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Review

Combination of Biomaterials and Extracellular Vesicles from Mesenchymal Stem-Cells: New Therapeutic Strategies for Skin-Wound Healing

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Abstract: Hard-to-heal chronic wounds associated with aging and high-prevalence pathologies, such as diabetes, are a global health problem. Therefore, it is necessary to advance effective treatments to accelerate wound healing. Among these potential treatments are new therapies based on mesenchymal stem cells (MSC) and their secretomes, including extracellular vesicles (EV). They have an important therapeutic potential for the treatment of chronic ulcers, due to their immunomodulatory activity, as well as their ability to induce angiogenesis, cell proliferation and cell migration. The use of MSC-derived EV in regenerative medicine involves cell-free therapies that decrease risks associated with cell therapies, such as the potential development of tumors. However, the short half-life of MSC-EV is a limitation for their clinical use. A therapeutic strategy to increase the regenerative efficiency of EV in wounds is to encapsulate them in biomaterials. The latter must protect and progressively release EV in damaged tissues, optimizing healing. Biomaterials that can be used include hydrogels. These, in addition to acting as a vehicle for sustained application of EV, can create favorable environments for wound healing. Thus, the aim of this review is to critically describe the latest advances in the development of such therapeutic strategies. It highlights the significance and clinical potential of these new therapies, as well as the need to develop clinical trials, to ascertain their performance.

Keywords: mesenchymal stem cells; extracellular vesicles; wound healing; skin; wound dressings; biomaterials; hydrogels; diabetes

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1. Introduction

The skin is the largest and arguably the most important organ of the body, representing 16% of body weight. Its integrity is essential for survival. Thus, it protects from external aggressions, such as ultraviolet radiation, abrasion and parasites, as well as prevents dehydration [1]. Three main layers are distinguished in the skin (from the outside to the inside): epidermis, dermis and hypodermis. The first consists mainly of keratinocytes. These are proliferative in the stratum basale and differentiate, as they replace those in the upper layers (spinosum, granulosum, lucidum and corneum; from the inside to the outside) [2]. The epidermis also shows invaginations, with hair follicles associated with sebaceous and sweat glands. On the other hand, the dermis is located below the anterior layer and is the most vascularized layer of the skin. The main dermal cells are fibroblasts. They are responsible for the synthesis of the extracellular matrix, mainly consisting of collagen. The dermis contains most of the dermal appendages, such as apocrine and eccrine

glands and hair follicles. The dermis also contains interdigitations called dermal papillae, located in the area of contact with the epidermis [2].

Among the pathologies that can affect the skin are wounds caused by trauma or damage. Under normal physiological conditions, the regenerative process for the formation of new tissue occurs after a series of phases, in which different cell types intervene, until tissue similar to the original is formed [3]. However, under certain conditions, the healing process slows down and becomes chronic, resulting in difficult-to-heal or chronic wounds. Among the main risks for this to occur are aging, diabetes and recalcitrant infections [4]. Also, excessive fibrosis in the later stages of healing can generate hypertrophic scars, that under more extreme conditions can degenerate into keloids [5,6].

The number of difficult-to-heal skin ulcers is increasing, mainly in the most economically developed countries. This is due in part to the aging of the population, and the increased prevalence of pathologies such as diabetes [7]. The latter is estimated that will be suffered by 629 million people worldwide by 2045, with an increase of 48% since 2017 [8]. That also leads to an increase in the number of skin ulcers. It is considered that open wounds represent a problem for 3% of the population over 65 years of age in the USA. So, with the aging of the population, the number of chronic wounds is expected to increase in the coming years [9]. This has a significant impact on healthcare spending, because these types of wounds require frequent care and treatment by physicians and nurses. Thus, the cost and management of diabetic foot ulcers, which have a 15–25% prevalence in diabetes, is between 9 and 13 million dollars in the USA [10].

Although conventional treatments, such as skin grafts, have reduced the mortality rate in patients, they are limited due to a lack of tissue sources. Also, the outcome of other treatments, such as surgical removal, dressing changes, hyperbaric-oxygen therapy, and negative-pressure wound therapy in many patients, is not satisfactory. Hence, the current quest for the development of more effective therapeutic approaches [11]. Taking into account the above data, in addition to a policy of prevention to reduce the appearance of chronic ulcers, it is necessary to develop more effective treatments than the current ones, to accelerate their healing. Among the new therapies that have emerged in recent years, for the treatment of this type of ulcer, is the use of mesenchymal stem cells (MSC) through cell therapy [12]. For this, as a general rule, it is necessary to isolate such cells, expand them in vitro and inoculate them into the damaged tissue to promote healing, thanks to the regenerative capacity of these cells [13].

The regenerative power of MSCs has been associated in part with their paracrine effects, rather than their ability to differentiate into different cell types. Thus, several studies have shown that conditioned media from MSC cultures have a regenerative capacity, similar, or even superior, to that of MSC [14,15]. Therefore, MSC secretomes may play a very important role in the therapeutic capacity of MSC. Within the secretomes, there are the vesicular fractions, formed by extracellular vesicles (EV). These may carry in their interior different types of molecules, with the capacity to induce physiological changes in the recipient cells [16]. Thus, it has been shown that MSC-derived EV have immunomodulatory properties, being able to favor proliferation, migration and vessel formation in different cell types. This gives them a high capacity to induce regenerative processes; for example, in skin ulcers [17]. However, the half-life of these vesicles can be short in damaged tissues. Therefore, treatments involving the direct application of these EV on wounds may not be very efficient. Thus, in recent years, much attention has been focused on combinations of MSC-derived EV with biocompatible materials, suitable for preserving their biological activity, further allowing their controlled release into wound areas. These strategies may increase the efficiency of the biological effects of EV, with the biomaterials providing appropriate microenvironments for healing, which may have a positive impact on both the speed and quality of wound healing [18].

In this scenario, the aim of this review is to critically describe the current research status and clinical potential of such a wound-healing strategy. That involves the development of complex biomaterials, with the ability to modulate the release of MSC-derived EV with

regenerative capacity into wounds, during the healing process. For this purpose, a literature search was carried out in PubMed https://pubmed.ncbi.nlm.nih.gov (accessed between 1 December 2022 and 12 January 2023), without temporal limits, using key words such as "extracellular vesicles and wound healing", "extracellular vesicles and pharmacokinetics", "extracellular vesicles and biomaterials and skin", "extracellular vesicles and hydrogels and skin", as well as "extracellular vesicles and wound dressings".

2. Physiology and Pathology of Cutaneous-Wound Healing

In the skin, tissue damage is repaired through complex biological processes, involving extensive cooperation of various cell types, growth factors and cytokines [19]. Restoring the integrity of all the layers of the skin (epidermis, dermis and hypodermis) are essential for its function, as a protective barrier against the damaging effects of abiotic external factors, as well as to prevent dehydration of the body, and to protect wounds from biotic aggressions (infections) [20]. A normal scar will form with loose fibrous connective tissue in a slow healing process, which consists of several phases. However, the damaged tissue will remain weaker and functionally deficient for at least some time, compared to uninjured tissue [19].

Thus, healing is a dynamic progression comprising four phases: hemostasis, inflammation, proliferation and maturation/remodeling. These phases may overlap, and even different areas of wounds may be in different stages of healing [17]. Within seconds or minutes after the wound originates, hemostasis begins with the action of platelets. The latter produces a blood clot that stops bleeding, further protecting the wound area from microbial invasion [21]. In addition, the formed fibrin network serves as a platform for the migration of vascular cells, leukocytes and fibroblasts [20]. Different cytokines, hormones and chemokines, necessary for the activation of subsequent healing phases, are also released [17].

In the second phase, an inflammatory reaction is induced in the area of ulceration, which aim is cleansing the damaged area of the skin. Within the first 24 h after the wound is produced, neutrophils synthesize proteases and antimicrobial compounds [2]. They kill microorganisms that are trapped after violation of the skin barrier [22]. At 48 h of wounding, the damaged zone gets cleaned by macrophages and lymphocytes, that engulf and digest the remains of the matrix and existing microorganisms. Then, they change phenotype, participating in the production of anti-inflammatory cytokines and extracellular matrix (ECM) [23]. These cytokines and chemokines activate the proliferation phase [24]. Therefore, after 48 h, the inflammatory process must be turned off to prevent the development of a pathological process.

The third phase comprises re-epithelialization, neovascularization and granulation tissue formation [21], which take place up to two to three weeks after injury. Re-epithelialization of the damaged skin occurs due to the differentiation and proliferation of keratinocytes, as well as regenerative epidermal stem cells. They originate from the interfollicular epidermis, hair follicles and sebaceous glands at the wound edge [25]. Cytokines and vascular growth factors, such as vascular endothelial growth factors (VEGF) and basic fibroblast growth factor (bFGF), induce angiogenesis, and thus the formation of new blood vessels [17]. This process is important for the progression of healing in the face of hypoxia and nutrient deprivation, occurring at the wound site [19]. Fibroblasts also proliferate and produce large amounts of ECM components. Some fibroblasts, under the action of transforming growth factor beta (TGF-β) differentiate into myofibroblasts. They have a contractile function: attach to the extracellular matrix via integrins, and contract via alpha smooth-muscle actin (α-SMA) [20]. Therefore, the size of the wound is reduced, contributing to its closure [19].

Finally, in the last and longer maturation/remodeling phase (for up to a year after skin injury), the tissue reaches its final appearance of healing [2]. After the closure of the skin wound, the ratio of fibrillar collagen I to collagen III in the temporal wound matrix (2:1) returns to the level of normal skin (5:1) [19]. This phase is characterized by the restructuring of the entire unorganized ECM [26], and the reduction of both dermis cellularity and blood-vessel density [17]. In general, after superficial injury, the scar is barely (or may not

even be) visible to the naked eye. In the case of a deeper wound, the scar is often visible, but is seen as a smooth, pale and flattened area known as a normotrophic scar.

As a result of this final stage, barely visible scars are generated for superficial injuries. They are usually visible as smooth, pale and flattened in deep trauma [27]. An incorrect sequence and timing of these phases could potentially result in abnormal healing [2]. This failure of tissue-repair processes can be associated with vascular diseases, diabetes mellitus, or aging. It can be also caused by genetic variations in genes responsible for such processes [20]. Ulcers may become chronic if the inflammatory phase takes more than three weeks [28]. On the other hand, an excess of fibrosis during the proliferation phase may generate hypertrophic scarring or keloids [29] (Figure 1).

Inflammatory responses are essential during wound-healing processes. Yet, a prolonged acute-inflammation phase may impair the proliferation of keratinocytes and recruitment of profibrotic macrophages [30]. An imbalance may occur between inflammatory and anti-inflammatory signals, and chronic inflammation may develop. Appropriate steps may not progress, leading to an imbalance in the environment, and disrupting wound healing [31]. Failure to proceed through the normal phases of healing generates chronic wounds [11]. This prolonged inflammation stage is caused by a local increase in levels of pro-inflammatory cytokines, like interleukin-1 beta (IL-1β) and tumor necrosis-factor alpha (TNF-α). All that delays wound healing [29]. In this scenario, the levels of matrix metalloproteinases (MMP) and serine proteases are significantly increased. They are capable of destroying components of the extracellular matrix, and worsening the migration of cells [32] (Figure 1b).

Chronic inflammation is one of the causes of poor healing in patients with diabetes. This pathology is responsible for the majority of non-traumatic lower-limb amputations. In fact, 15–20% of diabetic foot ulcers may require amputation [33]. Diabetes decreases the immune response, which facilitates infections. It also produces defects in microcirculation which, together with peripheral neuropathy and peripheral arterial disease, favor the appearance of skin ulcers. The healing of these ulcers is aggravated by hyperglycemia, which leads to the formation of advanced glycation end-products (AGE). They favor the chronification of inflammation. That is characterized by an increased presence of pro-inflammatory M1 macrophages, in relation to anti-inflammatory M2 ones, and increased levels of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-a (TNF-a) [33]. Age is also a risk factor that slows the rate of healing of skin wounds. With age, there are changes in the conformation of skin, that affect both the epidermis and dermis, making it more vulnerable to different types of damage. For example, the number of dermal-epidermal junctions in the epidermis decreases, as does the speed of keratinocyte migration. Additionally, in the dermis, there are fewer fibroblasts, macrophages, vessels and extracellular matrix components, such as collagen [34]. In addition, age may be also associated with increased malnutrition, sedentary lifestyle, decreased steroid hormones and increased medication, as a consequence of the appearance of pathologies associated with aging. All these factors also have a negative influence on healing capacity [34].

Defects in scar formation can lead to excessive accumulation of ECM in tissues (fibrosis) and cause pathological scarring, with loss of skin function. The most significant components of such process are TGF-β, connective tissue growth factor (CTGF), interleukin-4, 13 (IL-4, 13), platelet-derived growth factor (PDGF) and osteopontin [35]. Such abnormal scar overgrowth can lead to two different types of fibrotic-skin disorders: hypertrophic scars and keloids [19]. The former is an area of fibrous-connective tissue, formed by disordered collagen; mainly type III [20]. These scars grow rapidly for four to 12 weeks after injury. They tend to mature and flatten over time, without extending beyond the boundaries of the original wound area [17]. The latter, however, invade the surrounding skin and are slower to develop. They can appear even years after the trauma. Inadequate inflammatory responses can facilitate their development. They continue to grow as a benign fibroproliferative tumor [36]. Histologically, keloids differ from normal skin and hypertrophic scars in the

arrangement of collagen fibers, the presence of α-SMA, positive myofibroblasts and a higher degree of angiogenesis [37] (Figure 1c).

