Therapeutic Application of Cell Secretomes in Cutaneous Wound Healing

Daniel Bormann[1], Alfred Gugerell[1], Hendrik Jan Ankersmit[1,3] and Michael Mildner[2,3]

JID Open

Although the application of stem cells to chronic wounds emerged as a candidate therapy in the previous century, the mechanism of action remains unclear. Recent evidence has implicated secreted paracrine factors in the regenerative properties of cell-based therapies. In the last two decades, considerable research advances involving the therapeutic potential of stem cell secretomes have expanded the scope of secretome-based therapies beyond stem cell populations. In this study, we review the modes of action of cell secretomes in wound healing, important preconditioning strategies for enhancing their therapeutic efficacy, and clinical trials on secretome-based wound healing.

Journal of Investigative Dermatology (2023) **143,** 893–912; doi:10.1016/j.jid.2023.02.019

INTRODUCTION

Cutaneous wound healing refers to the tightly regulated, multistage processes that reestablish the physiological skin barrier and its homeostatic functions. Conceptually, this process can be subdivided into 3–5 canonical, overlapping stages. Immediately after injury, vasoconstriction and hemostasis lead to the formation of a stable fibrin clot, stopping blood loss and generating a scaffold for migrating immune cells. An inflammatory phase follows, characterized by the accumulation of immunomodulatory mediators in the wound bed, rapid recruitment of neutrophils, and subsequent infiltration of monocytes and activation of skin-resident immune cells (Ellis et al., 2018; Rodrigues et al., 2019). During the early inflammatory phase, the accumulation of proinflammatory cytokines (e.g., TNF-α and IL-1), derived from infiltrating neutrophils and skin resident cells, orchestrates the rapid response to intruding pathogens and subsequent wound maturation (Barrientos et al., 2008; Kanno et al., 2011). Beyond debridement and pathogen clearance, immune cells are critically involved in bridging the late-inflammatory and proliferative phases of wound healing through the release of trophic factors, such as epidermal GF, PDGF, and VEGF, as well as immunomodulatory molecules, such as IL-1Ra, IL-10, and TGF-β (Ellis et al., 2018; Rodrigues et al., 2019). During the proliferative phase, the formation of granulation tissue begins to re-establish mechanical stability, whereas myofibroblast differentiation advances wound closure, followed by the migration of epidermal stem cells from their respective stem cell niches and the proliferation and differentiation of transiently amplifying cells to re-establish barrier function (Pastar et al., 2014; Rangel-Huerta and Maldonado, 2017; Rodrigues et al., 2019). These processes critically depend on oxygen and nutrient delivery to the wound area, which is achieved by neovascularization (Rodrigues et al., 2019). During the remodeling phase, the newly formed vasculature is pruned, and the extracellular matrix (ECM) is extensively rearranged, resulting in the formation of rigid scar tissue (Gushiken et al., 2021; Rodrigues et al., 2019).

Disruption of these intricate cellular interactions results in failure of the physiological wound healing process (Bjarnsholt et al., 2008; DiPietro, 2016; Ellis et al., 2018; Pastar et al., 2014). In developed countries, the lifetime prevalence of chronic wounds is 1–2%, representing a severe burden for patients and a socioeconomic challenge (Olsson et al., 2019; Sen, 2019; Sen et al., 2009). The annual global market for wound care–related products is projected to reach $15–22 billion by 2024, corresponding to 2–3% of total healthcare budgets in developed countries, although current treatment strategies are often unsatisfactory (Frykberg and Banks, 2015; Sen, 2019).

In the quest to address the unmet needs for high-quality therapeutics for chronic wounds, the initial focus was on stem cell–based therapies and their tissue regenerative and immunomodulatory properties (Bian et al., 2022). The concept of modern cell-based tissue regenerative therapies traces to the 19th and early 20th centuries (Charitos et al., 2021), and in the 20th century, the prevailing hypothesis explaining their regenerative effects was the engraftment of stem cells into the damaged tissue and their subsequent differentiation. In 1994, Holzinger et al. (1994) challenged this hypothesis by showing that nonstem cells, particularly autologous activated blood mononuclear cells, improved wound healing in chronic or non-healing ulcers. Meanwhile, reports emerged describing poor homing and engraftment of systemically and topically applied stem cells, leading to a search for alternative mechanisms of action (Chan et al., 2022; Eggenhofer et al., 2012; Murry et al., 2004).

Work showed that stem cells release a plethora of cytoprotective, trophic, and immunomodulatory paracrine factors

[1]*Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Department of Thoracic Surgery, Medical University of Vienna, Vienna, Austria; and* [2]*Department of Dermatology, Medical University of Vienna, Vienna, Austria*

[3]*These authors contributed equally to this work.*

Correspondence: Michael Mildner, Department of Dermatology, Medical University of Vienna, Lazarettgasse 14, Vienna 1090, Austria. E-mail: michael.mildner@meduniwien.ac.at

Abbreviations: ADMSC, adipose tissue–derived mesenchymal stem/stromal cell; AFL, ablative carbon dioxide fractional laser; Akt, protein kinase B; AMSC, amniotic mesenchymal stem/stromal cell; ECM, extracellular matrix; EV, extracellular vesicle; GF, growth factor; GMP, good manufacturing practice; ICH, International Council for Harmonization; miRNA, microRNA; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; PI3K, phosphatidylinositol 3-kinase; RTK, receptor tyrosine kinase; Th, T helper; UCMSC, umbilical cord–derived mesenchymal stem/stromal cell

Received 12 December 2022; revised 2 February 2023; accepted 10 February 2023

that account for most of the regenerative effects related to therapies (Baraniak and McDevitt, 2010). The sum of these biologically active paracrine factors, historically focusing on secreted proteins, has been termed the cell secretome (Tjalsma et al., 2004) and has developed into the major focus of a growing number of recent studies, examining novel tissue regenerative approaches (Tables 1–4) (Bian et al., 2022; Fadilah et al., 2022; Prasai et al., 2022). Proteins, lipids, and extracellular vesicles (EVs) are the major fractions of cell secretomes (Figure 1). Currently, EVs are the most extensively researched secretome fraction and can be further subdivided by size into exosomes (70–150 nm) and microvesicles (100–1,000 nm) and further characterized by their membrane surface–bound ligands and cargo, which largely mediate their bioactive properties (Lener et al., 2015). Established modes of EV-mediated signaling include the direct interaction with membrane receptors of the target cell; the transfer of activated receptors; and the modulation of intracellular signaling through the delivery of proteins, lipids, and RNAs, including post-transcriptional regulation through noncoding RNAs (Prasai et al., 2022; Zhang et al., 2019).

Among the various stem cell populations used as sources of therapeutically applied secretomes in cutaneous wound healing, mesenchymal stem cells (MSCs) derived from adipose and amniotic tissue, the umbilical cord, and the bone marrow have been investigated most extensively (Figure 1 and Tables 1–3). However, recent research explored further putative secretome sources, such as stem cells derived from human urine, cells derived from skin and gingival tissue, as well as deer antlers (Figure 1 and Table 4).

In recent decades, cell populations beyond stem cells have increasingly been explored as sources of tissue-protective and -regenerative secretomes in wound healing (Figure 1 and Table 5). These populations include mucosal fibroblasts (Ahangar et al., 2020a), epithelial cells (Sjöqvist et al., 2019), and diverse blood cell populations (Hacker et al., 2020, 2016; Li et al., 2019b; Mildner et al., 2013). In parallel with this research, a steadily growing body of work focused on using various preconditioning strategies to augment the regenerative properties of cell secretomes. Candidate strategies include hypoxia (Zhang et al., 2021d), pharmacological pretreatment (Hu et al., 2021), ex vivo exposure to cytokines (Zhu et al., 2020), and irradiation (Hacker et al., 2020).

In this review, we summarize the ever-expanding wealth of novel approaches in secretome-based wound healing therapies, emphasizing their modes of action (Figure 2) and roles in clinical trials.

