the cultured epithelial autograft (CEA), Epicel® and EpiDex®. Both involve the culture of autologous keratinocytes (patient's own keratinocytes). Since these substitutes are very delicate to handle, a delivery system or a supporting dressing is required. (12,50).

Epicel® (Genzyme Corporation, USA) is an epidermal substitute composed of autologous keratinocytes transplanted as epidermal sheet using petrolatum gauze support. It is especially used for permanent coverage in patients with burns higher than 30% TBSA. (51)

Epidex® (EuroDerm Biotech & Aesthetics, Germany) is an epidermal substitute composed of autologous keratinocytes isolated from outer root sheath of scalp hair follicles, and supplied as epidermal sheet discs with a silicone membrane support (51). Epidex® seems to be a valuable epidermal equivalent to repair hard-to-heal small to medium-sized chronic wounds that have some granulation tissue but fail to re-epithelialize, particularly, chronic venous leg ulcers (VLU) (52).

6.1.2.2. Dermal Substitutes

Dermal substitutes are either composed of substances that are identical to those found in human dermis with processed skin or manufactured as dermal collagen matrix besides other matrix proteins. They increase wound stability and prevent its contraction. Products in this category include bovine collagen sheets and dermal matrices, human dermal matrices, and porcine collagen sheets (35,42,53).

Bovine Collagen Sheets - Kollagen® (Eucare Pharmaceuticals, India) is an acellular matrix derived from bovine collagen. It enhances epithelialization and protects the wound against infection and fluid loss (53). It has been used in the treatment of partial and full thickness burns. (54).

Porcine Collagen Sheets - Permacol® (Medtronic, USA) is a sterile, moist, and tough but flexible sheet of acellular cross-linked porcine dermal collagen and elastin fibers (55). Permacol® can play an important role in the short-term management of complex or contaminated abdominal wall defects. However, the lack of long-term studies and the high cost of the implant call for a medical cost-effectiveness assessment are some of its limitations (56). OASIS Wound Matrix® (Smith & Nephew, UK) is a dermal regeneration matrix derived from submucosal layers of the porcine jejunum (57). It is composed by glycosaminoglycans, fibronectin, proteoglycans, and growth factors. This skin substitute is commonly used in lower limb wound treatment, and it appears to be useful in DUs (57,58).

Bovine Dermal Matrices - Matriderm® (MedSkin Solution Dr. Suwelack AG, Germany) is a highly porous membrane consisting of type I, II and V collagen obtained from the bovine dermis coated with elastin derived from bovine *ligamentum nuchae* (59,60). It minimizes scar contracture while increasing skin flexibility and elasticity (61). Moreover, the risk of hematoma is decreased due its haemostatic properties (62). Studies have reported its efficacy in the treatment of full thickness burns associated with autologous split skin graft, hands and scalp, and in chronic wounds such as DUs (62–65).

Human Dermal Matrices - Alloderm® (Allergan, Ireland) is an acellular dermal matrix composed by biological components derived from cryopreserved human cadaveric dermis namely fibrillar collagen and collagen VI, elastin, hyaluronan, proteoglycans, fibronectin and vascular channels (41,49). It serves as a template for the ingrowth of host fibroblasts and vascular tissue, and supports the dermal tissue regeneration (66). However, the barrier function of human cadaver allografts, including Alloderm®, is not ideal since contamination of cadaver skin could be possible resulting in disease transmission (67). Notwithstanding, it has been shown to be a good option in acute full-thickness burns, facial soft-tissue defect augmentation and, more recently, it has been reported for a wide range of applications, including abdominal wall reconstruction and alloplastic breast reconstruction (68–71).

6.1.3. Class III - Composite Skin Substitutes

Class III skin substitutes include those materials that replace both dermal and epidermal layers, and any cellularized full-thickness substitutes. These can be human skin grafts (autografts, allografts and xenografts) or produced by tissue engineering (53).

6.1.3.1. Produced by Tissue Engineering

Integra® (Integra LifeSciences, USA) is one of the composite skin substitutes produced by tissue engineering and it consists of an acellular dermal regeneration template, composed of two layers, one a silicone protective sheet which prevents fluid escape, and the other one, a matrix composed of bovine collagen and glycosaminoglycans which seats underneath the silicone layer and provides a base for revascularization and neodermal formation (72,73). It was initially created out of necessity to provide temporary coverage for patients suffering from extensive burns (73). However, since then, its clinical applications have broadened, and nowadays, it is also commonly used in the treatment of partial and full thickness wounds and chronic wounds (74). Another commonly used synthetic bilayer for skin substitution is Biobrane® (Smith & Nephew, UK) that has been used since the late 1970s.

Regarding the tissue engineering, an alternative for the treatment of complex wounds is the association of skin substitutes with keratinocytes in culture. Examples of these types of skin composites include Orcel® and Apligraft®.

Orcel® (Fortificell Bioscience, USA) is a composite skin substitute that consists of fibroblasts seeded into a bovine type I collagen matrix (dermal side) and keratinocytes cultured at the epidermal side. This product stimulates the host cell migration by providing a favorable matrix (12). Since its approval, it has been mainly used in the treatment of VLUs and diabetic foot ulcers (DFUs) as well as in partial-thickness burns (75,76). Despite its benefits in wound care, it presents some clinical limitations since it cannot be used in infected wounds; in patients with allergy to any animal products due to increased risk of rejection and disease, and neither in patients who are allergic to penicillin, gentamycin, streptomycin, or amphotericin B. Another disadvantage associated with Orcel® is the necessity of being cryopreserved (42).

Apligraft® (Organogenesis Inc., USA) is similar to Orcel® in the sense that both contain living dermis and epidermis being composed of mixing living fibroblasts from neonatal foreskin with bovine type I collagen in the lower layer (dermal layer), and keratinocytes in the upper layer (epidermal layer). However, Apligraft® is subjected of another additional heating step in its production in order to obtain a loose matrix allowing a dermal fibrous network development. Afterwards, cells proliferate on this fibrous matrix (12). Like Orcel®, Apligraft® has been used in the treatment of VLUs, DFUs and partial-thickness burns (76). Disadvantages regarding its clinical uses are similar to those of Orcel® and, unlike the latest (which has a shelf-life of 9 months), Apligraft's® shelf- life is quite low (about 5-10 days) (12,42). Besides, although there is a chance of disease transmission owing to allogenic nature of Apligraf®, the risk is very low (12).

6.2. External Tissue Expanders

Due to its viscoelastic properties, skin has the capacity to be stretched. It is believed that when skin is stretched, the collagen fibers straighten along one another until the forces cause elongation, which is called mechanical creep (77). Skin stretching can cause either cell proliferation or apoptosis depending on the context. This is due to the fact that mechanical stimulation is capable of activating highly integrated signaling cascades modulating cell response as well as ion channels and second messengers' activity (78).

Hereupon, the goal of tissue expansion is to promote wound closure based on the utilization of the skin's viscoelasticity by mechanical creep (79) without the need of more complicated plastic surgical techniques and potentially reducing length of hospitalization and expenses since it requires less nursing care (80).