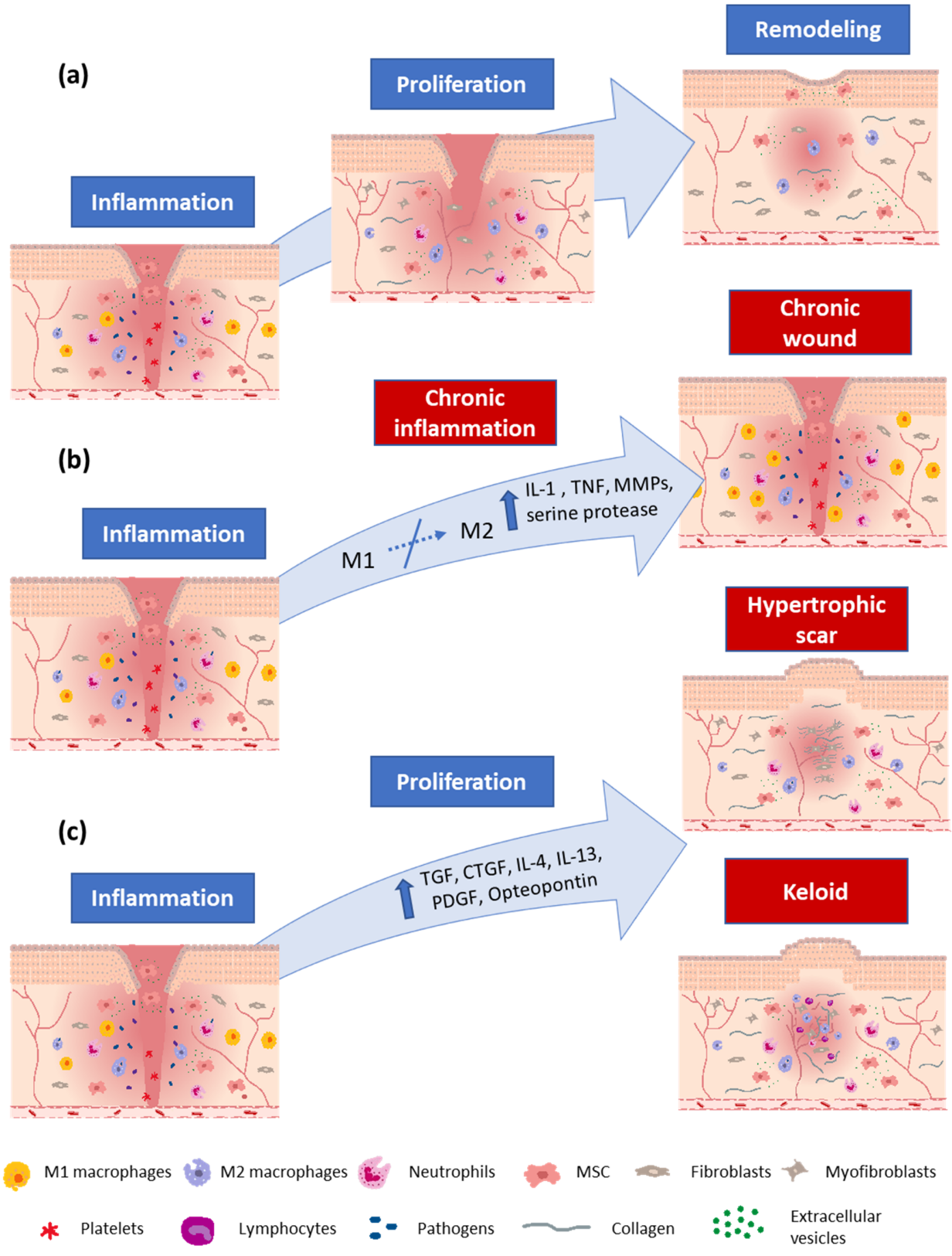

Figure 1. Pathological processes during wound healing. Compared to normal wound healing process (**a**), the ulcer may become chronic if the inflammatory phase lasts longer than three weeks (**b**). Excessive fibrosis during proliferative phase may lead to hypertrophic scars or keloids (**c**).

3. Mesenchymal Stem-Cells in Wound Healing

MSC are multipotent nonhematopoietic adult stem cells. In the last few years, they have acquired a lot of interest, due to their application in regenerative medicine. MSC can be isolated from various tissues such as umbilical cord blood, endometrium, bone marrow, fat tissues, Wharton's jelly, liver, amniotic fluid, placenta and dental pulp, among others [38]. Furthermore, they are characterized as plastic adherent cells, expressing the surface markers CD73, CD90 and CD105; not expressing CD14, CD34, CD45 and HLA-DR. Surprisingly, MSC have the possibility to differentiate into a wide variety of cells from mesenchymal (osteoblasts, chondrocytes, adipocytes, endothelial cells and cardiomyocytes), and non-mesenchymal (neurons, glial cells and hepatocytes) cell lineages [38–40]. They are known for their immunosuppressive, anti-inflammatory, tissue restoring, neuroprotection and differentiation properties [40].

These properties have made them an attractive therapeutic tool in cellular therapy and regenerative medicine. In fact, multiple clinical studies have been published about their applications to different diseases. They include a variety of tumors, multiple sclerosis, amyotrophic lateral sclerosis, acute and chronic heart failure, stroke, Crohn's disease, kidney and liver chronic disease, diabetes, osteoarthritis and rheumatoid arthritis, osteonecrosis, lumbar intervertebral disc degeneration, spinal cord contusions, sepsis, and critical limb ischemia [41]. MSC provide significant therapeutic potential, since they can expand in vitro, can be cryopreserved while maintaining their effectivity, express intermediate and low levels of the MHC class I and II molecules, and can be intravenously administered [17,39].

When an injury occurs, MSC are involved in the four wound-healing stages. They can reach the damaged tissue engrafting, differentiating into the convenient cells, as well as triggering anti-inflammatory responses, and promoting neovascularization for tissue regeneration [42]. However, this repair is not only due to MSC, but also to the trophic factors that they secrete, playing a paracrine effect. That is known as secretome and is composed of cytokines, growth factors, chemokines, inflammatory mediators, extracellular-matrix components, proteins and extracellular vesicles, which can mediate cell-to-cell communication, cell differentiation, chemoattraction, immunomodulation, inhibition of fibrosis and apoptosis, as well as secretion of pro-angiogenic factors [38,43].

In the case of a skin wound, the physiological response of MSC is the following: TNF-α and IL-1β are secreted by neutrophils, and MSC infiltrates the wound responding to the local inflammation. When MSC reach the wound, they produce pro-inflammatory cytokines like CCL2, CCL3, CSF2, IL-6 and IL-8, CCL2 and CCL3, recruiting neutrophils and macrophages. These are responsible for activating the adaptive immune system, and begin to degrade damaged tissue. Furthermore, MSC recruit plasmacytoid dendritic cells and limit the number of activated T cells, as well as other innate immune cells, to mediate the transition to the next phase. In the proliferation stage, MSC secrete some proteins and factors, promoting angiogenesis and re-epithelialization (EGF, HGF, VEGF, ANGPT1, PDGF-B and FGF). Also, they can promote resident macrophages to change from inflammatory (M1) toward wound healing (M2) phenotype. Finally, in the remodeling phase, MSC secrete matrix metalloproteinases, inducing matrix deposition and tissue inhibitors of metalloproteinases. That way, they avoid the deposition of extracellular matrix proteins, thus preventing the generation of scars [43].

4. MSC-Derived Extracellular Vesicles

An important fraction of the MSC secretome is composed of EV. They are defined as nano-sized particles surrounded by a lipid bilayer, without the capacity to replicate [44]. They can be released by the majority of cell types, and can be found in a variety of fluids [38]. There are three subtypes of EV: microvesicles, exosomes and apoptotic bodies. They are differentiated according to their size, biogenesis, content, release pathway and function [45]. Exosomes measure between 30 and 150 nm. They have an endosomal origin, through the fusion of plasma membranes with multivesicular bodies [46]. Microvesicles measure between 100 nm and 1 μm, and are formed by the evagination of the plasma membrane.

Finally, apoptotic bodies range from 50 nm up to 5000 nm in diameter. They are released by dying cells, due to the increase in hydrostatic pressure. Thus, cell contractions cause the separation of plasma membranes from cytoskeletons [45]. For more information about the biogenesis of EV, readers can consult some reviews [45,47].

Likewise, EV may contain active molecules like proteins, peptides, carbohydrates, lipids, and nucleic acids (miRNA, mRNA, non-coding RNA, double-stranded DNA, and mtDNA) that can be delivered into neighbor and even distant cells, modulating different biological process. These cargos can be different, depending on parent cells, biogenesis as well as physiological conditions at the time of formation [45,46,48]. They are intensively explored, because not only play an important role in the normal physiological process, but also in the development and progression of diseases [42,49]. Indeed, EV are involved in certain processes such as immune response, homeostasis maintenance, coagulation, inflammation, cancer progression, angiogenesis and antigen presentation [38].

In fact, in recent years, interest in MSC-derived EV (MSC-EV), as a regenerative therapeutic tool, has increased. Their effects have been studied in several important diseases involving the kidney, liver, cardiovascular system, wound healing, as well as neurological and neurodegenerative ones, among others [38,49]. Their use is considered a cell free-based therapy. Therefore, they overcome the safety limitations of whole-cell therapies. Among these limitations and undesirable effects xeno-contaminations caused by components of culture medium, including viruses. Likewise, unwanted physiological and differentiation changes may occur during in vitro expansion, prior to application. Also, the possibility of producing thromboembolism and fibrosis in the tissues where they are inoculated [50]. Conversely, MSC-EV offer the following advantages: (i) since they are not living and proliferative cell populations, problems related to immune compatibility, tumorigenicity, emboli formation and transmission of cell-borne infections are avoided; (ii) dosage and potency might be evaluated, such as conventional pharmaceutical agents; (iii) are stable and could be stored for long periods of time, without significant efficacy loss; (iv) collection processes are non-invasive, and different factors can be obtained changing culture media; (v) could be produced in large scales, using specific cell lines, in controlled conditions; (vi) could be engineered for carrying specific molecules, and labeled with certain surface proteins, to reach target cells; and (vii) show easy intravenous circulation, being even capable of crossing the brain barrier [17,51–53].

5. MSC-EV and Wound Healing

MSC-derived EV play crucial roles in the main wound-healing phases. Processes such as inflammation, induction of cell proliferation, formation of new tissues and maturation and transfer of certain biomolecules are some of them, in which MSC-EV are involved [48].

5.1. Hemostasis

In case of injury, the first stage is focused on the constriction of blood vessels and fibrin-clot formation, which prevents and protects the organism against blood loss [51]. MSC-EV are involved in blood coagulation. Thus, they decrease the time of such a process and increase the area of the clot. Among the proteins they present in their membrane, one of the most important is CD9, related to activation and stabilization of platelets, as well as improvement of fibrinogen binding. They also contain annexin V, which has anticoagulant activity. This shows that the coagulant properties of MSC-EV depend on an equilibrium between the activities of pro- and anti-coagulant proteins [54]. It has been found that EV play a role in coagulation processes independent of proinflammatory stimuli. In fact, they probably induce intrinsic and extrinsic pathways of coagulation, in which FXII and FVII are respectively involved [55].

5.2. Inflammation

In this phase, the wound bed is cleaned of cells and bacteria, by neutrophiles. Also, macrophages phagocytize tissue debris and produce some cytokines and growth factors,

enhancing the inflammatory response. In fact, macrophage polarization takes place. They change their inflammatory M1 into an anti-inflammatory M2 phenotype. In that case, MSC-EV might have an important role in promoting immunomodulatory effects, producing suitable wound-healing environments [39,51] (Figure 2a).

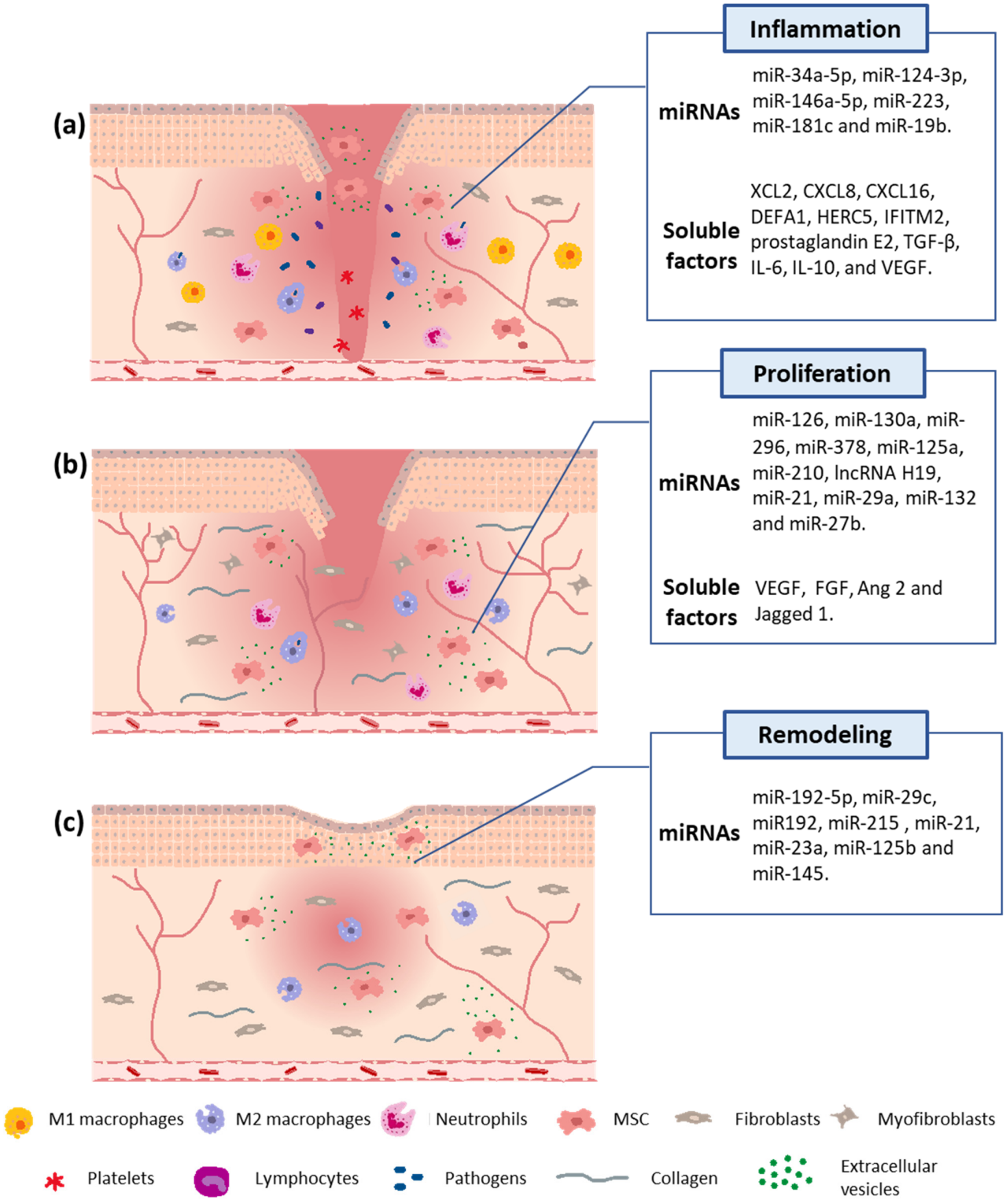

Figure 2. Role of extracellular vesicles during wound healing. EV may carry miRNA and proteins that can modulate a variety of physiological processes in different skin wound-healing phases. (**a**) In the inflammation phase, MSC-EV promote anti-inflammatory processes, suppressing apoptosis, inducing

macrophage phenotype change from pro-inflammatory (M1) to anti-inflammatory (M2) and decreasing reactive oxygen species (ROS) synthesis. (**b**) In the proliferation phase, they contribute to neovascularization and re-epithelialization, stimulating fibroblast migration and proliferation into wounds. (**c**) In the remodeling phase, EV increase collagen I production and promote scarless wound healing. In brackets are shown bibliographic references related to functions of these molecules in different wound healing phases.

Furthermore, it has been found that transmission of some miRNA could produce macrophage polarization. Among others, miR-34a-5p, miR-124-3p, miR-146a-5p and miR-223 [56,57]. MSC-EV miR-181c overexpression suppressed the TLR4 signaling pathway and alleviated inflammation in burned rats [58]. Proteomic analyses of MSC-EV have found that EV may contain chemoattractant proteins such as chemokine CXCL8, CXCL16, DEFA1, HERC5, IFITM2 and XCL2. They might enhance the immune response, protecting against infectious diseases [59].