IMMUNOMODULATORY PROPERTIES OF CELL SECRETOMES IN CHRONIC WOUND HEALING

The inflammatory response responsible for pathogen clearance and priming of the wound bed for granulation is necessarily acute. In contrast, the ongoing inflammation underlying chronic wound healing impairments is a well-established but mechanistically undercharacterized phenomenon (Ellis et al., 2018; Zhao et al., 2016). Immunomodulatory therapeutic approaches might serve to elucidate and target potential hallmarks of these impairments. Among these, various therapeutic secretomes modulate immune cell entry into the wound bed and adjacent tissues and influence the phenotype of infiltrating and resident immune cells and the cytokine milieu of the wound (Ahangar et al., 2020a, 2020b; De Gregorio et al., 2020; Irons et al., 2018; Li et al., 2019b; Wang et al., 2021). Macrophages appear to be a common target of various cell secretome products, and the accumulation of M2-like macrophages in the wound bed is induced in vivo by paracrine factors from bone marrow–derived MSCs (Ahangar et al., 2020b), amniotic mesenchymal stem/stromal cells (AMSCs) (Xiao et al., 2021), and gingival fibroblasts (Ahangar et al., 2020a). During physiological wound maturation, macrophages dynamically shift from an initial, predominately proinflammatory M1-like state, as defined by the release of ROS, nitric oxide, IL-1, IL-6, TNF-α, and high phagocytic activity, to an inflammation-resolving M2-like polarization, characterized by the release of proresolving cytokines such as IL-10, GFs such as VEGF, and ECM-remodeling matrix metalloproteinases (MMPs) (for review, see the studies by Krzyszczyk et al. [2018] and Lin et al. [2022]). Recent accumulating evidence suggests a more dynamic macrophage spectrum, including emerging M1/M2 hybrid macrophage phenotypes that distinctly modulate fibroblast biology and ECM remodeling (Direder et al., 2022; Witherel et al., 2021). The interrogation of M1/M2 hybrid macrophages might thus pose a promising novel target in future research on therapeutically applied secretomes.

In a mouse diabetic wound healing model, subcutaneous application of exosomes derived from the human macrophage cell line RAW 264.7 into the wound margins reduced the expression of the proinflammatory cytokines TNFα and IL-6 in the wound bed (Li et al., 2019b). In that study, however, the authors did not characterize macrophage phenotypes.

Other work has shown decreased IL-6 expression in wound tissue of diabetic nude mice treated with adipose stem cell–derived exosomes compared with that in sham-treated mice (Wang et al., 2021). Paradoxically, multiple proinflammatory cytokines (e.g., IL-1β, IL-6, and IL-8) are common in various stem cell and nonstem cell secretomes, even though their application triggers an anti-inflammatory response and improves wound healing (Ahangar et al., 2020a, 2020b; An et al., 2021; Hacker et al., 2020). Thus, in the context of wound healing, a suprasummative, anti-inflammatory effect of therapeutically applied secretomes appears to outweigh the proinflammatory influence of individual factors.

The overall milieu of chronic wounds, particularly in diabetic wound healing, appears to be an arrested state of nonresolving inflammation (Ellis et al., 2018; Zhao et al., 2016). The exact molecular processes governing the resolution of inflammation are not fully understood (Sugimoto et al., 2016), but apoptosis of immune cells in the wound tissue has been suggested to bridge the termination of the inflammatory phase and the initiation of the proliferative phase (Wu and Chen, 2014). In keeping with this proposed mechanism, paracrine factors released by PBMCs that are undergoing programmed cell death show strong anti-inflammatory properties, including reduced lipopolysaccharide-induced production of inflammatory mediators in macrophages, gingival fibroblasts, and monocyte-derived dendritic cells (Laggner et al., 2020; Panahipour et al., 2020). Furthermore, the PBMC-derived secretome diminishes dermal mast cell degranulation,

Table 1. ADMSC Secretomes

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing In Vivo	Proposed Mechanisms of Action	Reference
Wistar rats, aged 8 weeks at the beginning of experiments. Dorsal excisional wounds (full thickness), 4 weeks of high-fat diet and streptozotocin application to induce DM. Inoculation of wounds with Staphylococcus aureus and Pseudomonas aeruginosa in infected wound studies. Topical application of ADMSC-derived exosome incorporated into novel biomaterial wound-dressings.	Primary rat ADMSCs	Exosomes purified from CM, harvested after 48 hours of culture in a serum-free medium. Ascorbic acid based, antioxidant PUAO- and CPO-containing, oxygen-releasing PUAO CPO cryogel scaffolds were used as exosome carriers and wound dressing.	Accelerated wound closure at days 4, 8, and 14 of healing, increased re-epithelialization and epithelial maturation, granulation tissue formation, collagen I deposition, increased angiogenesis, and decreased oxidative stress at day 14 of healing in diabetic wounds treated with exosome-laden PUAO and PUAO CPO wound dressings. Infection- and ulceration-preventing effects, accelerated wound closure, increased collagen deposition, and re-epithelialization in infected diabetic wounds treated with exosome-laden PUAO CPO wound dressings.	Pleiotropic effects of exosome-laden PUAO cryogel formulations on infected and noninfected diabetic wounds through cytoprotection and oxidative stress reduction in a hyperglycemic milieu. Promotion of fibroblast and keratinocyte migration, collagen deposition, neovascularization, and antimicrobial effects.	Shiekh et al., 2020
ICR mice, male, approximately 30 g body weight. Dorsal excisional wounds (full thickness), Streptozotocin-induced DM. Topical administration of ADMSC-derived exosome on novel biomaterial wound dressing.	Primary murine ADMSCs	Exosomes purified from serum-supplemented CM. Exosomes were embedded in a newly developed polypeptide-based FHE hydrogel (F127/OHA-EPL) on the basis of oxidized hyaluronic acid, pluronic F127, and poly-ε-lysine.	Accelerated wound closure at days 7, 14, and 21 of wound healing; increased number of skin appendages; collagen deposition and angiogenesis; and enhanced re-epithelialization in wounds treated with exosome containing novel FHE hydrogel.	Pleiotropic, synergistic effect of self-healing, antimicrobial, stimulus-responsive exosome releasing FHE hydrogel and exosomes on wound healing.	Wang et al., 2019
Female diabetic, transgenic BKS.Cg-m+/+Lepr^{db} /J - mice (diabetic db/db and nondiabetic db/+ used as controls). Excisional dorsal feet wound (full thickness), mimicking diabetic ulcers. Systemic i.v. application of ADMSC CM.	Primary human ADMSCs	Primary ADMSCs sourced from fresh liposuction aspirates. CM harvested after 48-hour culture in a serum-free medium, with or without DFX hypoxia mimicking preconditioning. Filtration and elimination of DFX and 10× concentration of CM.	Accelerated wound closure in diabetic mice, normalization of aberrant epithelialization, and collagen deposition in diabetic mice, at day 14 of healing. Increased angiogenesis, peripheral nerve fiber density and vascular irrigation, reduced Schwann cell and neuronal apoptosis and inflammation, improved thermal and mechanical sensitivity in diabetic mice treated with ADMSC CM	Pleiotropic neurotrophic, angiogenic, and immunomodulatory effects	De Gregorio et al., 2020
Balb/c mice, aged 4 weeks. Dorsal excisional wounds (full thickness), 45% high-fat diet for 5 weeks, induction of DM with streptozotocin, and further high-fat diet for 4 weeks. S.c. Injection of exosomes at wound margins.	Primary human ADMSCs	Primary ADMSCs sourced from fresh liposuction aspirates. Exosome purification from serum-free CM harvested, after 24 hours of culture in normoxic or hypoxic conditions.	Accelerated wound closure, increased re-epithelialization, collagen deposition, and angiogenesis as well as immunomodulation in exosome-treated diabetic wounds, with superior outcomes in wounds treated with exosomes sourced from hypoxia preconditioned cells.	PI3K/Akt signaling mediated the increase of fibroblast proliferation and migration. The role of miRNAs isolated from exosomes is discussed descriptively.	Wang et al., 2021
Yorkshire Pigs Dorsal excisional wounds (full thickness), DM induction through streptozotocin injection. Local injection at wound side of either ADMSCs or topical application of ADMSCs CM.	Primary porcine ADMSCs	Harvesting of CM from ADMSCs not further described.	Accelerated wound closure, increased angiogenesis, diminished immune cell infiltration, and expression of inflammatory cytokines in the wound bed of ADMSC CM–treated wounds.	Pleiotropic angiogenic and immunomodulatory mechanisms implied.	Irons et al., 2018
Balb/c mice, male, aged 8 weeks. Dorsal excisional wounds (full thickness), Streptozotocin-induced DM. Topical application of exosomes or exosome-loaded hAAM scaffolds.	Primary human ADMSCs	Exosomes purified from CM, harvested after 48 hours of culture in a serum-supplemented medium. Exosomes were loaded onto hAAM scaffolds.	Accelerated wound closure at days 7, 10, and 14 of healing; decreased overall immune cell infiltration but increased M2 macrophage frequency in wounds; and increased angiogenesis and collagen deposition in diabetic wounds treated with exosomes or exosome-loaded hAAM, with the latter exhibiting superior effects.	Pleiotropic effects on wound healing, through enhanced angiogenesis, immunomodulation, and ECM production, with additive effects of hAAM scaffolds and ADMSC exosomes.	Xiao et al., 2021