External tissue expanders consist of skin-stretching devices proving to be more advantageous than internal traditional tissue expanders due to fewer complications (81). Most complications related to internal tissue expanders (including infection implant exposure and failure) are a result of their presence inside the tissue (81,82). Due to their potential, there has been critical innovation in this area; novel skin stretching devices/systems include Dermaclose®, Proxiderm®, Topclosure 3S System®, Silver Bullet Wound Closure®, Wisebands® and ABRA Dynamic Wound Closure®.

Dermaclose® (Wound Care Technologies, USA) is a continuous external tissue expander (CETE). It is applied through skin anchors connected to a controller, which provides a continuous force to slowly expand tissue allowing controlled and gradual tissue advancement hastening wound closure (80,83). This product is relatively simple and it can be quickly applied (80). Recently, it has been shown that it can be useful, not only in the closure of fasciotomy, DFUs and large Mohs surgical defect, but also in sternal wound dehiscence and in ankle arthroplasty (77,83–86).

Proxiderm® (Progressive Surgical Products, USA) is another CETE. This device is applied over the suture site with long hooks that are extended and inserted beyond the margins through normal dermis and fixed in the subcutaneous tissue near the wound margins. This allows the closing of deeper wounds (80).

Topclosure® 3S System (IVT Medical, Israel) is composed of a pair of attachment plates that are connected by a long flexible approximation strap. The plates adhere to the skin through a hypoallergenic and biocompatible adhesive located underneath them. Along the attachment plates there are multiple pairs of openings for invasive attachment to the skin using either

sutures or staples, if needed (87). It has a lock/release ratchet mechanism which allows the skin to be pulled together (80) being especially useful in large wounds' closure (88,89).

Silver Bullet Wound Closure Device® (Boehringer Laboratories, USA) is another recent device that is applied intraoperatively. Despite being an invasive device, it has been shown that it may be a more consistent, efficacious and faster way to manage upper extremity fasciotomy wounds when compared to other wound closing techniques such as the split thickness skin grafting (80,90). Although the device is reusable, it is still quite expensive. Other disadvantages include the possibility of causing numbness and persistent scar tenderness (90).

Wisebands® (Wisebands, UK) device consists of a tension feedback control mechanism device, a polypropylene band and a metal surgical needle. Both the band and the needle are brought through the wound edges, down to the underlying soft tissue under the wound. A feedback tension control device holds the band and, by rotating a knob on the unit, a load is transferred to the wound edges in a controlled manner. When the tension exceeds 1 kg/cm^2, the feedback control mechanism device releases and remains in the last safe position. It has been reported its benefits in complex wounds involving skin and soft tissues defects of various sizes and in various anatomical locations. However, minor wound complications such as pain, skin irritation or blanching, hematoma or neurapraxia can be caused. Other major complications have been detected such as wound infections or intractable pain when using this invasive device (91).

ABRA Dynamic Wound Closure Systems® (ACell, USA) includes two different systems: ABRA® Abdominal and ABRA® Surgical. As opposed to static devices, which apply a constant force, these ones induce a dynamic or cycling stretching. Cyclic stretching of tissue helps collagen fiber rotation hastening wound closure (92). The first one is based on elastomers that are fixed few centimeters away from the wound edge, allowing gradual approximation of abdominal midline muscles and fascia. These elastomers pass across the defect below the fascia and, then, exit on the opposite side from the wound edge in an equal distance back. It has been reported its advantages for the management of abdominal open wounds, either alone or in combination with other wound´s closure techniques (93–95). The technique involving the setup of this system is like the first one, but it is not designed specifically for primary closure for retracted, mid-line abdominal defects. Instead, it is designed to control, reduce or close retracted soft tissue defects, and some studies have shown its usefulness in fasciotomy wounds' closure (96,97).

6.3. Negative Pressure Wound Therapy

Negative pressure wound therapy (NPWT), sometimes also referred as vacuum-assisted closure (VAC) or topical negative pressure therapy, is another novel wound healing therapy which applies differential suction or vacuum to the wounds resulting in an enhanced wound healing. It has become commonly used in the treatment of acute and chronic wounds such as pressure wounds, DFUs, lower leg wounds, surgical incision, traumatic wounds, burns, infected wounds, necrotizing fasciitis, infected sternal wounds and after skin grafting. Notwithstanding, the most promising results of NPWT have been obtained on patients with lower leg ulcers that are vascular in origin as well as DFUs, in which there is a sufficient blood supply (98,99).

Although there are various and different NPWT devices, the fundamental components are similar. Essentially, these devices are composed of four components: 1) a porous material which fills the wound site; 2) a drainage port that is attached above the porous material and connected to a 3) controlled vacuum pump; 4) and an adhesive film dressing sealing the wound (100). The porous material typically consists of polyurethane foam with a general resting pore size ranging 400 to 600 µm, which seems optimal to maximize tissue regrowth, favoring pressure transmission within the wound by a evenly distributed way (100–102). Other porous material less used includes white polyvinyl alcohol or gauze (100). The adhesive dressing is usually made of polyurethane because of its occlusive properties, which not only allows the negative pressure to create an effective vacuum with minimal leak but also protects the wound from external contaminants (101). The vacuum pump usually applies pressures of 80-125 mmHg (103). This force seems to be ideal to maximize tissue regrowth and granulation formation (101).

6.3.1. Mechanisms of Action

Despite the fact that NPWT's mechanism of action is not totally understood, it is thought that it promotes wound healing through four primary mechanisms: 1) macrodeformation; 2) microdeformation; 3) wound fluid removal and reduction of edema; 4) alteration of wound environment (98,100).

6.*3.1.1. Macrodeformation*

Macrodeformation occurs when suction is applied to the foam causing the collapse of its pores. This process results in deformational forces exerted on the wound edges (100). During

macrodeformation, shearing forces affect the cytoskeleton initiating a signaling cascade that modulates cell response as well as ion channels and second messengers' activity stimulating production of granulation tissue, and ultimately, enhancing wound healing (78,98).

6.3.1.2. Microdeformation

The mechanical forces produced during NPWT not only cause macrodeformation of the wound, but also it can cause microdeformation. Microdeformation describes the mechanical deformations occurring at microscopic scale on the foam-wound interface when suction is applied to the porous material, whether it is foam or even a gauze. These tensile forces lead to pro-proliferative and pro-angiogenic responses (104). Moreover, when suction is applied, the microdeformation at the wound surface is maximized, further increasing the thickness of granulation tissue and angiogenesis (103). There are some factors that affect the efficiency of microdeformation such as: type of the tissue, the deformability of the surrounding tissues, the pore size and the consistency of the foam and the intensity of suction (100). Furthermore, microdeformational wound therapy has been shown to enhance neural growth and neuropeptide expression promoting neurogenesis in diabetic mice.