Additionally, MSC-EV contain prostaglandin E2, which regulate the migration of neutrophils and macrophages polarization. MSC-EV also carry IL-6, IL-10, TGF-β and VEGF, that act as chemotactic and anti-inflammatory factors [42]. Due to their action on dendritic cells, MSC-EV might shift the production of inflammatory T-cells towards FOXP3+ regulatory ones, reduce the secretion of inflammatory cytokines like IL-6, and expression of chemokine receptor CCR7. Furthermore, an increase in the secretion of anti-inflammatory TGF-β has been observed [60]. In another study, the HaCaT cell line, after hydrogen peroxide exposure treatment with MSC-EV, reduced expression of apoptotic and inflammatory proteins (caspase-3 and IL-6) and increased expression of Bcl-2 and IL-10 (anti-apoptotic and anti-inflammatory ones). Also, an increase of miRNA-19b levels was found in these cells. This miRNA binds to inflammatory factors, resulting in the activation of the TGF-β pathway, which inhibits inflammation [61]. Another study demonstrated that EV could modulate endothelial precursor cells. inhibiting ROS and inflammatory cytokine expression [62]. These data showed that MSC-derived EV had a high immunomodulatory capacity, through multiple mechanisms.

5.3. Proliferation

This phase involves fibroblast proliferation, production of collagen III matrix and creation of granulation tissue. At this moment, angiogenesis is extremely important. Wound environments need new blood vessels to transport the necessary nutrients, oxygen and factors for correct healing [39]. Interestingly, MSC-EV are involved in endothelial cell proliferation, migration, secretion of growth factors and vessel formation. It has been described that MSC-EV could transfer some miRNA to endothelial cells and promote angiogenesis (Figure 2b). In particular, miR-125a, miR-126, miR-130a, miR-296 and miR-378 [57,63]. Another study showed that MSC-EV contain miR-126, miR-210, miR-296 and miR-378 as well as some factors such as VEGF and FGF, necessary for this process [64]. In relation to proteins, it has been described that human umbilical cord-derived mesenchymal stem-cells (hUCMSC)-derived EV contain angiopoietin-2 (Ang-2), which is one of the main growth factors inducing new vessel formation [65].

Other cells involved in the proliferation phase are fibroblasts. MSC-EV promote their migration to the wound site, and improve granulation tissue formation. A study suggested that MSC-EV contain Jagged 1, which promotes fibroblast activity, via the Notch signaling pathway [66]. Additionally, lncRNA H19 has also been found in MSC-EV, inhibiting mir-19b expression and upregulating *SOX9*. This activates the Wnt/b-catenin pathway that promotes fibroblast proliferation, migration and invasion into wounds [67]. Also, the presence of miR-21, miR-29a and miR-132 in MSC-EV enhanced wound healing, promoting proliferation and migration of fibroblasts, in addition to extracellular matrix production [57].

Moreover, a greater proliferation and migration of keratinocytes has been observed after being treated with MSC-EV. This effect may be mediated by the transfer of miRNA from EV into tissues. Thus, it has been demonstrated that the transfer of miR-27b from hUCMSC-EV into wounds can activate ITCH/JUNB/IRE1α signaling, accelerating re-

epithelialization processes [68]. In general, MSC-EV could have effects on the proliferation, migration and angiogenesis of keratinocytes, fibroblasts and endothelial cells, modulating different signaling pathways, among which AKT/HIF-1α stands out, being related to cell proliferation [69].

5.4. Remodeling

In this stage, overexpression of collagen could produce the formation of large scars. To prevent this process, tissue maturation takes place, involving gradual replacement of collagen III with collagen I, as well as the formation of sweat glands and hair follicles [51]. Skin mechanical properties and reduction of scar formation can be enhanced by MSC-EV. They improve skin elasticity and barrier integrity, increasing some skin barrier proteins such as filaggrin and loricrin, as well as *AQP3* gene expression [70]. Additionally, another study showed that MSC-EV increase collagen III/collagen I ratios, ameliorate MMP3 expression, via protein kinase/mitogen-activated protein kinase (ERK/MAPK) signaling pathway, and regulate fibroblasts differentiation, to help scarless wound healing [71].

In the antifibrotic effect of MSC-EV application on wounds, an important role of different miRNA carried by these EV has been observed. Thus, MSC-EV could decrease collagen deposition, trans-differentiation of fibroblasts into myofibroblasts, and formation of fibrosis scars. MSC-EV transfer miR-192-5p into wounds, and this represses pro-fibrotic *IL-17RA/SMAD 2* axis [72]. Other MSC-EV miRNA, such as miR-29c, miR192 and miR-215 have also been involved in this phase [57]. Additionally, MSC-EV can deliver miR-21, miR-23a, miR-125b and miR-145, which inhibit TGF-β/SMAD2 signaling, suppressing myofibroblast formation [73] (Figure 2c).

The ability of MSC-EV to act on different phases of the healing process, through various mechanisms, has made them important candidates for the development of therapies, to promote the healing of difficult-to-heal wounds. However, when they are applied in vivo, topics such as clearance, maintenance and preservation of their viability and function must be considered [74]. Thus, currently available information on their pharmacokinetics will be reviewed in the following section. Then, how the use of different biomaterials can facilitate their application, stability and release, for skin-ulcer treatments.

6. Extracellular-Vesicle Pharmacokinetics

As explained in the previous section, different studies have shown that MSC-EV are involved in various biological processes. For this reason, since they are efficient vehicles to transport nucleic acids, proteins and other bioactive compounds, MSC-EV are expected to be used in therapeutic treatments, such as tissue regeneration. To develop therapies based on these vesicles, it is important to know their pharmacokinetics when they are applied in vivo. To elucidate the pharmacokinetics of MSC-EV, their tissue distribution must be evaluated. For this purpose, different methods of labeling exogenously administered EV have been used. One of them is the use of lipophilic fluorescent dyes to label EV for in vivo monitoring purposes. For example, it has been observed that EV derived from intravenously administered murine B16F10 melanoma cells accumulated in bone marrow, spleen, liver and lung, due to the lipophilic fluorescent dye PKH67. Furthermore, different studies in animal models observed that MSC-EV labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate (DiD), and intravenously injected, were distributed throughout spleen, liver and kidney [75].

Moreover, EV derived from different cells and labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) allowed us to elucidate the in vivo behavior of such vesicles [76]. Thus, EV were mainly distributed in the spleen, liver, lung and gastrointestinal tract when intravenously injected. Notably, tissue distribution of EV derived from HEK293T human embryonic kidney cells was different, depending on the injection method. They were mainly stored in the liver when intravenously applied. However, vesicles administered by subcutaneous or intraperitoneal injection accumulated in the pancreas, liver and gastrointestinal tract. The biodistribution of EV derived from

body fluids, such as milk, has also been studied [77]. These vesicles were labeled with DiR and orally or intravenously applied. They are distributed into various organs such as the lung, kidney, liver, spleen, pancreas, colon, brain and ovaries after four days, when orally administered. However, exosomes intravenously injected accumulated in the liver. Such a result is in line with what has been demonstrated in other studies of EV derived from cultured cells.

Another aspect to be considered for the study of pharmacokinetics is the time of elimination from the bloodstream, and accumulation in organs. It was observed by bioluminescence that the half-life of gLuc-LA-labeled B16BL6 EV was approximately two minutes after intravenous injection. Furthermore, it was found that these EV were mainly distributed in the lung, liver and spleen. Also, it was observed that EV derived from different mouse cell lines have a half-life of two to four minutes, after intravenous injection, being accumulated in the liver [78,79]. These studies demonstrate that there are several types of EV target tissues although, regardless of the method of exosome labeling, intravenously administered EV rapidly disappear from blood circulation, being mainly stored in the liver.

It is also important to identify which cell types recognize and take up EV, incorporating their cargos, for further advancement in the development of EV-based therapies. Different studies indicate that the primary cells that actively take up exogenous EV are macrophages. Thus, clearance of intravenously injected B16BL6 EV from the bloodstream was slower in mice without macrophages, thus marking the importance of these cells in the pharmacokinetics of exosomes. It has been observed that macrophages are responsible for hepatic and splenic uptake of B16BL6 EV, while in the lung they were mainly taken up by endothelial cells [78].

To address the problem of rapid clearance of EV when intravenously, subcutaneously or intraperitoneally administered, a combination of vesicles with biomaterials, such as hydrogels, has become the main research focus of exosome-based therapies. EV release can be controlled by hydrogel properties such as network morphology and cross-link density, as well as hydrogel degradability [18]. In fact, several studies have shown that encapsulation of EV in a biomaterial, such as a hydrogel, provides a sustained release of EV during hydrogel biodegradation. That improves the efficiency of the biological activity of such EV. For example, the release of M2 macrophage-derived VE from a hydrogel was slow over 21 days [80]. In another study, M2 macrophage-derived EV were encapsulated in hydrolytically-degradable polyethylene glycol hydrogels, for their application in skin-wound healing. The degradation time of hydrogels was adjustable from 6 to 27 days. These EV were released over a period of 11 days. After being released from hydrogel, they retained their physicochemical properties and biological functionality [81]. These studies show that the development of strategies that combine the application of MSC-EV and the use of wound dressing, with the capacity to maintain stability and functionality, favoring progressive release of these vesicles, have a high therapeutic potential for the treatment of skin ulcers.

7. Use of Biomaterials in the Release of Extracellular Vesicles for Treatment of Cutaneous Wounds

As discussed in the previous section, clearance of MSC-EV occurs rapidly when systemically delivered. Therefore, local administration of damaged tissues could improve their efficiency. However, proteases and pH changes in these tissues also produce a high degradation of MSC-EV. Those that remain are rapidly taken up by cells, such as endothelial ones and macrophages [82]. On the other hand, regenerative processes, such as skin healing, consist of different phases, as described above. Each of these phases needs a series of factors and molecules for its development. Therefore, a simple administration of MSC-EV for regenerative purposes may not be the most appropriate approach. This, together with the short half-life of EV forces the need for repeated applications, for the treatment to be efficient. However, high wound manipulation can be counterproductive, delaying the healing process. Therefore, in recent years, different types of biomaterials

have been developed and tested for their encapsulation, progressive release and creation of a suitable microenvironment that favors healing. Properties that these biomaterials should include an extracellular matrix-like, tissue-adhesive structure, high porosity and good permeability [83]. They are called modern wound dressings. Unlike more traditional or inert ones, such as cotton, gauze, pads and bandages, the new ones have better biocompatibility, degradability and moisture retention. This not only protects the wound from external factors, but also creates a more favorable microenvironment for healing [84].

Among the modern dressings, currently used in the clinic, are the following: (i) hydrogels, consisting of a three-dimensional network of hydrophilic polymers; (ii) hydrocolloids, made of hydrogel, mixed with adhesive materials and synthetic rubber; (iii) alginate, which is a polysaccharide derived from brown seaweed; (iv) foams from silicone or polyurethane; and (v) porous and transparent films based on polyurethane [84]. Synthetic and natural polymers have been used to make some of these dressings. Among the latter are alginate, chitosan, collagen, hyaluronic acid and gelatin [83].

Current treatments are still not sufficiently effective for healing difficult-to-heal skin ulcers. Thus, traditional clinical treatments of diabetic wounds include control of blood glucose, surgical debridement, graft transplantation, as well as a wound dressing. Although these measures can be successfully applied in the most severe cases, in many instances they are not sufficient, and the wounds do not heal, or heal after very long periods of time. It is, therefore, necessary to develop new treatments that favor tissue regeneration, with adequate progress of healing. Thus, it has been proposed that products to promote skin healing must meet one or more of the following properties: (i) stable under room temperature storage; (ii) suitable for topical or injectable delivery; (iii) antimicrobial, analgesic or hemostatic properties; and (iv) accelerate wound repair, by promoting conductive or inductive regenerative mechanisms [85]. The new products based on a combination of multifunctional wound dressings with EV meet these properties. Their potential clinical applications are expected to result in shortening the healing period, and improving the functionality of scar tissues. Among the dressings used as VE vehicles for wound treatment, hydrogels stand out. In addition to acting as a barrier to external wound agents, they are semi-permeable to water and oxygen, being biodegradable. They reduce pain in patients and dressing frequency, avoiding second trauma during dressing changes. Additionally, they reduce scar formation, promoting autolytic debridement [86]. In addition, active pharmaceutical ingredients or bioactive factors can be incorporated into hydrogels, which are thus more stable, and can be progressively released when the gel is biodegraded, as opposed to direct administration [87]. Another important characteristic of hydrogels is their porosity. It must be high, to facilitate the transport of nutrients, waste products and gas exchange, as well as the migration and survival of skin cells in wound beds [18]. Furthermore, because rheological properties of hydrogels are similar to those of the extracellular matrix of the skin, they can be used to mimic their functions [18]. Within the different types of hydrogels, injectable ones are well suited for wound treatment and nanoparticle or EV release. Injectable hydrogels are low viscosity fluids, with high penetration capacity into beds and crevices of ulcers. Once injected, chemical or physical hydrogel crosslinking can be induced in situ. This provides stable hydrogels, in intimate contact with damaged tissues [18].

The incorporation of MSC-EV into biomaterials depends on interactions between them. Therefore, one line of research is to explore new strategies that increase the loading and retention of MSC-EV in these biomaterials. EV can be incorporated into hydrogels in different ways: (i) combining EV with polymers, and then adding crosslinkers to induce gelation; (ii) making hydrogels first, and then incorporating EV through physical methods; and (iii) mixing polymers together with crosslinkers and EV [88]. Of the three possibilities, the second one has the advantage over the others in that polymerization conditions do not affect the properties of EV. Binding can occur through electrostatic or bioactive adhesion interactions [89]. In the former case, negatively-charged phospholipid membranes of MSC-EV and cationic (positively charged) delivery systems, such as those based on chitosan-containing hydrogels, retain MSC-EV through electrostatic forces. For bioactive

adhesion, considering that MSC-EV express adhesion molecules (such as CD29 CD44 and CD73, as well as some α-integrins), extracellular matrix components such as collagen I, fibronectin and hyaluronic acid are used for biomaterial fabrication [90].

The structure and porosity of the biomaterial matrix also play a very important role in the retention, stability and subsequent release of EV. A very promising technique for manufacturing nanofibers, with applications in regenerative medicine, is electrospinning. This consists of discharging a charged polymer solution through a syringe, at a given rate, in presence of high voltage. That causes electrostatic charges to accumulate at the tip of the polymer-droplet row, producing what is known as a Taylor cone. With increasing voltage, a polymer jet is generated and deposited on a collector, generating nanofibers to form non-woven mats with high porosity. They mimic the extracellular matrix, due to their small pore size [91]. With this technique, gelatin-based nanofibers, as well as others produced by mixing gelatin with other biopolymers, have been generated with the ability to improve wound healing [91]. In addition, it has been demonstrated that these nanofibers have a high capacity to store and release different drugs and bioactive agents, with therapeutic functions. Among these agents are EV. Thus, it has recently been described that electrospun polyvinylpyrrolidone-based nanofibers can preserve up to 12 weeks EV without significant loss of functionality [92]. So far, to our knowledge, no nanofibers-containing MSC-EV have been fabricated by this technique, for the treatment of cutaneous wounds. However, other preclinical studies have been performed using both in vivo and in vitro models. They have shown a high potential of this type of biomaterials in regenerative medicine when applied, for example, to urethral or bone regeneration [93,94]. Among the most used wound dressings to release MSC-EV are hydrogels based on chitosan, alginate, hyaluronic acid, collagen and Pluronic F127. Other biomaterials produced through tissue decellularization and from other compounds such as polyurethane also stand out, as described below.