(*continued*)

Table 1. Continued

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing In Vivo	Proposed Mechanisms of Action	Reference
Balb/c -nude mice, male, aged 8 weeks. Dorsal biopsy punch wounds. Topical secretome-loaded carbomer gel application.	Primary human ADMSCs	Conditioned media harvested after 7-day cell culture. Cell maturation in serum-containing or serum-free medium.	Accelerated wound closure, increased collagen deposition and number of skin appendages and enhanced ECM fiber alignment, as well as increased angiogenesis in ADMSC CM–treated wounds.	Pleiotropic effects implied. Proteomics analysis of secretome components is discussed descriptively	An et al., 2021
Balb/c, male, aged 8 weeks. Dorsal excisional wounds (full thickness). S.c. injection of MVs at wound margins.	Primary human ADMSCs	ASCs were purified from subcutaneous human fat tissue. MVs were purified from CM, harvested after 72-hour culturing intervals.	Accelerated wound closure at days 7, 10, and 13 of healing; increased angiogenesis; re-epithelialization; and collagen deposition at day 13 of healing.	Pleiotropic angiogenic, fibroblast, and keratinocyte proliferation and migration fostering and ECM remodeling effects, associated with Akt/ERK signaling.	Ren et al., 2019
Balb/c mice, male, aged 4 weeks. Dorsal excisional wounds (full thickness). S.c. injection of exosomes or ADMSCs at wound margins.	Primary human ADMSCs	Exosomes purified from CM of ADMSCs cultures, CM was collected every 72 hours, starting at passage 2 until passage 7, in a serum-supplemented medium	Equivalently accelerated wound closure after application of ADMSCs or ADMSC exosomes. Decreased number of infiltrating immune cells, increased collagen deposition.	Enrichment of lncRNA H19 in ADMSC exosomes. Inhibition of miR-19b by lncRNA H19, thereby disinhibition of SOX9-mediated activation of Wnt/β-catenin signaling, fostering proliferation, migration, and invasion of fibroblasts.	Qian et al., 2021
Sprague Dawley rats (270 g ∼ 290 g bodyweight). Dorsal excisional wounds (full thickness). S.c. and intradermal application of exosomes at wound margins.	Primary human ADMSCs	Exosomes purified from CM, harvested after 36 hours of culture in a serum-free medium.	Accelerated wound closure at days 3, 7, and 14 of healing; increased collagen deposition; but decreased α-SMA expression.	WNT/β-catenin signaling–mediated effect primarily on fibroblast migration and ECM formation in the context of wound healing.	Li et al., 2022

Abbreviations: ADMSC, adipose tissue–derived mesenchymal stem/stromal cell; Akt, protein kinase B; ASC, antler stem cell; CM, conditioned medium; CPO, calcium peroxide; DFX, deferoxamine; DM, diabetes mellitus; ECM, extracellular matrix; ERK, extracellular signal–regulated kinase; FHE, F127/oxidative hyaluronic acid/poly-ε-L-lysine; hAAM, human amniotic membrane; i.v., intravenous; lncRNA, long noncoding RNA; miRNA, microRNA; MV, microvesicle; PI3K, phosphatidylinositol 3-kinase; PUAO, polyurethane; S.c., subcutaneous; SMA, smooth muscle actin.

[1]In vivo wound healing model: Information on species; strain; sex; age or body weight; type of wounding; and further methods of inducing concomitant pathologies, such as DM, as well as the route through which secretome applications are given, where provided in the referenced study.

[2]Method of paracrine factor purification: Secretome compound used (e.g., exosomes) as well as details on the cell culture conditions from which the secretome compounds have been derived are summarized, where provided in the referenced study.

basophil activation (Laggner et al., 2022), dendritic cell differentiation, and the maturation and priming of T cells toward T helper Th1 and Th2 cells (Laggner et al., 2020). The secretome of stressed PBMCs inhibits NETosis (Klas et al., 2022), a distinct mode of programmed neutrophil cell death, contributing to first-line defense against pathogens by expelling chromatin and granular content (Thiam et al., 2020; Zhu et al., 2021). In line with the anti-NETotic action of PBMC-derived secretomes, paracrine factors from bone marrow–derived MSCs (Ahangar et al., 2020b) and gingival fibroblasts (Ahangar et al., 2020a) limit the excessive accumulation of neutrophil granulocytes during wound healing in vivo. However, NETosis also contributes to impaired wound healing, particularly in the context of diabetes (Wong et al., 2015). These findings highlight the need for further in vivo exploration of how therapeutic secretomes affect neutrophil biology during different stages of wound maturation.

Although cells of the adaptive immune system are less investigated, key players at the immune synapses, such as PD-1, might trigger potent immunomodulatory effects, aiding overall wound healing (Su et al., 2019). Release of exosomal PD-L1 is a systemic immunosuppressive mechanism for metastatic melanoma cell evasion of T-cell–mediated immune responses (Chen et al., 2018b; Su et al., 2019), and MSCs express and secrete PD-L1 and PD-L2 (Davies et al., 2017). However, further studies are needed to clarify whether PD-L1/PD1 signaling represents a common immunomodulatory mode of action of therapeutic secretomes.

Persisting pathogen colonization is a well-established driver of nonresolving inflammation in chronic wounds (Bjarnsholt et al., 2008; Ellis et al., 2018; Sugimoto et al., 2016), offering a target for a causal immunomodulatory approach. Secretome compounds derived from irradiated PBMCs (Kasiri et al., 2016), oral mucosal epithelial cells (Sjöqvist et al., 2019), and adipose tissue–derived mesenchymal stem/stromal cells (ADMSCs) incorporated in oxygen-releasing antioxidant wound dressing (Shiekh et al., 2020) or polypeptide-based F127/oxidative hyaluronic acid/poly-ε-L-lysine hydrogel (Wang et al., 2019) show antimicrobial properties. Furthermore, the secretomes of irradiated PBMCs and MSCs contain substantial amounts of antimicrobial peptides, such as calprotectin and cathelicidin (Harman et al., 2017; Kasiri et al., 2016). These factors could underlie their antimicrobial effects, but many studies have used antimicrobial wound dressings (Shiekh et al., 2020; Wang