6.3.1.3. Wound Fluid Removal and Reduction of Edema

Maintaining a controlled moist wound environment enhances wound healing process since it can prevents tissue dehydration and cell death, hastens angiogenesis, increases breakdown of dead tissue and fibrin and optimizes growth factors' interaction with their target cells (105). Meanwhile, its presence in an exaggerated way increases the risk of infection and abscesses (98). Moreover, fluid accumulation in the extracellular space elevates pressure in the interstitium inhibiting cell proliferation. Since the fluids in the extracellular space interact with the surface of the wound, suction facilitates removal of extracellular fluid (100). By removing fluid, the compression forces acting on the microvasculature allow higher localized blood flow, which increases the supply of oxygen and nutrients, and enhances formation of granulation tissue (98,106). This is correlated with an increase in interleukin 8 (IL-8) and Vascular Endothelial Growth Factor (VEGF) levels in case of wounds treated with NPWT (107). IL-8 plays a key role not only in the chemotaxis of granulocytes in the site of infections but also in the stimulation of angiogenesis (108). The fluid removal itself causes a reduction of edema. In addition, it has been noted, through morphological and quantitative analysis, that NPWT induces lymph vessel proliferation at the edge of the wounds reducing edema even though its effect depends on the presence of underlying diseases. Furthermore, an increase in the lymph

vessels' density seems to be correlated with a better clinical outcome, optimizing the healing process and reducing hospitalization time and the number of dressing changes, and thus providing greater patient comfort (99).

6.3.1.4. Alteration of Wound Environment

An ideal wound healing process results from a well-balanced presence between cytokines, growth factors and matrix MMPs. Wound exudate often contains high quantities of MMPs and low levels of their inhibitors promoting an unhealthy environment for wound healing. From the data available on the influence of NPWT on MMP expression, it appears to reduce expression of MMP-9 and increase tissue inhibitor of metalloproteinases 1 (TIMP-1) expression. MMP-9 is involved in the degradation of ECM, and it promotes angiogenesis and cell migration. TIMP-1 is responsible for balancing MMP-1's activity, promoting cell proliferation and reducing apoptosis effect (108,109).

6.3.2. Associated Risks

Although NPWT has been demonstrated to be effective in the treatment of numerous wounds as previously mentioned, there are some potential risks or complications associated with its use. Therefore, clinicians should be aware of strategies to prevent them. There are three major complications, which can potentially be life-threatening, such as bleeding, infection and retention of the foam dressing (102,110). The primary cause of bleeding is the mechanical damage of the underlying tissues and can be aggravated by infection, necrotic tissue or coagulopathy (110). Thereupon, it is not recommended NPWT's foam dressing to be in direct contact with exposed blood vessels, organ, nerves or anastomotic sites since the risk of fistulae formation in the presence of exposed organ or hemorrhage with exposed blood vessels is increased (102). Regarding the infections, the relation between infection and application of NPWT is not clearly defined although some authors have described chronic wound sepsis caused by retention of the foam dressing (111). Infections should be treated before NPWT application (102). Finally, retention of the foam in the wound may cause infection or bleeding since the dressing materials used with NPWT do not dissolve, and therefore, provoke an inflammatory reaction slowing the wound healing process down. Other complications include pain associated with dressing changes and patient's allergy to the adhesive dressing or the foam material (in these cases, NPWT is not recommended) (102).

6.3.3. Devices

Currently, there are numerous NPWT devices commercially available. The two most widely used systems are VAC® therapy (Kinetic Concepts Inc., USA) and the RENASYS® NPWT (Smith & Nephew, UK). Both systems are similar consisting of a portable pump capable of delivering negative pressure whose levels are adjustable. They are linked to the wound filler connected through tubes. The applied wound filler is the same for both, i.e., a black polyurethane foam equivalent. The main difference is related with the tubing that links the wound filler and the pump. While the VAC system incorporates traditional tubing, the RENASYS® system includes a soft and compressible device making it more comfortable for the patient (112).

6.4. Oxygen Therapy

Even though this form of wound healing therapy is not recent, there has been some innovation in this field due to research and technological advances. For a long time, the two primary methods of oxygen-based therapies used to treat wounds were Hyperbaric Oxygen and Topical Hyperbaric Oxygen therapies. More recently, another distinct class of oxygen generation and delivery wound care device, which provides continuous treatment of wounds with oxygen, has emerged. These are referred as Continuous Diffusion of Oxygen Therapy (CDOT) or Transdermal Oxygen Therapy. While all three methods are similar by using oxygen to promote wound healing, there are some technological and therapeutic differences (113).

6.4.1. Hyperbaric Oxygen Therapy (HBOT)

HBOT is a treatment in which a patient breathes 100% oxygen at higher than normal atmospheric pressure, i.e., higher than 1 atmosphere absolute using a treatment chamber (114). During treatment, arterial oxygen tension may reach 2000 mmHg and tissue oxygen tension of up to 500 mmHg (113). Benefits of HBOT includes improved neovascularization and ECM formation and decreased inflammation (115). It has been shown to be beneficial in the treatment of late radiation tissue injury, chronic ulcers, acute wounds and burns (116). Neovascularization occurs by two mechanisms simultaneously. Firstly, HBOT leads to an increased local production of reactive oxygen species (ROS) and reactive nitrogen species stimulating the production of some growth factors such as VEGF, transforming growth factor beta (TGF-β) and angiopoietin 2 (115). Secondly, it stimulates the recruitment and differentiation of circulating stem/progenitor cells (SPCs) from the bone marrow to form vessels in a process called vasculogenesis. This is due to the increased production of nitric oxide (NO) in the bone marrow which mobilizes SPCs in normal humans, diabetic patients and patients previously exposed to radiation. Furthermore, HBOT stimulates ECM formation by improving the synthesis of fibroblast growth factor (FGF) which, in consequence, leads to fibroblast migration and proliferation. In addition, increased oxygen stimulates the proliferating fibroblasts to produce collagen at increased rates and enhances collagen cross-linking to improve tissue tensile strength. Lastly, decrease in inflammation is achieved through reduction of edema, expression inhibition of pro-inflammatory cytokines by monocyte-macrophages, promotion of macrophages chemotaxis (which helps in the wound's cleaning), increased leukocytes' bactericidal activity, and blockage of neutrophil β2 integrin. The last one has been shown to decrease tissue reperfusion injuries without limiting the neutrophils' antibacterial function (115,117).

6.4.2. Topical Hyperbaric Oxygen Therapy (THOT)

THOT evolved from the HBOT and uses a chamber around the injured area sealing it tightly to prevent any leak which is then filled with oxygen from an external source at pressures slightly above atmospheric values but at high flow rates. This allows creating an oxygen rich environment at the wound surface. When compared to HBOT, THOT offers advantages including a lower risk profile of diverse therapeutic patient population by avoiding the potential side effects of high pressure and systemic oxygen (such as oxygen toxicity) besides it can be applied in more diverse settings at a lower cost and convenient way. However, it does not present the same oxygen potential as HBOT since collagen production by fibroblasts and collagen cross-linking is lower. In addition, ECM formation is not fully optimized and, therefore, the rate of wound closure is slower. Its benefits are similar to those of HBOT, and it has been shown to stimulate healing in chronic wounds (118,119). Moreover, THOT is FDA (Food and Drug Administration) approved for DUs, PUs, burns, venous insufficiency, postsurgical infections, gangrenous lesions, skin grafts, frostbite, and amputations (113,118). An example of a THOT device is Numobag® (Numotech, USA).