7.1. Chitosan Hydrogels

Chitosan is one of the most widely used polymers, as a base for manufacturing hydrogels with healing capacity. It is a poly-cationic biopolymer derived from a thermochemical deacetylation of chitin (a polymer found in the exoskeleton of arthropods). Chitosan has antimicrobial properties, promotes blood clotting, and is biocompatible and biodegradable. Therefore, it is a suitable substrate for the development of dressings, for the treatment of skin wounds [95]. In this regard, it has also served as a scaffold to include MSC and MSC-EV. Interestingly, a collagen- and chitosan-based hydrogel enriched in bone marrow-derived MSC or their EV, significantly improved wound healing in a rat model over hydrogel alone. This improvement was greater in wounds treated with a hydrogel containing MSC-EV. This was due to an increase in the deposition and organization of collagen in the extracellular matrix. That shows the therapeutic potential of cell-free therapies, based on hydrogels and VE, for the treatment of skin ulcers [96]. In this sense, the use of EV has the advantage that they can be enriched in different factors, through modifications such as genetic engineering. Thus, for example, in synovium mesenchymal stem cells (SMSC), miR-126-3p has been overexpressed, giving rise to EV enriched in such miRNA. Encapsulation of these EV in a chitosan-based hydrogel and their application to wounds in a diabetic rat model improved healing, by increasing granulation tissue formation and angiogenesis [97]. The rationale is that miR-126-3p induces angiogenesis, through phosphatidylinositol 3-kinase (PI3K)/AKT, as well as mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways [98].

A hydrogel based on chitosan and glycerol was enriched in EV, derived from human endometrial stem cells. It was used in an in vivo mouse model of skin healing, being compared against treatment with hydrogel without extracellular vesicles. Treatments were performed every three days, showing that hydrogel with MSC-EV had a greater capacity to promote wound closure (83.6% compared to 51.5% in the control), with a high degree of re-epithelialization and greater induction of angiogenesis and granulation tissue formation [99]. On the other hand, carboxymethyl chitosan is a derivative, that has been used

together with poloxamer 407 (P407), for the fabrication of a thermo- and pH-sensitive hydrogel, by crosslinking reactions with the natural nontoxic crosslinker genipin (GP) [100]. This hydrogel showed an adequate protective and moisturizing effect on wounds, without stimulating proinflammatory processes; in addition to being biodegradable. The incorporation of EV derived from hUCMSC provides a high healing capacity to the hydrogel, in a cutaneous wound rat model, when applied every three days. Thus, the hydrogel loaded with MSC-EV significantly improved wound closure, re-epithelialization, collagen deposition and the number of skin appendages with respect to hydrogel without MSC-EV, indicating a higher maturation of the regenerated tissue [100]. VE-loaded chitosan-based hydrogels can also exert their positive effect on healing, through the inhibition of apoptosis. Thus, a methylcellulose-chitosan hydrogel loaded with EV derived from placental-mesenchymal stem cells produced a positive effect on skin-wound healing in the db/db diabetic mouse model. That was partly due to its ability to promote angiogenesis and inhibit apoptosis. Indeed, this hydrogel increased the expression of VEGF and BCL-2 (anti-apoptotic) proteins, while *BAX* (pro-apoptotic) expression decreased, with respect to treatment with hydrogel or EV, separately [101].

Chitosan has also been used as a base for the manufacture of dressings with antimicrobial capacity. For instance, a chitosan-based dressing was developed, including silver nanoparticles (AgNP), attached to EV derived from hUCMSC. AgNP exhibits a broad antimicrobial spectrum, and is being widely used for the treatment of skin infections [102]. It has been combined with the regenerative effects of EV derived from MSC, and the moisturizing capacity of the dressing. Thus, in a mouse model of wound healing in which wounds were inoculated with *Pseudomonas aeruginosa*, the application of this multifunctional dressing inhibited bacterial growth and accelerated healing. That was accomplished by increasing collagen deposition, angiogenesis and nerve repair, to a greater extent than dressing containing the components separately [103].

In addition to chitosan, chitin nanofibers have also been used to develop hydrogels with good carrier properties, for sustained release of VE [88]. Recently, the fabrication of a β-chitin nanofiber (β-ChNF) hydrogel, which was loaded with EV derived from adipose-mesenchymal stem cells, extracted from C57BL/6 mice, has been described [104]. These MSC-EV increased proliferation and migration of the L929 mouse fibroblast line, when applied at 80 or 160 μg/mL. Furthermore, their incorporation into β-ChNF hydrogels accelerated the healing of cutaneous wounds, relative to the ones independently treated with just hydrogel or MSC-EV, in a rat wound-healing model. This process was mediated by a decrease in complement factor D (CFD) synthesis, with an increase in alpha-actinin-2 and aldolase A [104].

7.2. Alginate Hydrogels

Alginate has high biocompatibility, is inexpensive and easy to gel using divalent cations. In addition, alginate gels have a high capacity to absorb large amounts of wound exudate. These properties have allowed it to be used for drug delivery [105]. Thus, EV derived from cultured rat adipose-tissue-derived stromal cells (ADSC) have been encapsulated in this type of hydrogel at a concentration of 100 μg/mL. They were applied in a rat skin-wound model, where it significantly improved wound closure, collagen synthesis and vessel formation, with respect to treatment with alginate hydrogel only [106]. In such a study, the MSC-EV used were also found to induce human umbilical-cord-vein endothelial cell (HUVEC) migration in vitro. That could be related to their angiogenic activity observed in vivo [106]. Also, based on alginate, an innovative sponge-like wound dressing has been developed. In this case, under the principles of good manufacturing practices (GMP), it contained the total secretome derived from human AD-MSC, cultured for 48 h, at a concentration of 4×10^6 equivalent cells per mL. The resulting sponge-like wound dressing was freeze-dried (lyophilized). When applied to wounds in a mouse skin-ulcer model, it was hydrated with phosphate-buffered saline (PBS) buffer. That generated a gelatinous matrix, favoring the release of bioactive compounds from the secretome [107].

In treated wounds, such dressing induced increased levels of proteins involved in complement and coagulation cascades, cytoskeleton and extracellular matrix remodeling. Thus, in relation to the application of only alginate sponge-like wound dressing, incorporation of total secretomes produced an enhanced immune response. Additionally, it produced increased granulation tissue formation, more vascularization and collagen deposition in such in vivo model [107]. This study, therefore, shows the possibility of developing desiccated or lyophilized products based on biomaterials, containing MSC-EV and/or MSC-derived factors. This may facilitate their storage, transportation and clinical applications.

Alginate has also been used as a base for the development of more complex hydrogels, with layers of different resistance to degradation. This may allow the release of some particular type(s) of EV in the early stages of healing, and other(s) in later stages. Generally, the application of MSC-EV to wounds decreases inflammation, increases angiogenesis, promotes fibroblast proliferation and increases collagen deposition. These effects are mainly beneficial in the inflammatory and proliferative phases of healing. But high vascularization, fibroblast proliferation and collagen production may also favor the appearance of unwanted hyperplastic scar tissue.

Considering this, a bilayered thiolated alginate/PEG diacrylate hydrogel has been developed. Thus, they encapsulated BMMSC-derived EV in the first layer and BMMSC-derived miR-29b-3p-enriched EV, genetically engineered for miRNA overexpression, in the second layer [108]. Interestingly, it has been found that miR-29b mimics (mimicry) inhibited excessive blood vessel formation and collagen deposition, in endothelial cells and fibroblasts [109]. While BMMSC-derived EV promote angiogenesis and collagen formation through activation of PI3K/Akt, Erk1/2 and Smad3/TGFβ1 signaling pathways, miR-29b-3p-enriched EV inhibited these pathways. Application of hydrogel containing both (BMMSC-derived EV and miR-29b-3p-enriched EV) types of MSC-EV in different layers provide a temporally separated release. That allowed greater acceleration of healing in a full-thickness skin defect model of rat and rabbit ears. In the early phases, the hydrogel promoted angiogenesis and collagen deposition. In the later ones, as a consequence of the release of miR-29b-3p-enriched EV, the tissue formed was characterized by more uniform vascularization, better rearrangement of collagen fibers, and lower volume of hyperplastic scar tissue [108]. This study shows the therapeutic potential of this type of hydrogels, to design effective strategies for wound healing. In addition, it highlights the importance of knowing the mode of action of MSC-EV, to optimize their clinical application.

7.3. Hyaluronic-Acid Hydrogels

Hyaluronic acid is a natural linear polysaccharide, made of alternating units of d-glucuronic acid and N-acetyl-d-glucosamine, connected by β-1,3- and β-1,4-glycosidic bonds. Due to its biocompatibility, native biofunctionality, biodegradability, non-immunogenicity and versatility, hyaluronic acid is one of the most widely used polymers in the design of hydrogels, for biomedical applications. The incorporation of MSC-EV into an injectable hyaluronic acid hydrogel allows its progressive release for more than 90 h. Moreover, it favors the polarization of inflammatory macrophages M1, towards an anti-inflammatory and anti-fibrotic M2c phenotype in vivo. That decreases fibroblast activity in the late stages of healing, and thus such treatment produced scarless skin-wound healing in a mouse model of scarring, preventing fibrotic scar formation [110].

Also, hydrogels have been prepared from cross-linking hyaluronic acid with photocleavable linkers (PCL), being attached to EV from umbilical-cord-blood mononuclear cells. Their abilities to release EV after light activation have been evaluated. Thus, application of a low concentration of EV (0.2 μg) twice daily, for 10 days, significantly accelerated wound healing, in a diabetic mouse model. Surprisingly, in the same model with an application of injectable light-triggerable hydrogel, containing 2 μg of EV, and 1 min daily activation with light for 10 days, healing was faster than with bi-daily administration of EV, and with the application of the hydrogel without activation with light. Thus, this study shows the importance and clinical potential of the progressive release of MSC-EV through a hydrogel,

allowing us to considerably reduce the number of EV applications, to achieve the desired effect on healing [111].

7.4. Collagen Hydrogels

Collagen hydrogels are also widely used in regenerative medicine [112]. These hydrogels exhibit low cost, low immunogenicity, high versatility, biocompatibility and similarity to the natural extracellular matrix. Although collagen can be obtained from animal tissues, recombinant collagen is currently a good option to avoid some problems. They include batch-to-batch differences or risk of contamination with pathogens that animal-derived collagens may present [113]. Recently, recombinant human-collagen III protein (rhCol III) has been used to develop a hydrogel, in which hUCMSC-derived EV have been encapsulated [114]. This hydrogel sustainably releases MSC-EV in vitro, and has the capacity to induce M1 to M2 macrophage polarization, L929 fibroblast migration and angiogenesis in HUVEC. On the other hand, the application of such hydrogel every three days in a diabetic rat skin injury model promotes healing, due to its anti-inflammatory and angiogenesis-inducing capacity. Thus, levels of IL-6 decreased, while those of factor Ki67 proliferation biomarker, and those of vessel formation (CD31 and α-SMA), increased with respect to wounds treated with either rhCol III hydrogel or EV alone [114].

7.5. Pluronic F127 Hydrogels

Pluronic F127 (PF127) allows the manufacture of thermosensitive injectable hydrogels, because at certain concentrations the increase in temperature induces its gelation. Furthermore, the use of PF127 has been approved by the U.S. Food and Drug Administration (FDA) for the controlled release of drugs, due to its good biocompatibility and absorbability [115]. Thus, the fabrication of an injectable adhesive, with thermosensitive multifunctional polysaccharide-based hydrogel scaffolds has been described. It is generated by a reversible Schiff base reaction between PF127 grafting aldehyde pullulan and polyethyleneimine or PF127, oxidative hyaluronic acid and Poly-ε-L-lysine. Gelification is reached at temperatures above 30 °C. It exhibits antibacterial activity, good UV-shielding performance, fast hemostatic ability, tissue-adhesive and self-healing behavior. In addition, these biomaterials can be loaded with MSC-EV, through electrostatic interactions, and released in response to changes in pH [116,117]. Hydrogels were generated by incorporation of adipose-tissue MSC-derived EV, termed FEP@exo and FHE@exo. They showed increased proliferation, migration and formation of tubular structures in HUVEC, in vitro. In a diabetic full-thickness cutaneous-wound mouse model, the application of FEP@exo or FHE@exo accelerated wound healing. That promoted increased granulation tissue formation and collagen deposition, enhancing extracellular matrix remodeling, and favoring the appearance of skin appendages. Interestingly, unlike what happens when MSC-EV are directly applied to wounds, MSC-EV in these hydrogels were progressively released throughout the healing process for three weeks, maintaining their biological activity [116,117].

Type I and III collagens are the major components of the dermal extracellular matrix. While collagen I is the most abundant in normal skin, collagen III is produced during healing. Abundant deposition of the latter in the early stages of wound healing facilitates the generation of scarless skin [118]. In the case of PF-127 hydrogels loaded with EV derived from adipose-tissue MSC, increased production of Collagen III has been observed during healing, favoring the formation of scar-free wounds [119]. Also, these hydrogels decrease the inflammatory response by downregulating *TNF-α* and *IL-6*, as well as promoting polarization from M1 into M2 macrophages [119]. EV derived from hUCMSC have also been encapsulated in PF127-based hydrogels. They were evaluated in a streptozotocin-induced diabetic rat model of scarring. The PF127 hydrogel protected the biological activity of MSC-EV, increasing angiogenesis and granulation-tissue formation. That accelerated wound healing, relative to treatment with MSC-EV or hydrogel alone [120].

7.6. Decellularized Biomaterials

Wound dressings based on decellularized biomaterials have received much attention in regenerative medicine, due to their natural origin [121]. Recently, the development of hydrogels based on porcine small-intestinal submucosa (SIS), obtained by decellularization, has been described. This biomaterial is mainly composed of collagen, and is enriched in factors with the ability to promote tissue regeneration [122]. Such hydrogels were prepared with SIS, to which catecholamine was added to improve their properties. In addition, fusion peptides, consisting of collagen-binding peptides linked to CP05 peptide (CRHSQMTVT-SRL), were generated through genetic engineering. The latter recognizes the surface protein CD63, abundantly present on extracellular vesicles. Therefore, this modification allows the MSC-EV to remain anchored to the biomaterials, increasing their retention capacity. Thus, after seven days of maintenance of the hydrogel loaded with hUCMSC-derived EV in a culture medium, EV release was more than doubled in the hydrogel containing fusion proteins [123]. Moreover, this hydrogel increased the proliferation and migration of NIH3T3 fibroblasts in vitro, as well as angiogenesis of EA.hy926 endothelial cells, with respect to hydrogel without fusion proteins. On the other hand, application in wounds of a diabetic-rat model accelerated healing, promoting the formation of granulation tissue, collagen and skin appendages [123].