Table 2. UCMSC and AMSC Secretomes

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing in Vivo	Proposed Mechanisms of Action	Reference
Balb/c mice, male, aged 8 weeks. Dorsal excisional wounds (full thickness). S.c. injection of exosomes at wound margins.	Primary human UCMSC	Exosomes purified from serum-free CM after 48 hours of culture, from cultures in normoxic or hypoxic conditions.	Accelerated wound closure, enhanced skin cell proliferation, inhibited cell apoptosis, and increased angiogenesis, with most pronounced effects in wounds treated with exosomes purified from hypoxia-pretreated UCMSC.	Suppression of tumor protein p53 inducible nuclear protein 1 (TP53INP1) through miR-125b, thereby inducing cytoprotective effects in the hypoxic wound microenvironment.	Zhang et al., 2021d
Balb/c, female, aged 6–8 weeks. Dorsal excisional wounds (full thickness). Topical application of CM.	Primary human UCMSC	Collection of serum-free CM after 12 hours of culture, with or without prior inflammatory pretreatment of UCMSCs with IFN-γ and TNF-α. In some experiments, cells were additionally transfected with VEGFC-specific small interfering RNAs.	Accelerated wound closure at days 3–6 of healing, enhanced re-epithelialization, angiogenesis, and fostered collagen constriction in wounds treated with CM, harvested from inflammatory-pretreated UCMSCs.	IFN-γ– and TNF-α–mediated upregulation of VEGFC in UCMSCs, leading to VEGFC-mediated increase in angiogenesis, re-epithelialization, and collagen constriction.	Zhu et al., 2020
Wistar rats, male, aged 6 weeks. Dorsal full-thickness thermal burn model. Systemic i.v. application of exosomes, with or without temporary external magnetic guidance of Fe_3O_4 nanoparticle labeled exosomes.	Primary human UCMSC	Exosomes purified from CM, harvested after 48 hours of culture in a serum-supplemented medium. Some UCMSCs were labeled with superparamagnetic Fe_3O_4 nanoparticles, before exosome purification.	Accelerated wound closure at 1, 3, and 5 weeks of healing, increased re-epithelialization, collagen deposition, and angiogenesis at 5 weeks of healing, with superior effects of magnetically guided superparamagnetic nanoparticle labeled exosomes.	Increased targeting efficacy of systemically administered externally guided, superparamagnetic nanoparticle labeled exosomes, eliciting pleiotropic effects at the wound side. Cyclin A2 and D1, VEGFA, and CXCl2 upregulation and increased endothelial proliferation; migration; and tube formation are discussed as putative mechanisms of action.	Li et al., 2020
C57BL/6 mice, male, aged 8 weeks. Dorsal skin thermal burn injury model. S.c. application of AMSCs or AMSC CM at wound margins.	Primary human AMSC	CM was harvested after 48 hours of culture in serum-free, high glucose medium, final CM product was concentrated 10-fold.	Accelerated wound closure at 7, 14, and 21 weeks of healing, enhanced re-epithelialization, reduced number of apoptotic and increased number of proliferating skin cells, and increased angiogenesis, in both AMSCs and AMSC CM treated groups.	Prevention of heat stress–induced reduction of PI3K, Akt, mTOR, Gsk3β, and β-catenin phosphorylation, through AMSCs and their paracrine factors, eliciting antiapoptotic effects through PI3K/Akt-mediated signaling, associated with an increase in antiapoptotic protein Bcl-2 and decreased proapoptotic protein BAX expression. PI3K/Akt signaling mediated the phosphorylation of GSK3β and β-catenin, and PCNA upregulation was associated with keratinocyte and fibroblast proliferation. The role of putative PI3K/Akt signaling–activating paracrine factors, enriched in AMSC CM, such as PAI-1, G-CSF, periostin, and TIMP-1, is discussed.	Li et al., 2019a, 2019b
Sprague Dawley rats, male, 200–240 g body weight. Second-degree thermal burn injury model. S.c. injection of exosomes at wound margins.	Primary human UCMSC	Exosomes purified from serum-supplemented CM after 48 hours of culture of either untreated or transfected UCMSC.	Accelerated wound closure at day 13 of healing and increased angiogenesis in wounds treated with UCMSC exosomes. Effects were exerted by overexpression of angiopoietin-2 in UCMSCs before secretome harvest and exosome isolation.	Angiopietin-2–mediated proangiogenic effects.	Liu et al., 2021

(*continued*)

Table 2. Continued

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing in Vivo	Proposed Mechanisms of Action	Reference
ICR and Balb/c nude mice. Dorsal excisional wounds (full thickness). Topical application of exosomes in HydroMatrix.	Primary human UCMSC	Exosomes purified from serum-supplemented CM after 48 hours of culture.	Accelerated wound closure at days 14 and 25 of healing, reduced scar formation, and myofibroblast accumulation at day 25 of healing.	Suppression of TGF-β2/SMAD2 signaling through miR-21, -23a, -125b, and -145, thereby inhibiting myofibroblast formation and excessive α-SMA and collagen deposition during remodeling.	Fang et al., 2016
Sprague Dawley rats, female, aged 6–8 weeks. Dorsal excisional wounds (full thickness). I.v. application of exosomes through the tail vein.	Primary human UCMSC	Exosomes purified from serum-free CM after 48 hours of culture.	Accelerated wound closure at 1 and 2 weeks of healing, reduced scar formation and myo- and collagen fiber accumulation, increased number of cutaneous appendages, and increased angio- and neuritogenesis at 2 and 4 weeks of healing.	Suppression of TGF-β2/SMAD2 signaling through miR-21-5p and miR-125b-5p, thereby inhibiting myofibroblast formation and excessive α-SMA upregulation and collagen deposition during remodeling.	Zhang et al., 2021a
Sprague Dawley rats, female, aged 8 weeks, 200 g body weight. Dorsal excisional wounds (full thickness). S.c. injection of exosomes or AMSC cell suspensions at wound margins.	Primary human AMSC	Exosomes purified from CM, harvested after 48 hours of culture in a serum-supplemented medium.	Accelerated wound closure at days 7 and 14 of healing, increased number of skin appendages at day 28 of healing, enhanced reepithelialization, reduced collagen fiber deposition and myofibroblast accumulation at day 28 of healing, enhanced angio- and neuritogenesis at days 14 and 28 of healing.	Let-7–, miR-21–, miR-22–, miR23a–, and miR-27a–mediated inhibition of the TGF-β1–TGF-βR1 Smad signaling, inhibiting myofibroblast differentiation, thereby promoting scarless wound healing.	Zhang et al., 2021b

Abbreviations: AMSC, amniotic mesenchymal stem/stromal cell; Akt, protein kinase B; CM, conditioned medium; G-CSF, granulocyte colony-stimulating factor; i.v., intravenous; PI3K, phosphatidylinositol 3-kinase, S.c., subcutaneous; SMA, smooth muscle actin; UCMSC, umbilical cord–derived mesenchymal stem/stromal cell.

[1]In vivo wound healing model: information on species; strain; sex; age or body weight; type of wounding; and further methods of inducting concomitant pathologies, such as diabetes mellitus, as well as the route through which secretome applications are given, where provided in the referenced study.

[2]Method of paracrine factor purification: secretome compound used (e.g., exosomes) as well as details on the cell culture conditions from which the secretome compounds have been derived are summarized, where provided in the referenced study.

et al., 2019), complicating conclusions about the contribution of the incorporated secretome product.

NEOVASCULARIZATION: A COMMON TARGET OF THERAPEUTICALLY APPLIED CELL SECRETOMES

Pre-existing microvasculopathy and deficient neovascularization after wounding are well-established contributors to chronically impaired wound healing, in particular in patients with diabetes mellitus and obesity (Cheng and Ma, 2015). Angiogenesis promotion appears to be a common mode of action for the wound-healing effects of various cell secretomes because diverse stem cell and non–stem cell sources release many shared paracrine factors implicated in angiogenesis. For instance, VEGFs, a prototypical angiogenesis-promoting family of secreted polypeptides, are enriched in the secretomes of MSCs derived from bone marrow (Ahangar et al., 2020b), adipose tissue (An et al., 2021), and the umbilical cord (Zhu et al., 2020) as well as in the secretomes of gingival fibroblasts (Ahangar et al., 2020a), human adipose liquid extract (He et al., 2019), and PBMCs (Simader et al., 2019). Different cell secretomes also induce endogenous VEGF production in target-cell populations in the wounded area. For instance, exosomes derived from MSCs (Li et al., 2020) or macrophages (Li et al., 2019b) and adipose stem cell–derived microvesicles (Ren et al., 2019) upregulate VEGFA in endothelial cells. Moreover, the secretome of irradiated PBMCs potently upregulates VEGFA in monocytes (Copic et al., 2022) and ex vivo aortic rings (Wagner et al., 2018).