6.4.3. Continuous Diffusion of Oxygen Therapy (CDOT)

CDOT usually consists of devices that provide a continuous oxygen supply to the wound site. As opposed to HBOT and THOT, these devices are portable and deliver oxygen at normospheric pressure and slower flow rates directly to the wound bed that must be covered with a moist wound dressing to allow oxygen diffusion. In fact, without humidification, desiccation can occur preventing oxygen solubilization in the wound fluid, and consequently, reducing its transport into the tissue. Although THOT uses high flow rates (40 L/min) which can cause desiccation, these devices provide intermittent therapy (90 minutes/day, 5 days a week), and thus not require a moist environment. It has fewer reported risks and side effects than previous modalities and offers easy therapeutic delivery (113). Despite that studies regarding this new class of devices are limited, some have shown THOT could be beneficial in the healing of DFUs, sickle cell disease ulcers as well as in the treatment of recalcitrant and painful wounds as an adjunct (120–123).

6.5. Wound Dressings

Wound dressings have undergone an evolutionary process from materials that simply covered the wound to for materials that maintained a favorable wound moisturizing environment. More recently, materials that deliver active components and/or interact directly with cells or specific chemicals in the wound site (124). Nowadays, there are wound dressings available for all types of wounds but the selection of a dressing material for a particular wound is essential to achieve faster healing. An ideal wound dressing should be able to: provide or preserve a moist environment; enhance epidermal migration; promote angiogenesis and connective tissue synthesis; allow gas exchange between wounded tissue and environment; maintain favorable temperature in order to improve the blood flow to the wound bed; protect the wound bed against bacterial infection; not adhere to the wound and be easy to remove; stimulate debridement action to enhance leucocytes´ migration and support the accumulation of enzymes; be sterile, non-toxic and non-allergic and also cost-effective (125). Antimicrobial dressings have been the main target of great interest and innovation on this topic.

6.5.1. Antimicrobial Dressings

One of the more recent innovations in the area of wound dressings have been the development of antimicrobial dressings to prevent wound contamination since this has been associated, not only, with high rates of morbidity and mortality, but also, with increased expenditure on patient management and implementation of infection control measures (126). These dressings release antimicrobial agents continuously at the wound surface in order to provide a long-lasting antimicrobial action with maintenance of physiological moist environment (124). In order to improve dressing antimicrobial properties, different agents have been incorporated within their structure. Those antimicrobial agents usually consist of antibiotics (e.g. tetracycline, ciprofloxacin, gentamicin and sulfadiazine), silver (e.g. silver nanoparticles) and natural products (e.g. honey, essential oils and chitosan) (13).

6.*5.1.1. Antibiotics*

Until now aminoglycosides, beta-lactams, tetracyclines, glycopeptides, quinolones and sulphonamides have been used to display antimicrobial activity in wound dressings. These drugs interfere with a function/feature of the bacteria structure or on their metabolic pathways through one of the following four mechanisms: 1) inhibition of bacterial cell wall synthesis; 2) blockage of key metabolic pathways; 3) interference on protein synthesis; and 4) inhibition of

nucleic acids´ synthesis (13). Nevertheless, despite being useful in the treatment of infected wounds, repeated and/or improper usage of antibiotics can initiate bacterial resistance.

6.5.1.2. Silver Based Dressings

Silver has been used in wound care for a long time, but its antibacterial properties were only recognized after bacterial discovery. Silver has a broad spectrum of activity against both aerobic and anaerobic Gram-negative and Gram-positive bacterial strains (e.g. *E.coli*, *P.aeruginosa, methicillin resistant S.aureus and S.pyogenes*) (127). Elemental silver is chemically inactive, poorly absorbed by mammalian or bacterial cells, exerting its antibacterial activity when ionized in the presence of water, body fluids or tissue exudates. Silver ions exert its antimicrobial activity by: (a) binding to tissue proteins causing a structural change in the bacterial cell membranes; (b) blocking the respiratory chain acting on respiratory enzymes such as cytochrome oxidase and NADH-succinate dehydrogenase; (c) denaturizing bacterial DNA/RNA preventing replication; (d) interacting with bacterial proteins; (e) inducing production of ROS (128,129). Another mechanism by which silver may enhance wound healing is through the reduction in the activity of MMPs, which has been linked with delayed wound healing and inflammation due to its inhibitory effect on zinc activity. It also inhibits the release of tumor necrosis factor alpha (TNF-α) and other pro-inflammatory cytokines (34,130,131). Silver exhibits low toxicity in the human body, but chronic occupational exposure can lead to deposition of silver metal/silver sulphide particles in the skin (argyria), eye (argyrosis) and other organs (132). Despite these being not life-threatening conditions, they are cosmetically undesirable. Research indicates that silver dressing is effective for treating burns and chronic wounds (133). Also there is some evidence that it may improve the short-term healing of leg wounds and ulcers, although long-term results are nuclear (134). Due to its role in wound healing, silver has been impregnated in a variety of dressing materials such as hydrocolloid-based tulle dressing (Urgotul SSD®; Urgo Medical, UK), film dressing (Arglaes®; Maersk Medical, UK), foam dressing (Contreet Foam®; Coloplast, Denmark), hydrofiber alginates (Aquacel Ag®; ConvaTec, UK), low adherent materials (Acticoat®; Smith & Nephew, UK) and carbon fibers (Actisorb Silver 220®; Systagenix, UK) (131).

6.5.1.3. Natural Products

Nowadays, an increasing number of wound dressings has been functionalized with compounds obtained from natural sources, to improve their antimicrobial activity. As there is a

continuous need to search for new antibiotics, natural products are both fundamental sources of new bioactive agents and integral components of pharmaceutical compendium (13,135).

6.5.1.3.1. Honey

Honey's antimicrobial activity is attributed to its high sugar content, low water content, acidity and endogenous hydrogen peroxide which altogether inhibit the growth of micro-organisms (136,137). In addition, honey can also rapidly debride wounds and remove malodor; possess anti-inflammatory activity by increasing NO and decreasing prostaglandin levels; reduce edema and exudate and prevent/minimize hypertrophic scarring; stimulate granulation and epithelial tissues' growth hastening wound healing; and promote a non-adherent interface between the wound and the dressing facilitating its removal without pain or damaging newly regrowth tissue (13,137,138). Honey has shown to be beneficial in the treatment of burns and ulcers, including DFUs (137,139). Wound dressing containing honey as an antimicrobial agent are already available in the market, like non-adherent wound contact dressings and calcium alginate dressings (e.g. Medihoney Tulle Dressing® and Medihoney Apinate Dressing®, respectively; Comvita, New Zealand), gauze dressings, non-adherent soft alginate dressings and low-adherent light viscose net dressings (Activon Tulle®, Algivon® and Actilite®, respectively; Advances Medical, UK). (13)

6.5.1.3.2. Essential Oils (EO)

EO are natural products from the secondary metabolism of plants and present a broad spectrum of action, including antioxidant, antifungal and antibacterial activity. (140). Despite the fact that the antimicrobial mechanism of action of EO is not completely understood, it is believed that the activity is due to their hydrophobicity and different constituents like cinnamaldehyde, geraniol, thymol, menthol and carvacrol, among many others (141,142). It is thought that EO attack the cell membranes' phospholipids and the lipids available on the cell wall of the bacteria, leading to an increased permeability, and ultimately, to cell lysis which results in the cytoplasm leakage, pH decrease, and loss of cellular processes, like ATP biosynthesis, DNA transcription and protein synthesis (13). Due to its potential antimicrobial activity against Gram-positive and Gram-negative bacteria, EO based dressings have been studied (143). One study using alginate based films containing pepper essential oil showed antibacterial activity against *E. coli*, *S. aureus* (commonly found in hospital settings) and *B. cereus* (141). Another study, involving the encapsulation of EO in fibrous dressings, verified complete inhibition of *E. coli* growth even when low amounts of essential oils were used (142).