Decellularization of the human amniotic-membrane has also been used for the preparation of scaffolds for tissue and organ repair [124]. As with other non-cellular approaches, there are no ethical problems in its use. It is readily available without the high cost, being biocompatible. It also shows low immunogenicity, has adequate mechanical properties and good cell adhesion. Recently, EV derived from adipose-tissue MSC were incorporated into scaffolds, obtained from human acellular amniotic-membrane. They were used as dressings, for the treatment of diabetic skinulcers [125]. The MSC-EV used favored proliferation and migration of human dermal-fibroblasts, as well as angiogenesis of HUVEC in vitro. Dressing consisting of human acellular amniotic-membrane plus MSC-EV was applied every other day, three times in total, in an in vivo model of diabetic mouse, induced with streptozotocin. The results showed that with respect to the use of human acellular amniotic membrane dressing or VE in PBS separately, it accelerated healing. That was accomplished through a greater immunomodulatory effect, induction of angiogenesis and promotion of extracellular matrix production [125]. This confirms the high therapeutic potential of dressings, designed from human acellular amniotic-membrane, together with MSC-derived EV, for the treatment of chronic ulcers.

7.7. Complex Multifunctional Scaffolds

In order to optimize the functionality of dressing on wound healing, complex biomaterials are being developed. They combine EV with other biologically-active compounds or factors, that also contribute to improving and accelerating the healing process. Among these complex scaffolds are those based on polyurethanes. These constitute a family of polymers with a high spectrum of physicochemical properties. They are synthesized from reactions between polyols and diisocyanates. Currently, they have been developed with new biobased macromolecular architectures. That has allowed us to improve their biocompatibility for biomedical applications, including the development of wound dressings [126].

In this regard, the fabrication of a wound dressing called OxOband has recently been described. It is composed of oxygen-releasing antioxidant polyurethane (PUAO-CPO) scaffolds, embedded with exosomes derived from adipose-derived stem cells from rat fat-tissue [127]. This dressing is characterized by: (i) its antioxidant activity, as a consequence of the ascorbic acid incorporated in it [128], releasing O_2 for a period of more than 10 days, due to the incorporation of calcium peroxide [129]; and (ii) its regenerative capacity, including MSC-EV. Due to these properties, this biomaterial has the potential to decrease oxidative stress in wounds. Oxygen supply facilitates vascularization, further reducing the possibility of infection. Thus, in a diabetic animal healing model, treatment with OxOband, compared to control animals, accelerated wound closure and decreased inflammation and favored re-

epithelialization. Increased collagen formation and angiogenesis, promoting the formation of a mature epithelium with hair follicles, similar to that of healthy skin. These effects were also observed when wounds in the model were infected with *Staphylococcus aureus* and *Pseudomonas aeruginosa* [127].

Another possibility to enhance or complement the regenerative effects of MSC-EV is the incorporation of plant extracts, with positive effects on healing, into wound dressing. For example, the combination of MSC-EV and astragalus extract in gel improved the speed of skin healing in a murine model. This is partly enhanced by the anti-inflammatory effect of astragalus extract [130].

8. Conclusions and Future Perspectives

New therapies based on MSC and their secretome, to enhance tissue regeneration, have opened great expectations for chronic-wound treatments. Specifically, secretomes, and especially fractions formed by EV, have shown in recent years to have significant therapeutic potential in this regard. The rationale is that they carry molecules with immunomodulatory, angiogenic, proliferation- and migration-inducing properties. They modulate cells involved in healing, such as endothelial ones, fibroblasts and keratinocytes. However, the short half-life of MSC-EV, when they are directly applied to damaged tissues or via the bloodstream, complicates their clinical application. In order to overcome this limitation, numerous biomaterials have been designed with properties of retaining, protecting and progressively releasing MSC-EV. Thus, encapsulation of MSC-EV in these dressings has a high potential to facilitate tissue regeneration, for the treatment of cutaneous wounds as shown schematically in Figure 3.

However, currently, the studies performed with this strategy are mainly preclinical, using wound-healing animal models. On the other hand, these studies have been carried out under very different experimental conditions, in terms of cell type and culture conditions used to obtain EV. Other variables are EV concentrations and treatment patterns, as well as animal models used. Not all of them correspond to ulcers that are difficult to heal, such as diabetic ones (Table 1). Additionally, although such data are encouraging, most publications of translational studies are less than four years old, so they can still be considered preliminary. Thus, in clinical trials.gob <https://clinicaltrials.gov/> (accessed on 25 January 2023), we have found four studies have been reported proposing the use of EV for the treatment of skin ulcers: (i) NCT05475418 (without starting recruitment), in which they use adipose tissue-derived exosomes; (ii) NCT05078385 (without starting recruitment), which uses BMMSC-derived EV for treatment of burn wounds; (iii) NCT04134676 (completed), in which stem-cell-conditioned medium is used on chronic, ulcer-wounds, albeit without published results at the time of writing; and (iv) NCT02565264 (unknown status), to study the effect of plasma-derived exosomes on cutaneous-wound healing. EV will be applied in hydrogels, in studies NCT05475418 and NCT04134676, but their compositions are not specified.

Therefore, in order to understand the real clinical potential of such strategies, it is necessary to carry out clinical trials, to ascertain the promising results obtained so far in animal models. To this end, it is essential to advance mainly in the development of techniques for obtaining MSC-EV. In this regard, it is necessary to define which MSC sources, as well as which culture conditions or manipulations through bioengineering, would be the most appropriate to produce MSC-EV. They should have a high regenerative capacity, without posing a relevant risk to patients. Likewise, they should be obtained in sufficient quantities, to meet the demand required for clinical use. It is also important to standardize concentrations and purity of EV loaded in biomaterials, to guarantee consistent regenerative effects. That may be the main handicap to accelerating the progress of this approach, for the treatment of chronic ulcers in humans.

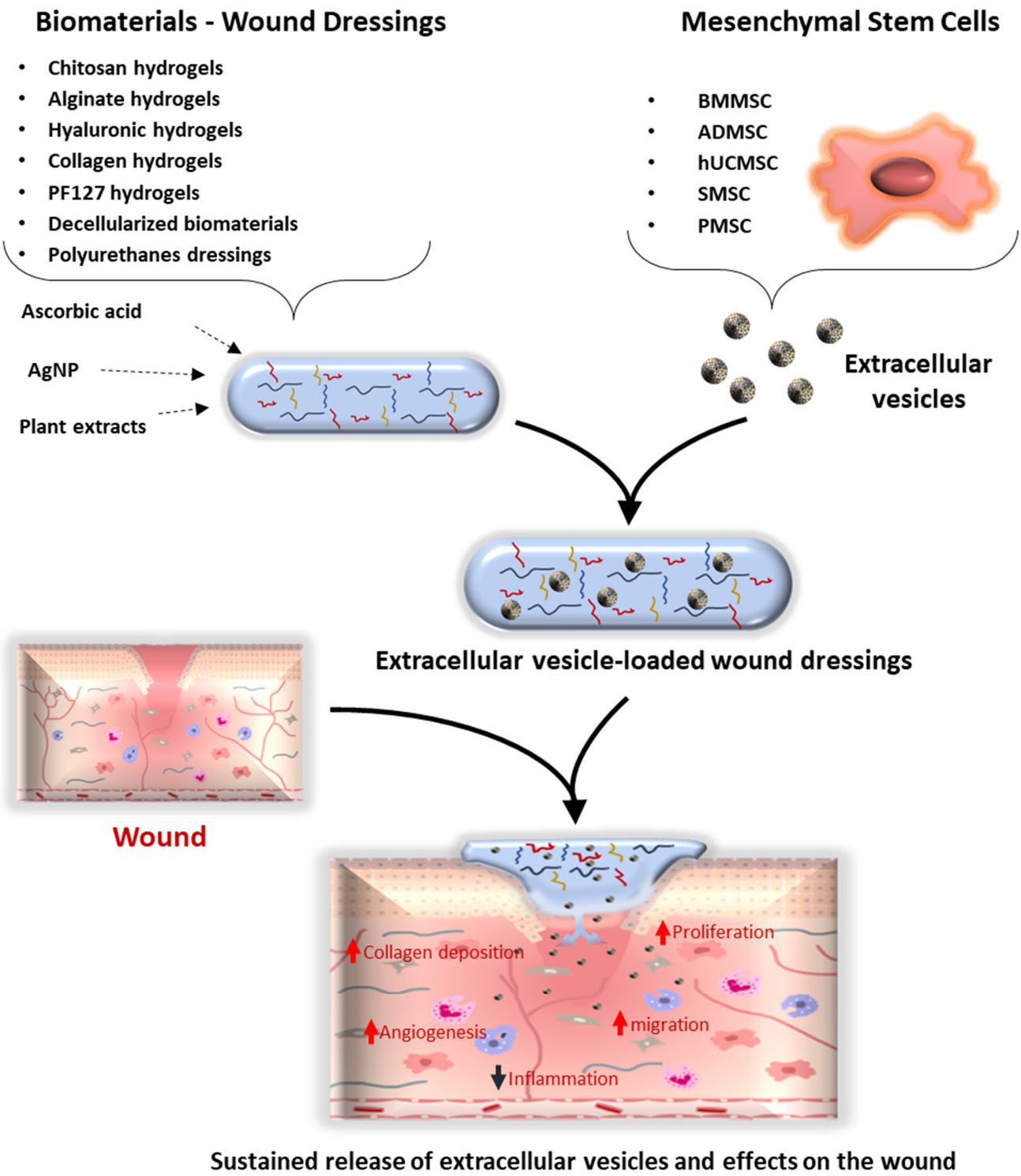

Figure 3. Scheme of therapeutic strategy for treatment of cutaneous wounds. It is based on combination of biomaterials or multifunctional wound-dressings with MSC-EV. Biomaterials, such as hydrogels, can be of different nature and can be supplemented, for example, with bioactive compounds such as ascorbic acid, AgNP and plant extracts. MSC-EV can be produced from MSC derived from different tissues and different culture conditions. Biomaterials allow the creation of adequate microenvironments for wound healing. That includes releasing EV in a sustained manner while biodegrading. EV accelerate wound healing through modulation of different physiological processes, related to tissue regeneration. AgNP: silver nanoparticles; BMMSC, ADMSC, hUCMSC, SMSC and PMSC: MSC derived from bone marrow, adipose tissue, umbilical cord, synovium and placenta, respectively. References are shown in brackets.

Table 1. Summary of the experimental conditions used in different in vivo studies, on the effects of combining biomaterials with MSC-EV. For each biomaterial, MSC origin of MSC used for EV production, culture condition, EV isolation method, EV concentration used, animal model and treatment regimen is specified, besides reference.

Biomaterial	Origin	Culture	Isolation	[EV]	Model	Treatment *	Ref.
Collagen chitosan scaffold	Rat Bone marrow MSC (BMMSC)	80% confluent cells in LG-DMEM serum-free medium for 48 h	Ultracentrifugation	EV isolated from 10^6 BMMSC	Sprague-Dawley rats	Not specified	[96]
Chitosan Hydrogel	Human Synovium MSC (SMSC) and SMSC overexpressing miR-126-3p	50–60% confluent cells in MesenGro hMSC medium for 48 h	Ultrafiltration-ultracentrifugation	Not specified	Diabetic sprague-Dawley rats (streptozotocin-induced)	Not specified	[97]
Chitosan Hydrogel	Human endometrial stem cell (hEnSC)	80% confluent cells in DMEM/F12 + 15% exo-free serum for 24 h	Ultracentrifugation	100 µg/mL (200 µL/treatment)	BALB/c mice	0, 3 and 7 days	[99]
Chitosan genipin crosslinked hydrogel	Human umbilical MSCs (hUCMSC)	Not specified	Ultracentrifugation	20 µg/mL (300 µL/ treatment)	Sprague-Dawley rats	every three days	[100]
Methylcellulose-chitosan hydrogel	Human placental MSC (hPMSC)	80% confluent cells. Medium and culture time are not specified	Exosome isolation reagent (Precipitation)	2×10^{12} EV/mL (100 µL/ treatment)	Diabetic mice (C57BLKS-Leprdb)	Not specified	[101]
Chitosan dressing	Human umbilical MSCs (hUCMSC)	Not specified	Size Exclusion Chromatography-ultrafiltration	Not specified	BALB/c mice	every two days	[103]
β-chitin nanofiber hydrogel	Mouse adipose MSC (AMSC)	Not specified	Not specified	200 µg/mL (400 µL/ treatment)	Sprague-Dawley rats	Not specified	[104]
Alginate hydrogel	Rat adipose MSC (AMSC)	80% confluent cells. Medium and culture time are not specified	Ultracentrifugation	100 µg/mL (300 µL/ treatment)	Wistar rats	Not specified	[106]
Alginate sponge-like dressing	Human adipose MSC (AMSC)	Sub-confluent cells in DMEM/F12 platelet lysate free for 48 h	Ultrafiltration (isolation of soluble proteins and EV)	EV isolated from 4×10^6 cells/mL (500 µL/ treatment)	Mice	Not specified	[107]
Bilayered Thiolated Alginate/Polyethylene Glycol Diacrylate Hydrogels	Human Bone marrow MSC (hBMMSC) and hBMSC overexpressing miR-29b-3p	80% confluent cells in LG-DMEM serum-free medium for 48 h	Ultracentrifugation	10^{11} EV/mL	Sprague-Dawley rats	Not specified	[108]
Hyaluronic acid hydrogel	Human placental MSC (hPMSC)	Confluent cell not specified. DMEM serum-free medium for 48 h	Ultracentrifugation	1000 µg/mL (100 µL/ treatment)	C57BL/6J mice	Not specified	[110]

Table 1. *Cont.*

Biomaterial	Origin	Culture	Isolation	[EV]	Model	Treatment *	Ref.
Hyaluronic acid-light-triggerable hydrogel	Human umbilical Mononuclear cells (hUCMNC)	2×10^6 cells/mL in X-VIVO 15 serum-free cell culture medium supplemented with Flt-3 and stem-cell factor in hypoxia (0.5% O_2) for 18 h	Ultracentrifugation	200 µg/ treatment	Diabetic C57BL/6 mice (streptozotocin-induced)	One time and light-activated each day for 1 min	[111]
RhCollagen III Hydrogel	Human umbilical MSCs (hUCMSC)	Not specified	Ultracentrifugation	Not specified	Diabetic sprague-Dawley rats (streptozotocin-induced)	every three days	[114]
Pluronic F127 hydrogel	Mouse adipose MSC (AMSC)	6 × 104 cells/cm^2 in DMEM + 3% exo-free serum for 48 h	Ultracentrifugation	10 µg/mL	Diabetic ICR mice (streptozotocin-induced)	Not specified	[116]
Pluronic F127-oxidized hyaluronic acid and poly-ε-lysine hydrogel	Mouse adipose MSC (AMSC)	Not specified	Ultracentrifugation	10 µg/treatment	Diabetic ICR mice (streptozotocin-induced)	Not specified	[117]
Pluronic F127 hydrogel	Human adipose MSC (AMSC)	80–90% confluent cells in α-MEM + 10% UltraGROTM-Advanced medium for 48 h	Ultracentrifugation	1000 µg/mL (100 µL/ treatment)	ICR mice	every three days	[119]
Pluronic F127 hydrogel	Human umbilical MSCs (hUCMSC)	Not specified	ExoQuick-TC	1000 µg/mL (100 µL/ treatment)	Diabetic sprague-Dawley rats (streptozotocin-induced)	Not specified	[120]
porcine small intestinal submucosa-based hydrogel	Human umbilical MSCs (hUCMSC)	Culture medium (DMEM + 10% exo-free serum) was collected when the cells reached 80–85% confluency.	Ultracentrifugation	Not specified	Diabetic sprague-Dawley rats (streptozotocin-induced)	Not specified	[123]
Human acellular amniotic membrane	Human adipose MSC (AMSC)	60–65% confluent cell in DMEM + 10% exo-free serum for 48 h	Exosome isolation reagent (Precipitation)	100 µg/ treatment	Diabetic BALB/c mice (streptozotocin-induced)	every other day, three times in total	[125]
Antioxidant polyurethane dressing	Rat adipose MSC (AMSC)	80% confluent cells in α-MEM serum-free medium for 48 h	Ultrafiltration	100 µg/ treatment	Diabetic wistar rats (streptozotocin-induced)	Not specified	[129]

* When it is not specified, we consider that the authors have only applied the treatment once at the time the wounds were made.