Several other angiogenesis-related GFs and cytokines appear to be present in secretomes from various cell populations. Among them, angiopoietins are canonical angiogenesis-modulating proteins (Karar and Maity, 2011) enriched in the secretome of MSCs (Li et al., 2019a; Liu et al., 2021) and gingival fibroblast populations (Ahangar et al., 2020a). Similarly, the angiogenesis-related HGF and PDGF are important components of MSC secretomes, lipid extracts derived from primary human emulsified adipose tissue, and gingival fibroblasts, whereas the secretomes of irradiated PBMCs, gingival fibroblasts, and MSCs include the angiogenic cytokine IL-8 (Ahangar et al., 2020a, 2020b; He et al., 2019; Li et al., 2019a; Simader et al., 2019).

Considering this overlap in GFs, diverse cell secretome products may share components of proangiogenic intracellular signaling cascades. For instance, upon binding, VEGF elicits dimerization and autophosphorylation of their respective target receptor tyrosine kinases (RTKs), activating various intracellular signaling molecules, such as phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), p38 MAPK, and phosphoinositide phospholipase C-γ (Hoeben et al., 2004; Karar

Table 3. BMMSC Secretomes

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing In Vivo	Proposed Mechanisms of Action	Reference
Sprague Dawley rats, 200–250 g body weight. Dorsal excisional wounds (full thickness) DM induction through Streptozotocin injection. S.c. injection of exosomes at wound margins.	Primary rat BMMSC	Exosomes purified from serum-supplemented conditioned medium after 48 hours of culture. Pretreatment of cells with pioglitazone before CM harvest and exosome purification in some experiments.	Accelerated wound closure, increased blood perfusion of the wound area, angiogenesis, and collagen deposition.	Enhanced wound healing through increased angiogenesis, through activation of the PI3K/Akt/eNOS pathway.	Hu et al., 2021
Balb/c, female, aged 10–12 weeks. Dorsal excisional wounds (full thickness). Intradermal injection of MAPC CM at wound margins.	Human MAPCs derived from bone marrow aspirates.	CM harvested after 24 hours of culturing under hypoxic conditions, in serum-free DMEM, filtration, 20× concentration of CM.	Reduction of average wound area and width at days 3, 7, and 14 of healing, accelerated re-epithelialization and resolution of inflammation, enhanced angiogenesis, and collagen I and III production.	Pleiotropic effects, through reduction of neutrophil infiltration and persistence, macrophage M2-like polarization, enhanced fibroblast collagen I and II biogenesis, and angiogenesis. Putative involvement of ECM proteins (e.g., MMP1), GFs (e.g., VEGF), adhesion molecules (VCAM-1), and inflammatory cytokines (MCP-1), all of which are enriched in the secretome, are discussed.	Ahangar et al., 2020b
C57BL/6J mice, aged 10–12 weeks. Dorsal excisional wounds (full thickness). Intradermal injection of exosomes or BMMSCs at wound margins.	Primary murine BMMSC	Exosome purification from the serum of adult (aged 12 weeks) and neonatal (aged 14 days) mice. Priming of BMMSCs with adult or neonatal-derived serum exosomes for 24 hours. Wash out of serum exosomes. Further culture of BMMSCs for 48 hours. Exosome purification from the conditioned medium of serum-primed and nonprimed BMMSCs.	Accelerated wound closure at days 7 and 14 of healing, enhanced re-epithelialization, and increased angiogenesis in exosome-treated wounds, with superior effect of exosomes derived from BMMSCs primed with neonatal mice serum exosomes.	Enhanced proliferative and sprouting capacities of endothelial cells, induced by BMMSC exosomes, harvested after *ex vivo* priming with neonatal serum secretome. The contributions of Akt and eNOS phosphorylation to the angiogenic effects are discussed.	Qiu et al., 2020
Sprague Dawley rats, 250–300 g body weight. Dorsal excisional wounds (full thickness), DM induction through Streptozotocin injection. S.c. injection of exosomes at wound margins.	Primary human BMMSC	Exosomes purified from conditioned medium, harvested after 48 hours culture in serum-supplemented medium, with or without DFX hypoxia mimicking preconditioning.	Accelerated wound closure at days 7 and 14 of healing, increased angiogenesis, decreased scar width after 14 days of healing in diabetic rats, most pronounced after application of exosomes from DFX pretreated BMMSC.	Enrichment of miR-126 in exosomes harvested from DFX pre-treated BMMSCs. Suppression of PTEN by miR-126, thereby promoting Akt phosphorylation and thus PI3K/Akt signaling, leading to increased angiogenesis.	Ding et al., 2019

(*continued*)

Table 3. Continued

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing In Vivo	Proposed Mechanisms of Action	Reference
New Zealand albino rabbits, female, 3–4 kg body weight. Circular full-thickness rabbit ear excisional wounds. Injection into lesion side.	Primary murine BMMSC	Conditioned media was harvested from BMMSC, BMC, or BMC-stimulated BMMSC after 48 hours of culture. CM was sterile filtered.	Accelerated wound closure and re-epithelialization in wounds treated with CM from BMC-stimulated BMMSCs, decreased formation of hypertrophic scar tissue, α-SMA, and collagen I and III expressions after 35 days of healing, in wounds treated with CM from BMC-stimulated MSCs.	Pleiotropic antifibrotic effects of BMC-induced BMMSC CM, through the regulation of ECM turnover, inhibition of myofibroblast generation, and reduction of profibrotic gene expression.	Hu et al., 2019
Sprague Dawley rats, female, aged 8 weeks. Dorsal excisional wounds (full thickness). Topical application of exosomes.	Primary human BMMSC	Exosomes purified from serum-free conditioned medium after 48 hours of culture.	Reduction of average wound area at days 4–16 of healing. Increased number of cutaneous appendages and increased angiogenesis at 16 days of healing.	Putative promotion of wound healing, through modulating TGF-β/Smad signaling. Decreased expression of TGF-β1, Smad2, Smad3, and Smad4 but increased TGF-β3 and Smad7 levels, induced by exosome treatment.	Jiang et al., 2020

Abbreviations: Akt, protein kinase B; BMC, bone marrow concentrate; BMMSC, bone marrow–derived mesenchymal stem/stromal cell; CM, conditioned medium; DFX, deferoxamine; DM, diabetes mellitus; eNOS, endothelial nitric oxide synthase; MAPC, multipotent adult progenitor cell; MCP-1, monocyte chemoattractant protein 1; miR, microRNA; MMP, matrix metalloproteinase; PI3K, phosphatidylinositol 3-kinase; S.c., subcutaneous.

[1]In vivo wound healing model: information on species; strain; sex; age or body weight; type of wounding; and further methods of inducing concomitant pathologies, such as diabetes mellitus, as well as the route through which secretome application is given, where provided in the referenced study.

[2]Method of paracrine factor purification: secretome compound used (e.g., exosomes) as well as details on the cell culture conditions from which the secretome compounds have been derived are summarized, where provided in the referenced study.