6.5.1.3.3. Chitosan

Chitosan is a cationic natural polymer and has been investigated as an antimicrobial agent for preventing and treating infections owing to its intrinsic antimicrobial properties despite other beneficial properties such as biocompatibility, biodegradability and non-toxicity (144,145). Accordingly, some mechanisms of action have been proposed: (a) the ionic surface interaction resulting in wall cell leakage; (b) the inhibition of the mRNA and protein synthesis through penetration of chitosan into the bacteria's nuclei; and (c) the formation of an external barrier, chelating trace metals and oligo-elements besides suppressing cell exchange and essential nutrients' absorption. All of these mechanisms may occur simultaneous but at different extensions (146). In addition, chitosan has been shown to be effective at hastening wound healing by enhancing the functions of inflammatory cells, such as polymorphonuclear leukocytes (PMN), macrophages, and fibroblasts, and thus, promoting granulation process. It has also been reported that chitosan could increase the tensile strength of wounds (144). Its clinical use has shown that mesh chitosan dressings promote efficient adherence, homeostasis, healing, and re-epithelialization of the wound while reducing itching and pain. Furthermore, histopathological analysis showed that the skin cell layers were repaired, and tissue architecture was reestablished after 10 days. Application of nonmesh chitosan based dressings is not recommended since it can cause blood accumulation under the dressing (147). For these reasons, chitosan based dressings have been used in order to treat moderately to heavily exuding open wound-infections and burns, including hydrogel dressings (e.g. HidroKi®; Ceramed, Portugal), hydrocolloid dressings (e.g. Tegasorb®; 3M, UK) and haemostatic gelling fiber dressings (e.g. KytoCel® Aspen Medical, Australia); which are commercially available (13).

6.6. Exogenous Growth Factor Based Therapy

It is well known that wound healing is a process that relies on a wide variety of growth factors. These are biologically active polypeptides that are involved in cell growth, differentiation, proliferation, migration and metabolism playing a key role in all phases of wound healing (haemostasis, inflammation, proliferation and remodeling phases) (148). More specifically, growth factors can enhance wound healing since they stimulate the formation of granulation tissue and ECM, modulate inflammatory response, promote angiogenesis being essential in the remodeling and re-epithelization processes (6). However, *in vitro* and *in vivo* studies have shown that some growth factors´ levels in non-healing acute and chronic wounds were decreased, such as the platelet derived growth factor (PDGF), FGF and epidermal growth factor (EGF). This led to an increased interest in using exogenous growth factors as a potential target for wound therapy (148). Despite their potential as therapeutic agents in wound healing, studies have shown that *in vivo* stability of both endogenous and exogenous growth factors is low in chronic wound fluids due to the high activity of MMPs which seems to degrade growth factors and their receptors. Also, it has been found that degradation can be blocked by the addition of MMP inhibitors (149). Considering this low *in vivo* stability, marketed medications require continuous administration or high doses to exert the desired effects. Consequently, side effects can occur if high systemic levels are reached which limit their clinical application. Another disadvantage of growth factor's use is the high cost (6).

6.6.1. PDGF Family

PDGF is one of several polypeptide growths factors which controls the growth, differentiation, and activation of a variety of cell types. It is known to be secreted from many activated cells, including macrophages, fibroblasts and endothelial cells. It plays an essential role in wound healing by: (a) stimulating directed and sequential migration of neutrophils, macrophages, and fibroblasts into the wound over the first days; (b) activating wound macrophages and fibroblasts leading to an increase in endogenous growth factor production, provisional ECM synthesis, fibroblast proliferation and collagen synthesis; (c) enhancing collagen remodeling of the wound since it has the ability to stimulate collagenase (150). Due to its benefits in wound healing, formulations using recombinant human-PDGF (rh-PDGF) have been developed.

In 1997 and 1999, the FDA and the European Medicines Agency (EMA), respectively, approved Regranex® (Smith *&* Nephew, UK), a gel containing becaplermin or rh-PDGF for DFUs. According to EMA, studies involving the use of Regranex®, applied topically on ulcers in diabetic adults, have found that it had healed about 10% more ulcers when compared with

placebo. In addition, in patients using Regranex®, 47% of ulcers with an area lower than 5 cm^2 were healed, compared with 35% of those treated with placebo (151).

Nevertheless, a post market study revealed that Regranex® increased five-fold the risk of death from cancer in diabetic patients who used three or more tubes when compared with patients who did not use it. This led FDA recommended its use only when the benefits are expected to outweigh the potential risk while EMA has withdrawn it from the market and is no longer used in the European Union (6,151).

6.6.2. FGF Family

The FGF family is composed of 23 members being the FGF-2, FGF-7, and FGF-10 the three most important members involved in the process of skin repair and regeneration. FGFs are produced by a variety of cell types like keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, and mast cells.

Regarding FGF-2, also named as basic FGF (bFGF), it was found that its levels are increased in acute wounds, playing an important role in the formation of granulation tissue and in the remodeling and re-epithelialization processes. *In vitro* studies have suggested that bFGF is able to: (a) stimulate keratinocytes migration during re-epithelization; (b) promote fibroblasts' migration and stimulate them to produce collagenase improving the remodeling phase; and (c) help to control the synthesis and deposition of various ECM components. Like PDGF, bFGF levels in chronic wounds are decreased (152). Thereafter, formulations using recombinant human-bFGF (rh-bFGF) have been developed.

In June 2001, Fiblast Spray® (Kaken Pharmaceutical, Japan), rh-bFGF or generic name trafermin, was launched and its indications include decubitus ulcers and skin ulcers (burn ulcers and leg ulcers) (6). One case-control studying the clinical impact of rh-bFGF on PUs found that it promoted granulation formation and re-epithelization in the early stages of treatment, and significantly reduced the volume of exudate and ulcer depth in the later stage of treatment accelerating the healing process (153). Another phase III clinical trial, in which 210 patients participated, has reported a faster wound closure rate of neuropathic DFUs treated with rh-bFGF for 12 weeks when compared to placebo. Also, it was observed a relative wound area regression of 40% or more on week 6, which was superior compared with placebo (154). Trafermin has also been shown to have a potential healing effect in PUs, DFUs and second degree burns (148). Also, a recombinant bovine bFGF is sold in China for wound healing proposes (155).

Despite its proven benefits in wound healing, trafermin is contraindicated in patients with malignant tumor or a history of malignant tumor at the application site (155).