Another important element for further developing this strategy is the biomaterial or dressing, used as scaffolds to apply MSC-EV. As described in this review, there are numerous biomaterials with well-proven properties. Their use in wounds is approved by health authorities, with commercial products available [84,85]. However, further progress is needed in the manufacture of new wound dressings at a low cost. They should have adequate properties to facilitate healing, with the capacity to encapsulate, protect and release EV in a sustained manner during the healing process. It is also important to note

that new wound dressings should not only serve the purpose of being a vehicle for MSC-EV, and create a barrier protecting wounds from external environments. They should also be biologically active, promoting healing. For example, they may have antimicrobial, antioxidant and wound pH-modulating activity, among sother properties.

Another important aspect to be taken into account is the possible cytotoxicity of biomaterials, their components, such as the crosslinkers, and their degradation products. For this it is necessary to evaluate their possible cytotoxicity in vitro, using suitable cellular models, such as fibroblasts and endothelial cells [131,132]. Thus, it may be convenient to evaluate, for instance, different crosslinkers when fabricating hydrogels, to obtain a product with low cytotoxicity [133]. Also, in relation to possible undesired effects in the application of MSC-EV encapsulated in biomaterials, it would be necessary to perform studies on possible adverse effects after the end of treatments. This is because the products derived from hydrogel degradation, and mainly the EV applied, can contain molecules with high biological activity. Therefore, it is important to ascertain potential undesired side-effects, as routinely done with pharmaceutical drugs-medicines. This could eventually manifest as alterations in immune responses, appearance of tumors or other consequences. In none of the studies reviewed has this type of research been carried out. Yet, this is an important aspect, in which progress must be made in the future.

In conclusion, the combination of MSC-EV with multifunctional wound dressings may have positive synergistic effects on cutaneous-wound healing. Current data show great potential for clinical use. However, further progress is needed for a better understanding of the molecular mechanism of action of MSC-EV, optimization of industrial production for clinical purposes, and standardization of methodologies. Likewise, for the development of suitable wound dressings for encapsulation, protection and release of MSC-EV. Increasing clinical trials of biomaterials and extracellular vesicles on wound healing should be one of the priorities in the coming years. That should allow us to ascertain the potential impact of this new therapeutic strategy in routine clinical practice.

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References

1. Takeo, M.; Lee, W.; Ito, M. Wound healing and skin regeneration. *Cold Spring Harb. Perspect. Med.* **2015**, *5*, a023267. [CrossRef]
2. Gantwerker, E.A.; Hom, D.B. Skin: Histology and physiology of wound healing. *Facial Plast. Surg. Clin. N. Am.* **2011**, *19*, 441–453. [CrossRef] [PubMed]
3. Rodrigues, M.; Kosaric, N.; Bonham, C.A.; Gurtner, G.C. Wound healing: A cellular perspective. *Physiol. Rev.* **2019**, *99*, 665–706. [CrossRef] [PubMed]
4. Beyene, R.T.; Derryberry, S.L.; Barbul, A. The Effect of Comorbidities on Wound Healing. *Surg. Clin. N. Am.* **2020**, *100*, 695–705. [CrossRef] [PubMed]
5. Velnar, T.; Bailey, T.; Smrkolj, V. The wound healing process: An overview of the cellular and molecular mechanisms. *J. Int. Med. Res.* **2009**, *37*, 1528–1542. [CrossRef]
6. Eming, S.A.; Martin, P.; Tomic-Canic, M. Wound repair and regeneration: Mechanisms, signaling, and translation. *Sci. Transl. Med.* **2014**, *6*, 265sr6. [CrossRef]

7. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* **2004**, *27*, 1047–1053. [CrossRef]
8. Glovaci, D.; Fan, W.; Wong, N.D. Epidemiology of Diabetes Mellitus and Cardiovascular Disease. *Curr. Cardiol. Rep.* **2019**, *21*, 21. [CrossRef]
9. Sen, C.K. Human Wounds and Its Burden: An Updated Compendium of Estimates. *Adv. Wound Care* **2019**, *8*, 39–48. [CrossRef]
10. Raghav, A.; Khan, Z.A.; Labala, R.K.; Ahmad, J.; Noor, S.; Mishra, B.K. Financial burden of diabetic foot ulcers to world: A progressive topic to discuss always. *Ther. Adv. Endocrinol. Metab.* **2018**, *9*, 29–31. [CrossRef]
11. Frykberg, R.G.; Banks, J. Challenges in the Treatment of Chronic Wounds. *Adv. Wound Care* **2015**, *4*, 560. [CrossRef] [PubMed]
12. Guillamat-Prats, R. The role of MSC in wound healing, scarring and regeneration. *Cells* **2021**, *10*, 1729. [CrossRef]
13. Kucharzewski, M.; Rojczyk, E.; Wilemska-Kucharzewska, K.; Wilk, R.; Hudecki, J.; Los, M.J. Novel trends in application of stem cells in skin wound healing. *Eur. J. Pharmacol.* **2019**, *843*, 307–315. [CrossRef] [PubMed]
14. Walter, M.N.M.; Wright, K.T.; Fuller, H.R.; MacNeil, S.; Johnson, W.E.B. Mesenchymal stem cell-conditioned medium accelerates skin wound healing: An in vitro study of fibroblast and keratinocyte scratch assays. *Exp. Cell Res.* **2010**, *316*, 1271–1281. [CrossRef]
15. Yew, T.-L.; Hung, Y.-T.; Li, H.-Y.; Chen, H.-W.; Chen, L.-L.; Tsai, K.-S.; Chiou, S.-H.; Chao, K.-C.; Huang, T.-F.; Chen, H.-L.; et al. Enhancement of wound healing by human multipotent stromal cell conditioned medium: The paracrine factors and p38 MAPK activation. *Cell Transplant.* **2011**, *20*, 693–706. [CrossRef]
16. Abels, E.R.; Breakefield, X.O. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. *Cell. Mol. Neurobiol.* **2016**, *36*, 301–312. [CrossRef] [PubMed]
17. Casado-Díaz, A.; Quesada-Gómez, J.M.; Dorado, G. Extracellular Vesicles Derived from Mesenchymal Stem Cells (MSC) in Regenerative Medicine: Applications in Skin Wound Healing. *Front. Bioeng. Biotechnol.* **2020**, *8*, e00146. [CrossRef]
18. Safari, B.; Aghazadeh, M.; Davaran, S.; Roshangar, L. Exosome-loaded hydrogels: A new cell-free therapeutic approach for skin regeneration. *Eur. J. Pharm. Biopharm.* **2022**, *171*, 50–59. [CrossRef] [PubMed]
19. Karppinen, S.M.; Heljasvaara, R.; Gullberg, D.; Tasanen, K.; Pihlajaniemi, T. Toward understanding scarless skin wound healing and pathological scarring. *F1000Research* **2019**, *8*, 787. [CrossRef]
20. Potekaev, N.N.; Borzykh, O.B.; Medvedev, G.V.; Pushkin, D.V.; Petrova, M.M.; Petrov, A.V.; Dmitrenko, D.V.; Karpova, E.I.; Demina, O.M.; Shnayder, N.A. The Role of Extracellular Matrix in Skin Wound Healing. *J. Clin. Med.* **2021**, *10*, 5947. [CrossRef]
21. Reinke, J.M.; Sorg, H. Wound Repair and Regeneration. *Eur. Surg. Res.* **2012**, *49*, 35–43. [CrossRef] [PubMed]
22. Curaj, A.; Schumacher, D.; Rusu, M.; Staudt, M.; Li, X.; Simsekyilmaz, S.; Jankowski, V.; Jankowski, J.; Dumitraşcu, A.R.; Hausenloy, D.J.; et al. Neutrophils Modulate Fibroblast Function and Promote Healing and Scar Formation after Murine Myocardial Infarction †. *Int. J. Mol. Sci.* **2020**, *21*, 3685. [CrossRef]
23. Ha, D.H.; Kim, H.K.; Lee, J.; Kwon, H.H.; Park, G.H.; Yang, S.H.; Jung, J.Y.; Choi, H.; Lee, J.H.; Sung, S.; et al. Mesenchymal Stem/Stromal Cell-Derived Exosomes for Immunomodulatory Therapeutics and Skin Regeneration. *Cells* **2020**, *9*, 1157. [CrossRef]
24. Gurtner, G.C.; Werner, S.; Barrandon, Y.; Longaker, M.T. Wound repair and regeneration. *Nature* **2008**, *453*, 314–321. [CrossRef]
25. Pastar, I.; Stojadinovic, O.; Yin, N.C.; Ramirez, H.; Nusbaum, A.G.; Sawaya, A.; Patel, S.B.; Khalid, L.; Isseroff, R.R.; Tomic-Canic, M. Epithelialization in Wound Healing: A Comprehensive Review. *Adv. Wound Care* **2014**, *3*, 445–464. [CrossRef]
26. Oliveira, G.V.; Hawkins, H.K.; Chinkes, D.; Burke, A.; Tavares, A.L.P.; Ramos-E-Silva, M.; Albrecht, T.B.; Kitten, G.T.; Herndon, D.N. Hypertrophic versus non hypertrophic scars compared by immunohistochemistry and laser confocal microscopy: Type I and III collagens†. *Int. Wound J.* **2009**, *6*, 445–452. [CrossRef]
27. van den Broek, L.J.; Limandjaja, G.C.; Niessen, F.B.; Gibbs, S. Human hypertrophic and keloid scar models: Principles, limitations and future challenges from a tissue engineering perspective. *Exp. Dermatol.* **2014**, *23*, 382–386. [CrossRef] [PubMed]
28. Zhao, R.; Liang, H.; Clarke, E.; Jackson, C.; Xue, M. Inflammation in Chronic Wounds. *Int. J. Mol. Sci.* **2016**, *17*, 2085. [CrossRef] [PubMed]
29. Eming, S.A.; Wynn, T.A.; Martin, P. Inflammation and metabolism in tissue repair and regeneration. *Science* **2017**, *356*, 1026–1030. [CrossRef]
30. Wilgus, T.A. Inflammation as an orchestrator of cutaneous scar formation: A review of the literature. *Plast. Aesthetic Res.* **2020**, *7*, 54. [CrossRef]
31. Eming, S.A.; Koch, M.; Krieger, A.; Brachvogel, B.; Kreft, S.; Bruckner-Tuderman, L.; Krieg, T.; Shannon, J.D.; Fox, J.W. Differential proteomic analysis distinguishes tissue repair biomarker signatures in wound exudates obtained from normal healing and chronic wounds. *J. Proteome Res.* **2010**, *9*, 4758–4766. [CrossRef] [PubMed]
32. Stojadinovic, O.; Minkiewicz, J.; Sawaya, A.; Bourne, J.W.; Torzilli, P.; de Rivero Vaccari, J.P.; Dietrich, W.D.; Keane, R.W.; Tomic-Canic, M. Deep Tissue Injury in Development of Pressure Ulcers: A Decrease of Inflammasome Activation and Changes in Human Skin Morphology in Response to Aging and Mechanical Load. *PLoS ONE* **2013**, *8*, e69223. [CrossRef] [PubMed]
33. Baltzis, D.; Eleftheriadou, I.; Veves, A. Pathogenesis and Treatment of Impaired Wound Healing in Diabetes Mellitus: New Insights. *Adv. Ther.* **2014**, *31*, 817–836. [CrossRef]
34. Sgonc, R.; Gruber, J. Age-related aspects of cutaneous wound healing: A mini-review. *Gerontology* **2013**, *59*, 159–164. [CrossRef] [PubMed]
35. Friedman, S.L.; Sheppard, D.; Duffield, J.S.; Violette, S. Therapy for Fibrotic Diseases: Nearing the Starting Line. *Sci. Transl. Med.* **2013**, *5*, e3004700. [CrossRef]