Table 4. Secretomes Derived from Dermal and Gingival MSCs and Non-MSC Stem Cell Populations

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing In Vivo	Proposed Mechanisms of Action	Reference
C57BL/6 mice, female, aged 3 months. Dorsal excisional wounds (full thickness), induction of DM through streptozotocin injection. S.c. injection of exosomes at wound margins.	Primary human USC	Exosomes purified from serum-supplemented conditioned medium, after 48 hours of culture. Some cultures were transfected with shRNAs against the target: deleted in malignant brain tumors 1 (DMBT1) (shDMBT1 #1).	Accelerated wound closure, increased re-epithelialization, the proliferation of skin cells, angiogenesis, and decreased scar formation. Less pronounced effect of exosomes sourced from shDMBT#1 transfected cells.	Pleiotropic effects of USC exosomes on angiogenesis, proliferation, and migration of keratinocytes and fibroblasts, largely mediated by DMBT1.	Chen et al., 2018a
Sprague Dawley rats, female, aged 8 weeks. Full-thickness skin defect wound. Topical application of exosomes in HydroMatrix.	EPSCs	Exosomes purified from a serum-free conditioned medium, after 48 hours of culture.	Reduction of average wound area at days 7 and 14 of healing. Modulation of skin remodeling 4 weeks after wounding. Increased number of skin appendages, fewer myo- and collagen fibers, smaller Col I/III ratio, increased angio- and neuritogenesis.	Reduced scar formation through TGF-β suppression through miR-425-5p and miR-142-3p, thus a reduction in SMAD2 dependent α-SMA and collagen I expression.	Duan et al., 2020
Sprague Dawley rats, male, 280–320 g body weight. Dorsal excisional wounds (full thickness), high sucrose and high-fat diet for 10 weeks and streptozocin injection to induce DM. Topical application of exosomes in chitosan/silk hydrogel matrix.	Primary human GMSCs	Exosomes purified from conditioned medium, harvested after 48 hours of culture in serum-supplemented medium. Exosomes were loaded onto a chitosan/silk hydrogel sponge.	Accelerated wound closure at weeks 1 and 2 of healing, increased re-epithelialization, collagen deposition, angiogenesis, and neuritogenesis.	Pleiotropic effect on wound healing through re-epithelialization, collagen deposition, angiogenesis, and neuritogenesis.	Shi et al., 2017
C57BL/6 mice, aged 4–5 months. Dorsal excisional wounds (full thickness). Topical application of CM in hydrogel or without carrier gel.	Primary human SDMSCs	SDMSCs sourced from human facelift skin fragments. CM from a culture of SDMSCs over 10 days was harvested, sterile filtered, and concentrated.	Increased angiogenesis, but no significant beneficial effect on overall wound closure, reepithelialization, or leukocyte infiltration *in vivo*.	Proangiogenic effect, putatively through the enrichment of proangiogenic proteins, as suggested by mass spectrometry proteomics analysis.	Robert et al., 2019
Sprague Dawley rats, aged 6–8 weeks. Dorsal excisional wounds (full thickness). Topical application of UBMSCs and ASC CM.	Primary ASCs	Purification of ASCs from deer antlers, isolation of human UCMSCs from umbilical cords. Harvest and filtration of CM after 48 hours of culture of either ASCs or UCMSCs in serum-free medium.	Reduction of average wound area at 4, 8, 12, and 16 days of healing in UCMSC CM– and ASC CM–treated wounds. Increased angiogenesis and number of appendages, decreased the number of proliferating cells after 16 days of healing.	Increase in Col3a1/ Col1A2, TGF-β3/ TGF-β1, MMP1/TIMP1, MMP3/ TIMP1 mRNA expression ratios. Putative fetal transcriptional profile and function of wound fibroblasts.	Rong et al., 2019

Abbreviations: ASC, antler stem cell; CM, conditioned medium; Col, collagen, DM, diabetes mellitus, EPSC, epidermal stem cell; GMSC, gingival mesenchymal stem cell; MMP, matrix metalloproteinase; S.c., Subcutaneous; SDMSC, skin-derived multipotent stem/stromal cell; shRNA, short hairpin RNA, SMA, smooth muscle actin; UCMSC, umbilical cord mesenchymal stem cell; USC, urine-derived stem cell.

[1]In vivo wound healing model: information on species; strain; sex; age or body weight; type of wounding; and further methods of inducing concomitant pathologies, such as diabetes mellitus, as well as the route through which secretome application are given, where provided in the referenced study.

[2]Method of paracrine factor purification: secretome compound used (e.g., exosomes) as well as details on the cell culture conditions from which the secretome compounds have been derived are summarized, where provided in the referenced study.

and Maity, 2011). Indeed, activation of PI3K and phosphorylation of Akt/PKB and its targets, such as endothelial nitric oxide synthase, are described as crucial for mediating the proangiogenic and cytoprotective potential of the secretome of bone marrow–derived stem cells, MSCs derived from urine and adipose tissue, and leukocytes (Chen et al., 2018; Hu et al., 2021; Li et al., 2019b; Lichtenauer et al., 2011; Qiu et al., 2020; Ren et al., 2019). In addition to GF-initiated activation of the PI3K–Akt/PKB pathway, MSC exosomes appear to modulate this pathway downstream of RTK interactions by