Other important members of the FGF family include FGF-7 and FGF-10, also known as keratinocyte growth factor 1 (KGF-1) and keratinocyte growth factor 2 (KGF-2), respectively. *In vitro* studies have shown that both FGF-7 and FGF-10 stimulate keratinocytes' proliferation and migration which are essential in the re-epithelialization process; and promote transcription of factors that are involved in the detoxification of ROS helping to reduce induced apoptosis of keratinocytes in the wound bed preserving these cells for re-epithelialization (152).

Repifermin is a recombinant human KGF-2 (rhKGF-2) which has been clinically used. A randomized clinical trial, where repifermin was topically applied on chronic VUs for 12 weeks, has shown that repifermin was well tolerated, with no differences when compared to placebo. In addition, it decreased the risk of infections and, most importantly, accelerated wound healing by achieving 75% wound closure in the group treated with repifermin over placebo (156).

6.6.3. EGF Family

Perhaps the best-characterized growth factors in wound healing are those from the EGF family with EGF, transforming growth factor alpha (TGF-α) and heparin binding EGF (EGF-HB) being the members more involved in the process of wound healing. In healthy humans, epidermal growth factor receptors (EGFR) can be localized throughout the entire epidermis and its activation leads to various cell proliferation and migration, including glial cells, fibroblasts and, most importantly, keratinocytes, playing an important role in re-epithelialization according to *in vitro* studies (152).

In what concerns to EGF, there are three types of medicines based on the administration of recombinant human EGF (rhEGF): Heberprot-P®, Regen-D® 150 and Easyef® (6).

Heberprot-P® (Genetic Engineering and Biotechnology, Cuba) is an intralesional injectable for the treatment of DFUs with high risk of amputation. It was launched in Cuba but it has been registered in 15 other countries (157). Several studies have shown the potential of Heberprot-P® as a potential wound healing agent. It seems that its benefits in skin repair are associated with its capacity to stimulate the formation of granulation tissue and hastening wound closure (157–159). Thereafter, it has been reported that it lowers amputation risk. Despite its positive effects on wound healing, side effects (tremors, chills, pain and burning at the administration site, and local infection) have been reported (158).

Regen-D® 150 (Bharat Biotech, India) is a gel commercialized in India for the treatment of grade I or II DUs (6). A study conducted in India showed that application of this gel in patients with DFU resulted in a faster wound closure (average wound healing was 86% and average wound healing time was 4.8 weeks) since it promoted granulation and re-epithelization which, in turn, led to a decrease in wound healing duration when compared to placebo. Also, no side effects were observed (160).

Easyef® (Daewoong Pharmaceutical, South Korea) is a spray used for the complete healing of DFU launched in Korea in 2001, and nowadays, it is commercialized in South America, China and Vietnam. Studies involving the use of Easyef® in the treatment of DFUs for 8 weeks observed significant benefits in wound healing while being well tolerated with no side effects reported. It was shown that relative wound sizes were reduced by at least 50% and 80% in the eighth week. This is due to EGF's effect on promoting epidermal regeneration and corneal re-epithelialization by stimulating the production of proteins like fibronectin increasing the number of fibroblasts, enhancing the proliferation and migration of epithelial cells (161).

6.7. Systemic Therapy

Although several systemic agents have been studied in wound healing, including low-molecular-weight heparin, oral pentoxifylline and vildagliptin, there is still a lack of evidence showing their efficacy. Meanwhile, a particular interest in vitamins and supplements as potential therapeutic agents for wound healing has been growing, such as magnesium, zinc and vitamin D (162)**.**

6.7.1. Magnesium Supplementation

Magnesium plays a key role in several biological functions and its low levels (hypomagnesaemia) are associated with insulin resistance and type 2 *Diabetes Mellitus* (DM) among other conditions as dyslipidemia and hypertension (163). Moreover, hypomagnesaemia not only can impair glucose homeostasis and insulin sensitivity in type 2 DM patients which can lead to neuropathy development, but also has been associated with abnormal platelet activity. Thereafter, its low levels represent a risk factor for the development of DFU. On the other side, supplementation of magnesium leads to lower risk of type 2 DM development and improved biomarkers of inflammation and oxidative stress (163,164).

A randomized clinical trial using magnesium supplementation in patients with DFU for 12 weeks reported magnesium benefits in wound healing. Some of its benefits included: (a) a significant reduction in ulcer length, width and depth; (b) an increased insulin metabolism which delays vascular complications in DFU patients; (c) a decreased high-sensitivity C-reactive protein (hs-CRP) which might suggest an anti-inflammatory effect even though other inflammatory markers did not change; and (d) an increased plasma total antioxidant capacity (TAC) probably due to a decrease in ROS production and an increase in glutathione-peroxidase activity (164). Other study involving the co-supplementation of magnesium and vitamin E in DFU patients for 12 weeks also reported the same previous results besides the benefits on lipid profile that were also observed (165).

6.7.2. Vitamin D Supplementation

Vitamin D has been widely studied and is essential in various biological processes. Recent evidence has shown that levels of VU and PU patients' vitamin D are low which suggests that vitamin D might play an important role in the wound healing process (166).

One study reported vitamin D beneficial effects on wound healing when supplemented for 12 weeks in patients with DFU. It was found that vitamin D accelerated wound closure since ulcer

regression in terms of depth, width and length was superior when compared to placebo. Also, vitamin D seemed to improve lipid profile by reducing total cholesterol, LDL and total/HDL ratio; and glycemic control. These could help wound healing by an indirectly way. Furthermore, other vitamin D findings included a decrease in serum hs-CRP levels; increase in plasma TAC and cellular total glutathione (GSH) levels which, altogether, might suggest the benefits of vitamin D on oxidative stress (167).

6.7.3. Zinc Supplementation

Zinc is one of the most important micronutrients in the human body. It is a key factor in innumerous biological functions including wound healing. Zinc is present in all stages of wound repair and participates in the majority of the biological events including: platelet activation; PMN influx; bacterial clearance; apoptosis/phagocytosis; anti-inflammatory activity; tissue debris removal; migration of fibroblasts and keratinocytes, and thus, being important in re-epithelization; angiogenesis and SPCs activation; and, ECM remodeling (168). Thereafter, its low levels can impair proper wound healing.

As example, one randomized clinical trial evaluating the effects of zinc sulfate supplementation on wound healing and metabolic status in patients with DFU for 12 weeks concluded that, similarly to vitamin D, zinc had beneficial effects on parameters of ulcer size, glucose metabolism, HDL, total-/HDL-cholesterol ratio, hs-CRP, plasma TAC and GSH (169).

7. RECENT TRENDS

Due to the technological and research advances in the past decades, new modalities or categories of wound care have been emerging. In this section, two of these promising novel therapies, that might have a very important impact in the field of wound care, are following presented. The first one regards the development of particles at nanoscale that have been widely studied including its potential benefits in wound care. Since nanotechnology is quite recent; the development of new formulations using these particles will be certainly successful. The other therapy refers to topical insulin as a wound healing agent.