36. Berman, B.; Maderal, A.; Raphael, B. Keloids and hypertrophic scars: Pathophysiology, classification, and treatment. *Dermatol. Surg.* **2017**, *43*, S3–S18. [CrossRef]
37. Stoica, A.E.; Grumezescu, A.M.; Hermenean, A.O.; Andronescu, E.; Vasile, B.S. Scar-Free Healing: Current Concepts and Future Perspectives. *Nanomaterials* **2020**, *10*, 2179. [CrossRef] [PubMed]
38. Keshtkar, S.; Azarpira, N.; Ghahremani, M.H. Mesenchymal stem cell-derived extracellular vesicles: Novel frontiers in regenerative medicine. *Stem Cell Res. Ther.* **2018**, *9*, 63. [CrossRef]
39. Bray, E.R.; Oropallo, A.R.; Grande, D.A.; Kirsner, R.S.; Badiavas, E.V. Extracellular vesicles as therapeutic tools for the treatment of chronic wounds. *Pharmaceutics* **2021**, *13*, 1543. [CrossRef]
40. Yu, B.; Zhang, X.; Li, X. Exosomes derived from mesenchymal stem cells. *Int. J. Mol. Sci.* **2014**, *15*, 4142–4157. [CrossRef] [PubMed]
41. Han, Y.; Li, X.; Zhang, Y.; Han, Y.; Chang, F.; Ding, J. Mesenchymal Stem Cells for Regenerative Medicine. *Cells* **2019**, *8*, 886. [CrossRef] [PubMed]
42. Hade, M.D.; Suire, C.N.; Mossell, J.; Suo, Z. Extracellular vesicles: Emerging frontiers in wound healing. *Med. Res. Rev.* **2022**, *42*, 2102–2125. [CrossRef]
43. Riedl, J.; Popp, C.; Eide, C.; Ebens, C.; Tolar, J. Mesenchymal stromal cells in wound healing applications: Role of the secretome, targeted delivery and impact on recessive dystrophic epidermolysis bullosa treatment. *Cytotherapy* **2021**, *23*, 961–973. [CrossRef] [PubMed]
44. Théry, C.; Witwer, K.W.; Aikawa, E.; Alcaraz, M.J.; Anderson, J.D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-Smith, G.K.; et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J. Extracell. Vesicles* **2018**, *7*, 1535750. [CrossRef] [PubMed]
45. Doyle, L.; Wang, M. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells* **2019**, *8*, 727. [CrossRef]
46. Lu, S.; Lu, L.; Liu, Y.; Li, Z.; Fang, Y.; Chen, Z.; Zhou, J. Native and engineered extracellular vesicles for wound healing. *Front. Bioeng. Biotechnol.* **2022**, *10*, 1053217. [CrossRef]
47. Van Niel, G.; D'Angelo, G.; Raposo, G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 213–228. [CrossRef]
48. Akbar, A.; Malekian, F.; Baghban, N.; Kodam, S.P.; Ullah, M. Methodologies to Isolate and Purify Clinical Grade Extracellular Vesicles for Medical Applications. *Cells* **2022**, *11*, 186. [CrossRef]
49. Rani, S.; Ryan, A.E.; Griffin, M.D.; Ritter, T. Mesenchymal stem cell-derived extracellular vesicles: Toward cell-free therapeutic applications. *Mol. Ther.* **2015**, *23*, 812–823. [CrossRef]
50. Baranovskii, D.S.; Klabukov, I.D.; Arguchinskaya, N.V.; Yakimova, A.O.; Kisel, A.A.; Yatsenko, E.M.; Ivanov, S.A.; Shegay, P.V.; Kaprin, A.D. Adverse events, side effects and complications in mesenchymal stromal cell-based therapies. *Stem Cell Investig.* **2022**, *9*, 7. [CrossRef]
51. Narauskaitė, D.; Vydmantaitė, G.; Rusteikaitė, J.; Sampath, R.; Rudaitytė, A.; Stašytė, G.; Calvente, M.I.A.; Jekabsone, A. Extracellular vesicles in skin wound healing. *Pharmaceuticals* **2021**, *14*, 811. [CrossRef]
52. Ramos-Zaldívar, H.M.; Polakovicova, I.; Salas-Huenuleo, E.; Corvalán, A.H.; Kogan, M.J.; Yefi, C.P.; Andia, M.E. Extracellular vesicles through the blood–brain barrier: A review. *Fluids Barriers CNS* **2022**, *19*, 60. [CrossRef] [PubMed]
53. Vizoso, F.J.; Eiro, N.; Cid, S.; Schneider, J.; Perez-Fernandez, R. Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine. *Int. J. Mol. Sci.* **2017**, *18*, 1852. [CrossRef] [PubMed]
54. Silachev, D.N.; Goryunov, K.V.; Shpilyuk, M.A.; Beznoschenko, O.S.; Morozova, N.Y.; Kraevaya, E.E.; Popkov, V.A.; Pevzner, I.B.; Zorova, L.D.; Evtushenko, E.A.; et al. Effect of MSCs and MSC-derived extracellular vesicles on human blood coagulation. *Cells* **2019**, *8*, 258. [CrossRef] [PubMed]
55. Fiedler, T.; Rabe, M.; Mundkowski, R.G.; Oehmcke-Hecht, S.; Peters, K. Adipose-derived mesenchymal stem cells release microvesicles with procoagulant activity. *Int. J. Biochem. Cell Biol.* **2018**, *100*, 49–53. [CrossRef]
56. He, X.; Dong, Z.; Cao, Y.; Wang, H.; Liu, S.; Liao, L.; Jin, Y.; Yuan, L.; Li, B.; Bolontrade, M.F. MSC-Derived Exosome Promotes M2 Polarization and Enhances Cutaneous Wound Healing. *Stem Cells Int.* **2019**, *2019*, 7132708. [CrossRef]
57. Heo, J.S.; Kim, S.; Yang, C.E.; Choi, Y.; Song, S.Y.; Kim, H.O. Human Adipose Mesenchymal Stem Cell-Derived Exosomes: A Key Player in Wound Healing. *Tissue Eng. Regen. Med.* **2021**, *18*, 537–548. [CrossRef]
58. Li, X.; Liu, L.; Yang, J.; Yu, Y.; Chai, J.; Wang, L.; Ma, L.; Yin, H. Exosome Derived from Human Umbilical Cord Mesenchymal Stem Cell Mediates MiR-181c Attenuating Burn-induced Excessive Inflammation. *EBioMedicine* **2016**, *8*, 72–82. [CrossRef]
59. Mardpour, S.; Hamidieh, A.A.; Taleahmad, S.; Sharifzad, F.; Taghikhani, A.; Baharvand, H. Interaction between mesenchymal stromal cell-derived extracellular vesicles and immune cells by distinct protein content. *J. Cell. Physiol.* **2019**, *234*, 8249–8258. [CrossRef]
60. Favaro, E.; Carpanetto, A.; Caorsi, C.; Giovarelli, M.; Angelini, C.; Cavallo-Perin, P.; Tetta, C.; Camussi, G.; Zanone, M.M. Human mesenchymal stem cells and derived extracellular vesicles induce regulatory dendritic cells in type 1 diabetic patients. *Diabetologia* **2016**, *59*, 325–333. [CrossRef]
61. Cao, G.; Chen, B.; Zhang, X.; Chen, H. Human adipose-derived mesenchymal stem cells-derived exosomal microRNA-19b promotes the healing of skin wounds through modulation of the CCL1/TGF-β signaling axis. *Clin. Cosmet. Investig. Dermatol.* **2020**, *13*, 957–971. [CrossRef] [PubMed]

62. Li, X.; Xie, X.; Lian, W.; Shi, R.; Han, S.; Zhang, H.; Lu, L.; Li, M. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. *Exp. Mol. Med.* **2018**, *50*, e0585. [CrossRef] [PubMed]
63. Liang, X.; Zhang, L.; Wang, S.; Han, Q.; Zhao, R.C. Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a. *J. Cell Sci.* **2016**, *129*, 2182–2189. [CrossRef]
64. Krawczenko, A.; Bielawska-Pohl, A.; Paprocka, M.; Kraskiewicz, H.; Szyposzynska, A.; Wojdat, E.; Klimczak, A. Microvesicles from Human Immortalized Cell Lines of Endothelial Progenitor Cells and Mesenchymal Stem/Stromal Cells of Adipose Tissue Origin as Carriers of Bioactive Factors Facilitating Angiogenesis. *Stem Cells Int.* **2020**, *2020*, 1289380. [CrossRef]
65. Liu, J.; Yan, Z.; Yang, F.; Huang, Y.; Yu, Y.; Zhou, L.; Sun, Z.; Cui, D.; Yan, Y. Exosomes Derived from Human Umbilical Cord Mesenchymal Stem Cells Accelerate Cutaneous Wound Healing by Enhancing Angiogenesis through Delivering Angiopoietin-2. *Stem Cell Rev. Rep.* **2021**, *17*, 305–317. [CrossRef] [PubMed]
66. Wang, X.; Jiao, Y.; Pan, Y.; Zhang, L.; Gong, H.; Qi, Y.; Wang, M.; Gong, H.; Shao, M.; Wang, X.; et al. Fetal dermal mesenchymal stem cell-derived exosomes accelerate cutaneous wound healing by activating Notch signaling. *Stem Cells Int.* **2019**, *2019*, 2402916. [CrossRef]
67. Qian, L.; Pi, L.; Fang, B.R.; Meng, X.X. Adipose mesenchymal stem cell-derived exosomes accelerate skin wound healing via the lncRNA H19/miR-19b/SOX9 axis. *Lab. Investig.* **2021**, *101*, 1254–1266. [CrossRef]
68. Cheng, S.; Xi, Z.; Chen, G.; Liu, K.; Ma, R.; Zhou, C. Extracellular vesicle-carried microRNA-27b derived from mesenchymal stem cells accelerates cutaneous wound healing via E3 ubiquitin ligase ITCH. *J. Cell. Mol. Med.* **2020**, *24*, 11254–11271. [CrossRef]
69. Ren, S.; Chen, J.; Duscher, D.; Liu, Y.; Guo, G.; Kang, Y.; Xiong, H.; Zhan, P.; Wang, Y.; Wang, C.; et al. Microvesicles from human adipose stem cells promote wound healing by optimizing cellular functions via AKT and ERK signaling pathways. *Stem Cell Res. Ther.* **2019**, *10*, e1152. [CrossRef]
70. Zhou, Y.; Zhao, B.; Zhang, X.L.; Lu, Y.J.; Lu, S.T.; Cheng, J.; Fu, Y.; Lin, L.; Zhang, N.Y.; Li, P.X.; et al. Combined topical and systemic administration with human adipose-derived mesenchymal stem cells (hADSC) and hADSC-derived exosomes markedly promoted cutaneous wound healing and regeneration. *Stem Cell Res. Ther.* **2021**, *12*, 257. [CrossRef]
71. Wang, L.; Hu, L.; Zhou, X.; Xiong, Z.; Zhang, C.; Shehada, H.M.A.; Hu, B.; Song, J.; Chen, L. Exosomes secreted by human adipose mesenchymal stem cells promote scarless cutaneous repair by regulating extracellular matrix remodelling. *Sci. Rep.* **2017**, *7*, e12919. [CrossRef]
72. Li, Y.; Zhang, J.; Shi, J.; Liu, K.; Wang, X.; Jia, Y.; He, T.; Shen, K.; Wang, Y.; Liu, J.; et al. Exosomes derived from human adipose mesenchymal stem cells attenuate hypertrophic scar fibrosis by miR-192-5p/IL-17RA/Smad axis. *Stem Cell Res. Ther.* **2021**, *12*, 221. [CrossRef] [PubMed]
73. Fang, S.; Xu, C.; Zhang, Y.; Xue, C.; Yang, C.; Bi, H.; Qian, X.; Wu, M.; Ji, K.; Zhao, Y.; et al. Umbilical Cord-Derived Mesenchymal Stem Cell-Derived Exosomal MicroRNAs Suppress Myofibroblast Differentiation by Inhibiting the Transforming Growth Factor-β/SMAD2 Pathway during Wound Healing. *Stem Cells Transl. Med.* **2016**, *5*, 1425–1439. [CrossRef]
74. Nallakumarasamy, A.; Jeyaraman, M.; Maffulli, N.; Jeyaraman, N.; Suresh, V.; Ravichandran, S.; Gupta, M.; Potty, A.G.; El-Amin, S.F.; Khanna, M.; et al. Mesenchymal Stromal Cell-Derived Extracellular Vesicles in Wound Healing. *Life* **2022**, *12*, 1733. [CrossRef] [PubMed]
75. Grange, C.; Tapparo, M.; Bruno, S.; Chatterjee, D.; Quesenberry, P.J.; Tetta, C.; Camussi, G. Biodistribution of mesenchymal stem cell-derived extracellular vesicles in a model of acute kidney injury monitored by optical imaging. *Int. J. Mol. Med.* **2014**, *33*, 1055–1063. [CrossRef]
76. Wiklander, O.P.B.; Nordin, J.Z.; O'Loughlin, A.; Gustafsson, Y.; Corso, G.; Mäger, I.; Vader, P.; Lee, Y.; Sork, H.; Seow, Y.; et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. *J. Extracell. vesicles* **2015**, *4*, e26316. [CrossRef] [PubMed]
77. Munagala, R.; Aqil, F.; Jeyabalan, J.; Gupta, R.C. Bovine milk-derived exosomes for drug delivery. *Cancer Lett.* **2016**, *371*, 48–61. [CrossRef]
78. Charoenviriyakul, C.; Takahashi, Y.; Morishita, M.; Matsumoto, A.; Nishikawa, M.; Takakura, Y. Cell type-specific and common characteristics of exosomes derived from mouse cell lines: Yield, physicochemical properties, and pharmacokinetics. *Eur. J. Pharm. Sci.* **2017**, *96*, 316–322. [CrossRef]
79. Takahashi, Y.; Nishikawa, M.; Shinotsuka, H.; Matsui, Y.; Ohara, S.; Imai, T.; Takakura, Y. Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. *J. Biotechnol.* **2013**, *165*, 77–84. [CrossRef]
80. Xiong, Y.; Chen, L.; Liu, P.; Yu, T.; Lin, C.; Yan, C.; Hu, Y.; Zhou, W.; Sun, Y.; Panayi, A.C.; et al. All-in-One: Multifunctional Hydrogel Accelerates Oxidative Diabetic Wound Healing through Timed-Release of Exosome and Fibroblast Growth Factor. *Small* **2022**, *18*, e202104229. [CrossRef]
81. Kwak, G.; Cheng, J.; Kim, H.; Song, S.; Lee, S.J.; Yang, Y.; Jeong, J.H.; Lee, J.E.; Messersmith, P.B.; Kim, S.H. Sustained Exosome-Guided Macrophage Polarization Using Hydrolytically Degradable PEG Hydrogels for Cutaneous Wound Healing: Identification of Key Proteins and MiRNAs, and Sustained Release Formulation. *Small* **2022**, *18*, e202200060. [CrossRef]
82. Imai, T.; Takahashi, Y.; Nishikawa, M.; Kato, K.; Morishita, M.; Yamashita, T.; Matsumoto, A.; Charoenviriyakul, C.; Takakura, Y. Macrophage-dependent clearance of systemically administered B16BL6-derived exosomes from the blood circulation in mice. *J. Extracell. Vesicles* **2015**, *4*, e26238. [CrossRef]
83. Dhivya, S.; Padma, V.V.; Santhini, E. Wound dressings—A review. *BioMedicine* **2015**, *5*, 24–28. [CrossRef]