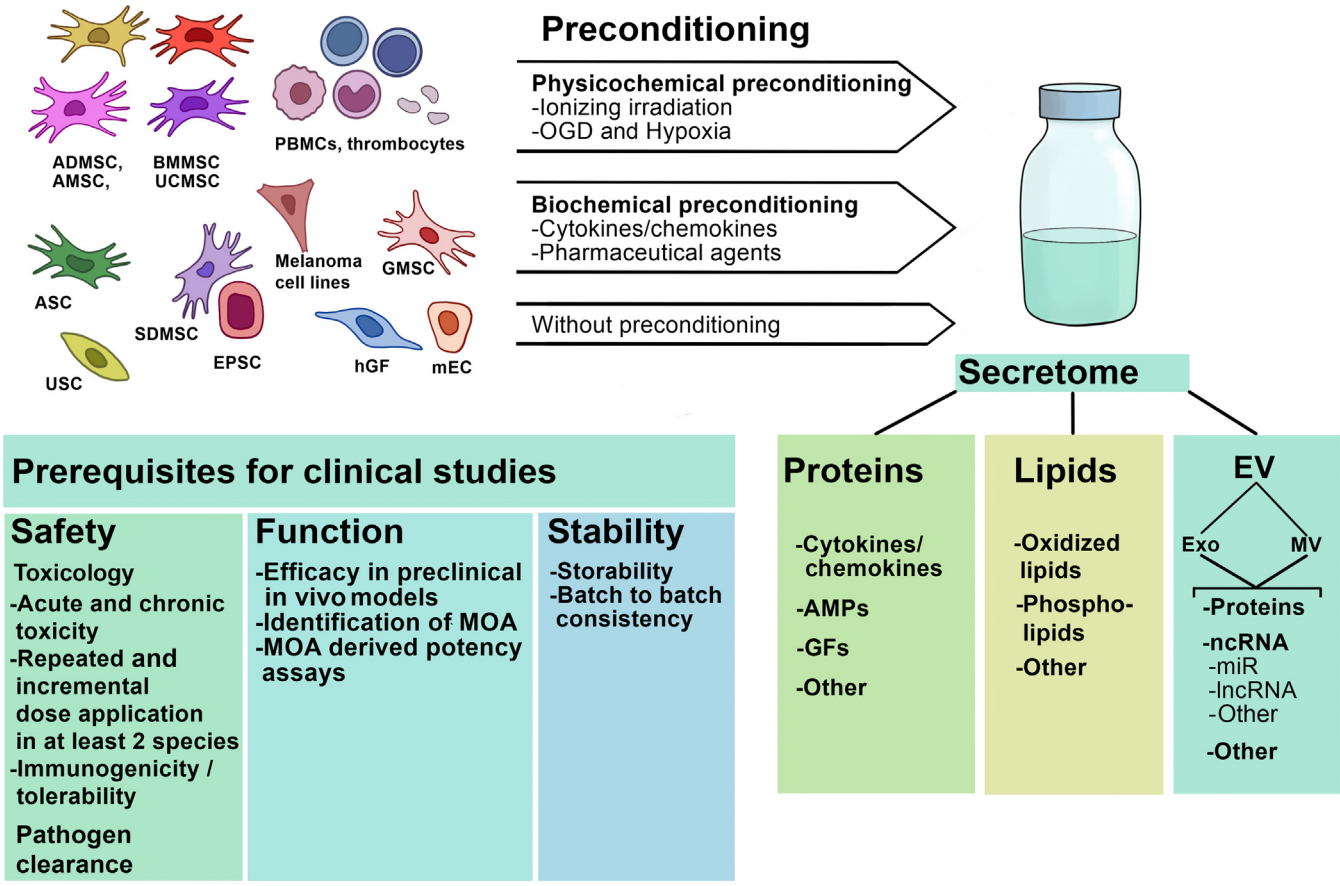

Figure 1. Overview of the generation of therapeutic secretomes and its components and regulatory prerequisites for clinical studies. The upper row of the figure depicts cellular sources of secretomes and established preconditioning strategies. Selected regulatory demands and considerations for the advancement of secretome-based therapies into clinical trials are depicted in the left lower row, and the most investigated secretome fractions are listed in the right lower row. ADMSC, adipose tissue–derived mesenchymal stem/stromal cell; AMP, antimicrobial peptide; AMSC, amniotic mesenchymal stem/stromal cell; ASC, antler stem cell; BMMSC, bone marrow–derived mesenchymal stem/stromal cell; EPSC, epidermal stem cell; Exo, exosome; GMSC, gingival mesenchymal stem cell; hGF, human gingival fibroblast; lncRNA, long noncoding RNA; mEC, mucosal epithelial cell; miR, microRNA; MOA, mechanism of action; MV, microvesicle; ncRNA, noncoding RNA; OGD, oxygen glucose deprivation; SDMSC, skin-derived multipotent stem/stromal cell; UCMSC, umbilical cord–derived mesenchymal stem/stromal cell; USC, urine-derived stem cell.

downregulating phosphatase and tensin homolog through microRNAs (miRNAs), such as miR-126 and miR-21-3p (Hu et al., 2018). Extracellular signal–regulated kinase is another cell survival- and proliferation-associated signaling hub downstream of various RTKs, and its phosphorylation is an event elicited by various cell secretomes (Hoeben et al., 2004; Hu et al., 2018; Mildner et al., 2013; Ren et al., 2019).

These signaling pathways culminate in proliferative, vessel-sprouting, and migration-inducing effects in endothelial cells (Tables 1–5). Moreover, various MSC and non–stem cell secretomes are enriched in MMPs (e.g., MMPs 1, 2, 3, and 9) or elicit MMP upregulation in the wound tissue, implicating MMP involvement in the ECM reorganization required for neovascularization during wound healing and subsequent wound remodeling (Ahangar et al., 2020a, 2020b; Ren et al., 2019; Rong et al., 2019; Simader et al., 2019). Although a limited and temporally restricted elevation of MMPs might foster angiogenesis, prolonged and excessive MMP production is a hallmark of chronic wounds (Krishnaswamy et al., 2017).

THE INFLUENCE OF CELL SECRETOMES ON TISSUE REMODELING AND SCAR FORMATION

Maturation of granulation tissue into scar tissue marks the most common endpoint of dermal wound healing (Rodrigues et al., 2019). The composition and function of the newly formed tissue range from essentially scarless regeneration, as in early fetal skin and oral mucosa, to the pathological formation of hypertrophic scars and keloids (Moore et al., 2018; Sjöqvist et al., 2019). Signaling through the TGFβ–SMAD pathway is closely linked to ECM protein synthesis and deposition by dermal fibroblasts and to myofibroblast differentiation, all involved in physiological wound healing and in hypertrophic scarring (Penn et al., 2012). This influence has attracted growing interest in the putative influence of various cell secretomes on TGFβ–SMAD signaling during wound healing. Paracrine factors secreted by epidermal, umbilical cord–, bone marrow–, and human amniotic fluid–derived stem cells diminish profibrotic TGFβR1–SMAD2 signaling in dermal fibroblasts, reducing excessive collagen I deposition and myofibroblast differentiation (Duan et al., 2020;

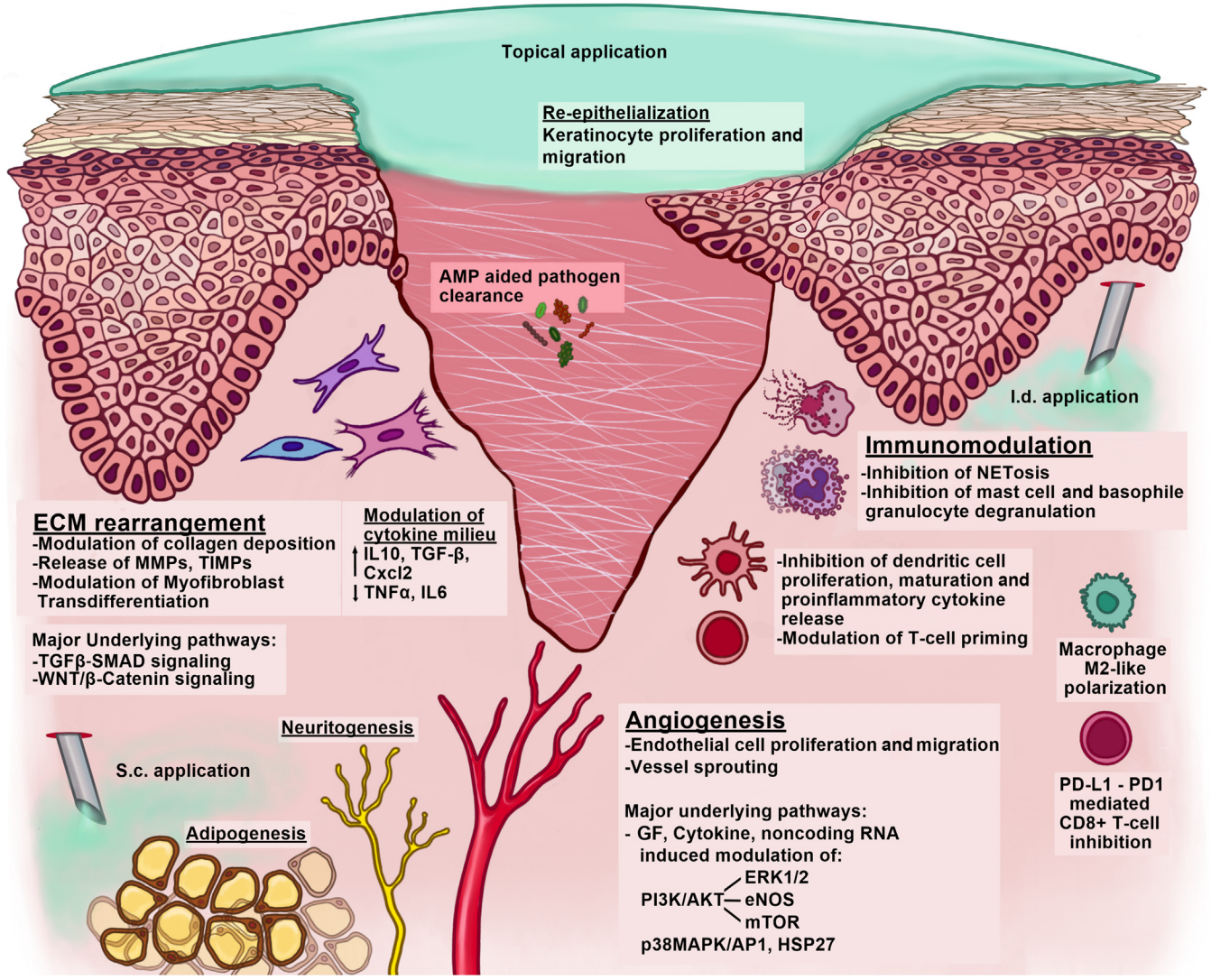

Figure 2. Major mechanisms of action underlying the therapeutic effect of secretomes on dermal wound healing. Depiction of common routes of secretome application, important mechanisms of action, and corresponding signaling pathways underlying the wound healing improving effects of topically, intradermally, or subcutaneously applied therapeutic secretomes. ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal–regulated kinase; i.d., intradermal; MMP, matrix metalloproteinase; S.c., subcutaneous.