7.1. Nanoparticles (NPs)

In some cases, NPs can prevent microbial drug resistance which is the primary reason why NPs have been considered as promising alternatives to traditional antibiotics. This is an essential benefit since multidrug-resistant bacteria have increased at an alarming rate over recent decades leading to an increase in morbidity and mortality (170,171). In order to exhibit their antibacterial function, NPs need to be in contact with the bacterial cell. This contact can be achieved through electrostatic attraction, van der Waals forces, receptor-ligand and hydrophobic interactions. Thereafter, NPs cross the bacteria's membrane, interacting with the bacterial cellular components, such as DNA, enzymes, ribosomes and lysosomes. Also, it affects metabolic pathways, or even target mitochondria causing its disruption. This leads to excessive production of ROS favoring oxidation stress which damages the individual components of bacterial cells. Moreover, it changes the cell membrane's permeability resulting in bacterial membrane damage. It increases the gene expression levels of oxidative proteins, which is an essential mechanism in bacterial cell apoptosis (171). From all the NP formulations developed, metallic nanoparticles (such as silver, gold and zinc oxide NPs) have been of particular interest. Besides providing support for tissue repair, nanofibrous materials can also act as delivery systems for drugs, proteins and growth factors among other agents.

7.1.1. Silver Nanoparticles (AgNPs)

Advances in nanotechnology allowed the interest in producing silver particles at nanoscale (29). In fact, silver loaded nanoparticles are more efficient as an antimicrobial agent than the free silver from a conventional formulation, since that advanced delivery system presents a larger active surface providing better contact with microorganisms (128). Silver based nanoparticles act primarily in three ways: 1) attach to the surface of the cell membrane disturbing its functions; 2) penetrate inside the bacteria, causing further damage possibly by

interacting with cellular components such as the DNA; and 3) release silver ions which further contributes to the bactericidal effect (172).

It has been shown that AgNPs can induce rapid healing by decreasing the inflammatory stage through fibrogenic cytokine modulation, being a viable option for the treatment of all wounds (173,174). It is suggested that this decrease in inflammatory activity is not due to an increase in apoptosis of inflammatory cells but rather to an interruption in the signaling pathway of inflammation, more specifically, the TNF-α pathway (174). Additionally, it was observed higher expression of IL-10, VEGF and interferon γ (IFN-γ) when compared to silver sulfadiazine (173).

Another AgNPs´ benefit consists on achieving better skin resemblance with less hypertrophic scarring and nearly normal hair growth on the wound surface in mice model. It has been demonstrated that AgNPs induce higher mRNA levels of TGF-β1 in the initial period of healing when compared to topical sulfadiazine. However, the same is not observed during the latter phase of healing (173). TGF-β1 plays an important role in tissue fibrosis and its mRNA over-expression has been found in both keloids and hypertrophic scars (173,175). In contrast, a lack of TGF-β results in scarless healing of a fetal wound, with the exception of TGF-β3 (176).

Also, it has been observed, through histological analysis, that AgNPs promote the proliferation, migration and maturation of keratinocytes from wound edge toward the wound center enhancing wound healing by accelerated re-epithelialization. However, the exact underlying mechanism of action is still unclear. Moreover, AgNPs can significantly decrease the amount of hydroxyproline and collagen proteins, suggesting an alteration in fibroblasts' phenotype. Finally, it seems that AgNPs can decrease type I collagen, and increase α-SMA, supporting its relevance regarding the differentiation of fibroblasts into myofibroblasts. Overall, this mechanism allows an accelerated wound healing (29).

Although AgNPs have an enormous potential to be used as an effective antibacterial agent in treatment of wounds*, in vitro* studies revealed an alteration in mitochondrial activity in human fibroblasts. Nevertheless, these nanoparticles do not seem to affect cell viability. Despite their mitochondrial toxicity, it seems they do not cause cell death although there's some evidence suggesting the opposite (177). Since clinical trials regarding the possible toxicity via dermal exposure are not sufficient enough, more clinical studies are strongly recommended (178).

7.1.2. Gold Nanoparticles (AuNPs)

AuNPs are biocompatible and biodegradable, and hence they can be extensively used in wound healing (179). Like AgNPs, the antibacterial effect of AuNPs justifies its potential complementary use with antibiotics. They exert the antibacterial activity through the formation

of holes in the bacterial cell wall, which leads to cell death due to the loss of cell contents. Furthermore, AuNPs bind to bacterial DNA blocking the transcription process and preventing the uncoiling of DNA during transcription (180). Despite its antibacterial properties, several studies have demonstrated other benefits in wound healing context (180–184).

An *in vivo* study conducted in rats has reported that the application of AuNPs on cutaneous wound showed greater beneficial effects on wound healing compared to the control group. Moreover, when combined with photo biomodulation therapy, it even has greater benefits by stimulating angiogenesis and triggering inflammatory response at the early stage (181). Also, topically applied AuNPs with antioxidants (epigallocatechin gallate and α-lipoic acid) presented high anti-inflammatory and antioxidant activity resulting in significant wound healing acceleration (182). Furthermore, AuNPs seemed to decrease the secretion of IL-6, IL-12 and TNF-α levels which reflects its anti-inflammatory activity which can be helpful in wound care (183). It also seems that AuNPs can hasten DFUs healing when associated with essential oils such as nerolidol (184). Lastly, an important study showed that AuNPs thermoresponsive gels allowed to obtain further improved effects (bioavailability, skin permeation, antibacterial and anti-inflammatory activity) when compared to AuNPs suspensions (180).

Despite AuNPs' toxicity is relatively low, they can penetrate deeper into the skin and other organs which may result in the manifestation of some adverse side effects (183). Also, its toxicity is dose dependent. One study concluded that low AuNPs concentrations could stimulate keratinocytes´ proliferation but high concentrations could be cytotoxic (185).

7.1.3. Zinc Oxide Nanoparticles (ZnONPs)

It is already known that ZnONPs inhibit the growth of microorganisms. It has been proposed two mechanisms of action: (1) it damages the bacterial cell membrane due to the release of zinc ions, and then, permeates into cells; and (2) it generates ROS (186). A study in mice observed that ZnONPs disrupt bacterial cell membrane integrity and induce ROS, reduce cell surface hydrophobicity and downregulate the transcription of oxidative stress-resistance genes in bacteria, and therefore, stimulate intracellular bacteria death (187). Also, according to other studies, ZnONPs could induce only keratinocytes´ proliferation contributing to the re-epithelialization process. Also, it was reported that this improvement was dose and size-dependent (188,189). For these reasons, ZnONPs may seem to be a therapeutic agent with great potential in wound care (190–192).

Although FDA recognizes ZnONPs as "safe", the tissue tolerance of absorbed NPs by the living body should be carefully considered. Its presence at low concentrations avoids toxicity

since they are generally cleared by constant keratinocytes' replacement or the lymphatic system and, even if they penetrate into circulation, they are metabolizable (190). However, its high concentrations are associated with mitochondrial dysfunction in keratinocytes and, since they produce ROS while inhibiting the expression of superoxide dismutase and glutathione peroxidase genes in human keratinocytes, they can induce cell membrane oxidative stress and, consequently, apoptosis. (179).