84. Shi, C.; Wang, C.; Liu, H.; Li, Q.; Li, R.; Zhang, Y.; Liu, Y.; Shao, Y.; Wang, J. Selection of Appropriate Wound Dressing for Various Wounds. *Front. Bioeng. Biotechnol.* **2020**, *8*, 182. [CrossRef]
85. Frazier, T.; Alarcon, A.; Wu, X.; Mohiuddin, O.A.; Motherwell, J.M.; Carlsson, A.H.; Christy, R.J.; Edwards, J.V.; Mackin, R.T.; Prevost, N.; et al. Clinical translational potential in skin wound regeneration for adipose-derived, blood-derived, and cellulose materials: Cells, exosomes, and hydrogels. *Biomolecules* **2020**, *10*, 1373. [CrossRef] [PubMed]
86. Boateng, J.; Catanzano, O. Advanced Therapeutic Dressings for Effective Wound Healing—A Review. *J. Pharm. Sci.* **2015**, *104*, 3653–3680. [CrossRef] [PubMed]
87. Rice, J.J.; Martino, M.M.; De Laporte, L.; Tortelli, F.; Briquez, P.S.; Hubbell, J.A. Engineering the regenerative microenvironment with biomaterials. *Adv. Healthc. Mater.* **2013**, *2*, 57–71. [CrossRef]
88. Khayambashi, P.; Iyer, J.; Pillai, S.; Upadhyay, A.; Zhang, Y.; Tran, S.D. Hydrogel encapsulation of mesenchymal stem cells and their derived exosomes for tissue engineering. *Int. J. Mol. Sci.* **2021**, *22*, 684. [CrossRef] [PubMed]
89. Huang, J.; Xiong, J.; Yang, L.; Zhang, J.; Sun, S.; Liang, Y. Cell-free exosome-laden scaffolds for tissue repair. *Nanoscale* **2021**, *13*, 8740–8750. [CrossRef]
90. Brennan, M.; Layrolle, P.; Mooney, D.J. Biomaterials functionalized with MSC secreted extracellular vesicles and soluble factors for tissue regeneration. *Adv. Funct. Mater.* **2020**, *30*, e201909125. [CrossRef] [PubMed]
91. Li, T.; Sun, M.; Wu, S. State-of-the-Art Review of Electrospun Gelatin-Based Nanofiber Dressings for Wound Healing Applications. *Nanomaterials* **2022**, *12*, 784. [CrossRef] [PubMed]
92. Németh, K.; Kazsoki, A.; Visnovitz, T.; Pinke, B.; Mészáros, L.; Buzás, E.I.; Zelkó, R. Nanofiber formation as a promising technology for preservation and easy storage of extracellular vesicles. *Sci. Rep.* **2022**, *12*, 22012. [CrossRef]
93. Wang, L.; Cheng, W.; Zhu, J.; Li, W.; Li, D.; Yang, X.; Zhao, W.; Ren, M.; Ren, J.; Mo, X.; et al. Electrospun nanoyarn and exosomes of adipose-derived stem cells for urethral regeneration: Evaluations in vitro and in vivo. *Colloids Surf. B Biointerfaces* **2022**, *209*, 112218. [CrossRef] [PubMed]
94. Nieuwoudt, M.; Woods, I.; Eichholz, K.F.; Martins, C.; McSweeney, K.; Shen, N.; Hoey, D.A. Functionalization of Electrospun Polycaprolactone Scaffolds with Matrix-Binding Osteocyte-Derived Extracellular Vesicles Promotes Osteoblastic Differentiation and Mineralization. *Ann. Biomed. Eng.* **2021**, *49*, 3621–3635. [CrossRef]
95. Elangwe, C.N.; Morozkina, S.N.; Olekhnovich, R.O.; Krasichkov, A.; Polyakova, V.O.; Uspenskaya, M.V. A Review on Chitosan and Cellulose Hydrogels for Wound Dressings. *Polymers* **2022**, *14*, 5163. [CrossRef] [PubMed]
96. Abolgheit, S.; Abdelkader, S.; Aboushelib, M.; Omar, E.; Mehanna, R. Bone marrow-derived mesenchymal stem cells and extracellular vesicles enriched collagen chitosan scaffold in skin wound healing (a rat model). *J. Biomater. Appl.* **2021**, *36*, 128–139. [CrossRef]
97. Tao, S.-C.; Guo, S.-C.; Li, M.; Ke, Q.-F.; Guo, Y.-P.; Zhang, C.-Q. Chitosan Wound Dressings Incorporating Exosomes Derived from MicroRNA-126-Overexpressing Synovium Mesenchymal Stem Cells Provide Sustained Release of Exosomes and Heal Full-Thickness Skin Defects in a Diabetic Rat Model. *Stem Cells Transl. Med.* **2017**, *6*, 736–747. [CrossRef] [PubMed]
98. Huang, F.; Fang, Z.F.; Hu, X.Q.; Tang, L.; Zhou, S.H.; Huang, J.P. Overexpression of miR-126 promotes the differentiation of mesenchymal stem cells toward endothelial cells via activation of PI3K/Akt and MAPK/ERK pathways and release of paracrine factors. *Biol. Chem.* **2013**, *394*, 1223–1233. [CrossRef]
99. Nooshabadi, V.T.; Khanmohamadi, M.; Valipour, E.; Mahdipour, S.; Salati, A.; Malekshahi, Z.V.; Shafei, S.; Amini, E.; Farzamfar, S.; Ai, J. Impact of exosome-loaded chitosan hydrogel in wound repair and layered dermal reconstitution in mice animal model. *J. Biomed. Mater. Res.* **2020**, *108*, 2138–2149. [CrossRef]
100. Li, Q.; Gong, S.; Yao, W.; Yang, Z.; Wang, R.; Yu, Z.; Wei, M. Exosome loaded genipin crosslinked hydrogel facilitates full thickness cutaneous wound healing in rat animal model. *Drug Deliv.* **2021**, *28*, 884–893. [CrossRef]
101. Wang, C.; Liang, C.; Wang, R.; Yao, X.; Guo, P.; Yuan, W.; Liu, Y.; Song, Y.; Li, Z.; Xie, X. The fabrication of a highly efficient self-healing hydrogel from natural biopolymers loaded with exosomes for the synergistic promotion of severe wound healing. *Biomater. Sci.* **2020**, *8*, 313–324. [CrossRef] [PubMed]
102. Xu, L.; Wang, Y.Y.; Huang, J.; Chen, C.Y.; Wang, Z.X.; Xie, H. Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics* **2020**, *10*, 8996–9031. [CrossRef] [PubMed]
103. Qian, Z.; Bai, Y.; Zhou, J.; Li, L.; Na, J.; Fan, Y.; Guo, X.; Liu, H. A moisturizing chitosan-silk fibroin dressing with silver nanoparticles-adsorbed exosomes for repairing infected wounds. *J. Mater. Chem. B* **2020**, *8*, 7197–7212. [CrossRef]
104. Liu, Y.; Liu, Y.; Zhao, Y.; Wu, M.; Mao, S.; Cong, P.; Zou, R.; Hou, M.; Jin, H.; Bao, Y. Application of adipose mesenchymal stem cell-derived exosomes-loaded β-chitin nanofiber hydrogel for wound healing. *Folia Histochem. Cytobiol.* **2022**, *60*, 167–178. [CrossRef]
105. Zhang, M.; Zhao, X. Alginate hydrogel dressings for advanced wound management. *Int. J. Biol. Macromol.* **2020**, *162*, 1414–1428. [CrossRef]
106. Shafei, S.; Khanmohammadi, M.; Heidari, R.; Ghanbari, H.; Taghdiri Nooshabadi, V.; Farzamfar, S.; Akbariqomi, M.; Sanikhani, N.S.; Absalan, M.; Tavoosidana, G. Exosome loaded alginate hydrogel promotes tissue regeneration in full-thickness skin wounds: An in vivo study. *J. Biomed. Mater. Res.* **2020**, *108*, 545–556. [CrossRef]

107. Bari, E.; Di Silvestre, D.; Mastracci, L.; Grillo, F.; Grisoli, P.; Marrubini, G.; Nardini, M.; Mastrogiacomo, M.; Sorlini, M.; Rossi, R.; et al. GMP-compliant sponge-like dressing containing MSC lyo-secretome: Proteomic network of healing in a murine wound model. *Eur. J. Pharm. Biopharm.* **2020**, *155*, 37–48. [CrossRef]
108. Shen, Y.; Xu, G.; Huang, H.; Wang, K.; Wang, H.; Lang, M.; Gao, H.; Zhao, S. Sequential Release of Small Extracellular Vesicles from Bilayered Thiolated Alginate/Polyethylene Glycol Diacrylate Hydrogels for Scarless Wound Healing. *ACS Nano* **2021**, *15*, 6352–6368. [CrossRef] [PubMed]
109. Zhu, Y.; Li, Z.; Wang, Y.; Li, L.; Wang, D.; Zhang, W.; Liu, L.; Jiang, H.; Yang, J.; Cheng, J. Overexpression of miR-29b reduces collagen biosynthesis by inhibiting heat shock protein 47 during skin wound healing. *Transl. Res.* **2016**, *178*, 38–53.e6. [CrossRef] [PubMed]
110. Yang, S.; Jiang, H.; Qian, M.; Ji, G.; Wei, Y.; He, J.; Tian, H.; Zhao, Q. MSC-derived sEV-loaded hyaluronan hydrogel promotes scarless skin healing by immunomodulation in a large skin wound model. *Biomed. Mater.* **2022**, *17*, 034104. [CrossRef]
111. Henriques-Antunes, H.; Cardoso, R.M.S.; Zonari, A.; Correia, J.; Leal, E.C.; Jiménez-Balsa, A.; Lino, M.M.; Barradas, A.; Kostic, I.; Gomes, C.; et al. The Kinetics of Small Extracellular Vesicle Delivery Impacts Skin Tissue Regeneration. *ACS Nano* **2019**, *13*, 8694–8707. [CrossRef]
112. Sarrigiannidis, S.O.; Rey, J.M.; Dobre, O.; González-García, C.; Dalby, M.J.; Salmeron-Sanchez, M. A tough act to follow: Collagen hydrogel modifications to improve mechanical and growth factor loading capabilities. *Mater. Today Bio* **2021**, *10*, 100098. [CrossRef]
113. Liu, W.; Lin, H.; Zhao, P.; Xing, L.; Li, J.; Wang, Z.; Ju, S.; Shi, X.L.; Liu, Y.; Deng, G.; et al. A regulatory perspective on recombinant collagen-based medical devices. *Bioact. Mater.* **2021**, *12*, 198–202. [CrossRef]
114. Xu, L.; Liu, Y.; Tang, L.; Xiao, H.; Yang, Z.; Wang, S. Preparation of Recombinant Human Collagen III Protein Hydrogels with Sustained Release of Extracellular Vesicles for Skin Wound Healing. *Int. J. Mol. Sci.* **2022**, *23*, 6289. [CrossRef]
115. Diniz, I.M.A.; Chen, C.; Xu, X.; Ansari, S.; Zadeh, H.H.; Marques, M.M.; Shi, S.; Moshaverinia, A. Pluronic F-127 hydrogel as a promising scaffold for encapsulation of dental-derived mesenchymal stem cells. *J. Mater. Sci. Mater. Med.* **2015**, *26*, 153. [CrossRef]
116. Wang, M.; Wang, C.; Chen, M.; Xi, Y.; Cheng, W.; Mao, C.; Xu, T.; Zhang, X.; Lin, C.; Gao, W.; et al. Efficient Angiogenesis-Based Diabetic Wound Healing/Skin Reconstruction through Bioactive Antibacterial Adhesive Ultraviolet Shielding Nanodressing with Exosome Release. *ACS Nano* **2019**, *13*, 10279–10293. [CrossRef] [PubMed]
117. Wang, C.; Wang, M.; Xu, T.; Zhang, X.; Lin, C.; Gao, W.; Xu, H.; Lei, B.; Mao, C. Engineering Bioactive Self-Healing Antibacterial Exosomes Hydrogel for Promoting Chronic Diabetic Wound Healing and Complete Skin Regeneration. *Theranostics* **2019**, *9*, 65–76. [CrossRef]
118. Monavarian, M.; Kader, S.; Moeinzadeh, S.; Jabbari, E. Regenerative Scar-Free Skin Wound Healing. *Tissue Eng. Rev.* **2019**, *25*, 294–311. [CrossRef]
119. Zhou, Y.; Zhang, X.L.; Lu, S.T.; Zhang, N.Y.; Zhang, H.J.; Zhang, J.; Zhang, J. Human adipose-derived mesenchymal stem cells-derived exosomes encapsulated in pluronic F127 hydrogel promote wound healing and regeneration. *Stem Cell Res. Ther.* **2022**, *13*, 407. [CrossRef]
120. Yang, J.; Chen, Z.; Pan, D.; Li, H.; Shen, J. Umbilical cord-derived mesenchymal stem cell-derived exosomes combined pluronic F127 hydrogel promote chronic diabetic wound healing and complete skin regeneration. *Int. J. Nanomed.* **2020**, *15*, 5911–5926. [CrossRef]
121. Guruswamy Damodaran, R.; Vermette, P. Tissue and organ decellularization in regenerative medicine. *Biotechnol. Prog.* **2018**, *34*, 1494–1505. [CrossRef]
122. Andrée, B.; Bär, A.; Haverich, A.; Hilfiker, A. Small intestinal submucosa segments as matrix for tissue engineering: Review. *Tissue Eng. Rev.* **2013**, *19*, 279–291. [CrossRef]
123. Ma, S.; Hu, H.; Wu, J.; Li, X.; Ma, X.; Zhao, Z.; Liu, Z.; Wu, C.; Zhao, B.; Wang, Y.; et al. Functional extracellular matrix hydrogel modified with MSC-derived small extracellular vesicles for chronic wound healing. *Cell Prolif.* **2022**, *55*, e13196. [CrossRef]
124. Fénelon, M.; Catros, S.; Meyer, C.; Fricain, J.C.; Obert, L.; Auber, F.; Louvrier, A.; Gindraux, F. Applications of human amniotic membrane for tissue engineering. *Membranes* **2021**, *11*, 387. [CrossRef]
125. Xiao, S.; Xiao, C.; Miao, Y.; Wang, J.; Chen, R.; Fan, Z.; Hu, Z. Human acellular amniotic membrane incorporating exosomes from adipose-derived mesenchymal stem cells promotes diabetic wound healing. *Stem Cell Res. Ther.* **2021**, *12*, 255. [CrossRef]
126. Wendels, S.; Avérous, L. Biobased polyurethanes for biomedical applications. *Bioact. Mater.* **2020**, *6*, 1083–1106. [CrossRef]
127. Shiekh, P.A.; Singh, A.; Kumar, A. Exosome laden oxygen releasing antioxidant and antibacterial cryogel wound dressing OxOBand alleviate diabetic and infectious wound healing. *Biomaterials* **2020**, *249*, 120020. [CrossRef]
128. Shiekh, P.A.; Singh, A.; Kumar, A. Engineering Bioinspired Antioxidant Materials Promoting Cardiomyocyte Functionality and Maturation for Tissue Engineering Application. *ACS Appl. Mater. Interfaces* **2018**, *10*, 3260–3273. [CrossRef]
129. Shiekh, P.A.; Singh, A.; Kumar, A. Oxygen-Releasing Antioxidant Cryogel Scaffolds with Sustained Oxygen Delivery for Tissue Engineering Applications. *ACS Appl. Mater. Interfaces* **2018**, *10*, 18458–18469. [CrossRef]
130. Wang, J.; Zhang, D.; Zhu, Y.; Mo, X.; McHugh, P.C.; Tong, Q. Astragalus and human mesenchymal stem cells promote wound healing by mediating immunomodulatory effects through paracrine signaling. *Regen. Med.* **2022**, *17*, 219–232. [CrossRef]
131. Rodríguez-Rodríguez, R.; García-Carvajal, Z.Y.; Jiménez-Palomar, I.; Jiménez-Avalos, J.A.; Espinosa-Andrews, H. Development of gelatin/chitosan/PVA hydrogels: Thermal stability, water state, viscoelasticity, and cytotoxicity assays. *J. Appl. Polym. Sci.* **2019**, *136*, 47149. [CrossRef]

132. Tyliszczak, B.; Drabczyk, A.; Kudłacik-Kramarczyk, S.; Bialik-Wąs, K.; Kijkowska, R.; Sobczak-Kupiec, A. Preparation and cytotoxicity of chitosan-based hydrogels modified with silver nanoparticles. *Colloids Surf. B Biointerfaces* **2017**, *160*, 325–330. [CrossRef]
133. Ye, J.; Xiao, Z.; Gao, L.; Zhang, J.; He, L.; Zhang, H.; Liu, Q.; Yang, G. Assessment of the effects of four crosslinking agents on gelatin hydrogel for myocardial tissue engineering applications. *Biomed. Mater.* **2021**, *16*, 045026. [CrossRef]