7.2. Topical Insulin

Insulin is a peptide hormone and growth factor which regulates blood glucose levels. Additionally, several studies, either in animal or humans, have shown that insulin could be a potential therapeutic agent in wound care management (193–198). Since insulin can potentially help restoring the integrity of damaged skin, and also considering its low cost relatively to other growth factors, it is more likely to be considered for incorporation into wound dressings, bio adhesive films and hydrogels (199). Regarding wound care, it is known that insulin stimulates the growth and development of different cell types, and affects proliferation, migration, and secretion by keratinocytes, endothelial cells and fibroblasts (196). Despite its benefits in promoting wound healing, the exact underlying mechanism is still unclear. Some recent studies using topical insulin were able to give some clues about these mechanisms.

One study has reported an increase in keratinocytes´ migration in a time and dose dependent manner when insulin was topically applied (193). Moreover, it has verified that topical insulin accelerated and improved the quality of healing reflected by a decreased wound area at several different time points. It is thought that insulin-accelerated wound healing involves increased expression of the integrin α3β1 in keratinocytes as well as increased levels of laminin 332 (LN332) present in the basement membrane, resulting in full epidermal differentiation. Furthermore, insulin has also shown to promote attachment of the epidermis to the dermis and the appearance of a well-organized epidermis (193).

It has been suggested that insulin through insulin-like growth factor-1 (IGF-1) stimulates ECM production, induces keratinocytes and fibroblasts´ proliferation, inhibits apoptosis pathways and attenuates anti-inflammatory cytokine production. Furthermore, the levels of IGF-1 in wound fluid demonstrate a positive correlation with the success of wound healing in burn patients (194).

Other study, using topical insulin cream, has shown it normalized the wound healing time in diabetic animals and increased the wounds' tissue expression of IR (insulin receptor), IRS-1 (insulin receptor substrate 1), IRS-2 (insulin receptor substrate 2), SHC (Src

homology/collagen proteins), ERK (extracellular signal-regulated kinases), and AKT (serine-threonine kinases) compared to intact skin (196). This might suggest that the insulin signaling pathway may have an important role in wound healing. Furthermore, it has been noticed an increased expression of other proteins, such as: endothelial nitric oxide synthase (eNOS) in the bone marrow leading to an increased mobilization of SPCs; VEGF promoting angiogenesis; and stromal cell-derived factor 1 alpha (SDF-1α) enhancing wound healing (196,197). In a similar study (195), the use of IGF-1 based cream induced a higher expression of myofibroblasts in the wound bed, and as a consequence, a wound healing improvement was observed in both diabetic and non-diabetic animals. Also, when treated with IGF-1, the myofibroblasts' expression in non-diabetic rats was higher than in diabetic rats which might indicate a decrease in IGF-1 receptor expression (195).

Another important and quite recent study has evaluated the effects of a new nano-insulin formulation consisting of insulin-coated silver nanoparticles (IAgNPs), in diabetic and non-diabetic wound healing (198). The study has reported an increase in the percentage of wound closure in both diabetic and non-diabetic groups when treated with IAgNPs. This might be due to: (a) its effect on the balance of pro- and anti-inflammatory cytokines since a rapid decrease of pro-inflammatory cytokines (IL-6 and TNF-α) and an increase of anti-inflammatory cytokines (IL-10) were observed accelerating the inflammatory phase; (b) higher human epidermal keratinocyte (HEKa) migration in both diabetic and non-diabetic groups being the extent of migration directly increased with time and IAgNPs´ concentration; (c) decreased level of leukocytes´ infiltration associated with prolonged inflammatory phase in chronic wounds slowing down the process of skin regeneration; (d) faster deposition of collagens and rapid re-epithelization which hastens wound healing.

8. CONCLUSION

This review explores the benefits and associated risks of the various therapies as well as their clinical uses and their availability in the market. It is observed that each therapy is different in its characteristics presenting a unique set of benefits, risks and clinical uses.

Thus, the selection of an appropriate therapy or combination of therapies for a specific wound is an essential clinical step to achieve better outcomes.

Most of the technological/therapeutic advances that have occurred in the last two decades have been made from pre-existing therapies not having been developed many innovative therapies.

Nevertheless, improved outcomes in wound care, both in quality and in treatment time, have been achieved. Despite the positive impact of these technological/therapeutic advances, very few studies evaluated their cost-effectiveness which is of particular interest since costs associated with wound care have been raising and will continue to raise, due to population aging, representing a significant burden on national health systems.

Therefore, more cost-effectiveness analysis studies are recommended in order to make better decisions improving the quality of wound care in a sustainable manner.

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10. ATTACHMENTS

A1. Skin substitutes - Classes and types of skin substitutes; products available on the market; characteristics; and clinical uses.

SKIN SUBSTITUTES

Substitute Type	Product	Characteristics	Clinical Uses	References
Class I - Temporary Impervious Dressing Materials				
Single Layer Natural Materials	Biomembrane®	Human amniotic membrane	Burn wounds	(42)
	Hevea brasiliensis biomembranes	Vegetal biomembranes	Chronic venous ulcers	(46)
Single Layer Synthetic Materials	Mepilex®	Biocellulose layer derived from silicon foam	Chronic wounds, particularly ulcers and burn wounds	(35)
	Nexfill®	Biosynthetic cellulose layer		
	Veloderm®	Crystallin biocellulose layer		
	Tegaderm®	Synthetic polymer film	Partial skin lesions	
Double Layer Materials	Transcyte®	Sheet of neonatal human foreskin fibroblast cells and a polymer membrane	Excised and partial thickness burns	(12,47,48)
Class II - Single Layer Durable Skin Substitutes				
Epidermal Substitutes	Epicel®	Sheet of autologous keratinocytes attached to a petrolatum gauze support	Full thickness burns comprising a TBSA > 30%	(51)
	Epidex®	Sheet of autologous keratinocytes attached to a silicone membrane support	Small to medium-sized chronic wounds	(51,52)

Dermal Substitutes	Alloderm®	Acellular dermal matrix with biological components derived from cryopreserved human cadaveric dermis	Acute full-thickness burns; facial soft-tissue defect augmentation; abdominal wall and alloplastic breast reconstruction	(41,49,68-71)
	Kollagen®	Acellular matrix derived from bovine collagen	Partial and full thickness burns	(53,54)
	Matriderm®	Porous membrane of type I, II and V collagen	Full thickness burns and chronic wounds	(59,60,62-65)
	OASIS Wound Matrix®	Dermal matrix derived from submucosal layers of the porcine jejunum	Lower limb wound; and diabetic ulcers	(57,58)
	Permacol®	Sheet of acellular cross-linked porcine dermal collagen	Complex or contaminated abdominal wall defects	(55,56)
Class III - Composite Skin Substitutes				
Produced by Tissue Engineering	Apligraft®	Bovine type I collagen matrix seeded with fibroblasts (dermal side) and keratinocytes (epidermal side)	Partial thickness burns; diabetic foot ad venous leg ulcers	(12,76)
	Integra®	Acellular dermal regeneration template (two sheets: a silicone sheet: silicone protective a bovine collagen matrix)	Extensive burns; partial and full	(72-74)
	Orcel®	Bovine type I collagen matrix seeded with fibroblasts and keratinocytes (similar to Apligraft®)	Partial thickness burns; diabetic foot ad venous leg ulcers	(12,75,76)