Fang et al., 2016; Hu et al., 2019; Zhang et al., 2021a, 2021b). Myofibroblast differentiation and collagen accumulation are described as profibrotic during the remodeling phase of wound healing, although these processes constitute pivotal steps during the proliferative phase (Rodrigues et al., 2019). Of interest, paracrine factors derived from deer velvet antler tissue, which exhibits scarless tissue regeneration, also appear to elicit their antifibrotic effects during wound healing through TGFβR1–SMAD2 signaling inhibition (Rong et al., 2019; Zhang et al., 2021c).

Exosomes released by different cell populations used in generating wound healing therapeutics share a common set of noncoding RNAs with antifibrotic properties. Mechanistically, these exosomal miRNAs, including miR-21-5p, miR-125b-5p, and let-7, target and downregulate either TGFβR1 or TGFβR2 in fibroblasts, inhibiting SMAD2 phosphorylation and translocation and thus transcription of its downstream targets (2021a, 2021b, 2021c). However, contradicting this implied profibrotic and potentially deleterious role of TGFβR1–SMAD2 signaling in wound remodeling, Shi et al. (2021) reported that TGF-β–enriched platelet exosomes upregulate TGF-β targets beyond SMAD2, such as RAS, RHOA, MKK3, periostin, and p38, resulting in enhanced wound healing and restoration of dermal architecture in vivo.

Apart from TGFβ–SMAD signaling, the WNT/β-catenin pathway has been implicated as a key target of ADMSC-derived exosomes. In one study, the application of these exosomes upregulated WNT2b and β-catenin expression in the wound bed, accompanied by enhanced collagens I and III deposition and improved wound closure (Li et al., 2022). Of note, the concomitant application of the selective WNT/β-catenin inhibitor XAV939 diminished these effects. Mechanistically, the long noncoding RNA H19, which is enriched in adipose-derived stem cell exosomes, might mediate these effects by binding miR-19b and thus disabling miR-19b downregulation of SOX9 translation and activating the Wnt/β-catenin pathway (Qian et al., 2021).

Table 5. Secretomes Derived from Non−Stem Cell Populations or Mixed Populations

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing In Vivo	Proposed Mechanisms of Action	Reference
C57BL/6J mice, aged 3 months. Dorsal excisional wounds (full thickness). Topical administration of PBMC secretome in hydrophilic cream-based emulsion.	Primary human PBMCs	CM harvested after 24 hours of PBMC culture. CM was dialyzed, filtered, and lyophilized.	Accelerated wound closure, increased re-epithelialization, and angiogenesis at 3 and 7 days of healing.	Pleiotropic angiogenic and fibroblast and keratinocyte migration promoting effects. Putative involvement of CREB, ERK1/2, cJun, Akt, and HSP27 signaling.	Mildner et al., 2013
Sprague Dawley rats, male, 422 ± 30 g body weight. Epigastric flap wound healing model. Topical administration of PBMC secretome in fibrin sealant.	Primary human PBMCs	CM harvested after 24 hours of culturing 60 Gy irradiated PBMCs. CM was sterile filtered, viral clearance was performed using methylene blue plus light treatment, additional γ-irradiation after lyophilization of secretome product was performed.	Decreased tissue necrosis, seroma formation, improved flap adherence in the transitional and ischemic flap zones, decreased tissue edema formation in the transitional zone, improved recovery of blood perfusion in the ischemic zone, at day 7 after injury, and increased overall angiogenesis in flaps treated with PBMC secretome supplemented fibrin.	Pleiotropic angiogenesis-driven effects.	Hacker et al., 2020
DanBred pigs, female, aged 12 weeks. Thermal full-thickness burn injury and skin grafting model. Topical administration of PBMC secretome in a hydrogel-based vehicle.	Primary human PBMCs	CM harvested after 24 hours of culture of either 60 Gy irradiated or non-preconditioned PBMCs. CM was sterile-filtered and lyophilized.	Enhanced re-epithelialization, increased angiogenesis, and blood vessel maturation in burn wounds treated with the secretome of irradiated, apoptotic PBMCs.	Pleiotropic effect on angiogenesis and re-epithelialization.	Hacker et al., 2016
Sprague Dawley rats. Dorsal excisional wounds (full thickness): DM induction through streptozotocin injection. S.c. injection of exosomes at wound margins.	RAW 264.7 cells	Exosomes purified from serum-supplemented CM after 24 hours of culture.	Accelerated wound closure, increased collagen fiber deposition, increased angiogenesis, and decreased inflammation in the wound area in diabetic rats treated with RAW 264.7 exosomes.	Pleiotropic angiogenic and immunomodulatory effects. Involvement of VEGFA and pAkt signaling is discussed.	Li et al., 2019b
C57BL/6 mice, male, aged 12 weeks. Dorsal excisional wounds (full thickness). S.c. injection of exosomes at wound margins.	Human UCB plasma	Exosomes purified from human umbilical cord blood plasma.	Accelerated wound closure 2, 5, and 8 days after wounding, increase re-epithelialization and angiogenesis as well as decreased scar formation.	Inhibition of PTEN and SPRY1 through miR-21-3p and concomitantly increased phosphorylation-dependent PI3K/Akt and ERK1/2 signaling. In sum, proangiogenic modulation of endothelial cells and fostering of fibroblast motility and proliferation.	Hu et al., 2018
New Zealand white rabbits, female, aged approximately 6 months. Circular full-thickness excisional wounds in rabbit ear, induction of ischemia by ligation of vascular bundles of the ear. Topical application of platelet exosomes in fibrin sealant.	Primary human platelets	PEP (Rion LLC, Rochester, MN) loaded into injectable surgical fibrin sealant (TISSEEL).	Accelerated wound closure, restoration of cutaneous homeostasis, decreased rate of ulceration at 28 days of healing, decreased scar formation, improved tensile strength, and proangiogenic and immunomodulatory effects observed in wounds treated with PEP-loaded TISSEEL.	Delivery of TGF-β to wound side, eliciting the upregulation of SMAD2, RAS, and MKK3 expression in both Keratinocytes and fibroblasts, and RHOA, p38, periostin, and ERK1 and TAK1 expression in keratinocytes and fibroblasts respectively, fostering epithelial trans-differentiation, fibroblast activation and collagen synthesis. Pleiotropic in vivo effects on wound healing linked to the upregulation of transcripts related to ECM organization, fibroblast and keratinocyte proliferation and metabolic state of skin cells, immunomodulation, and angiogenesis in wound tissue in vivo.	Shi et al., 2021

(*continued*)

Table 5. Continued

In Vivo Wound Healing Model[1]	Cell Source	Method of Paracrine Factor Purification[2]	Effect of Secretome on Wound Healing In Vivo	Proposed Mechanisms of Action	Reference
Balb/c mice, female, aged 10–12 weeks. Dorsal excisional wounds (full thickness). Intradermal injection of hGFs and hGF-CM at wound margins.	Primary hGFs	CM, harvested after 24 hours of culturing in serum-free medium, sterile filtration, 20× concentration.	Reduction of average wound area and width at days 3, 7, and 14 of healing, accelerated re-epithelialization and resolution of inflammation, enhanced angiogenesis, and collagen deposition.	Pleiotropic effects through the dampening of inflammatory response (e.g., increased macrophage M2 polarization, a decrease of TNFa in the wound, an increase of IL-10 in the wound bed, migration, proliferation, and collagen deposition of dermal fibroblasts) through enrichment of the secretome in ECM proteins (e.g., MMP-2), GFs (e.g., VEGF, FGF-2, Ang-1&2), adhesion molecules (VCAM-1), and cytokines (MCP-1, IL-6, IL-8, IL-23) and Arginase.	Ahangar et al., 2020a
Sprague Dawley rats, 248 ± 26 g body weight. Dorsal Excisional wounds (full thickness). Topical application of exosomes.	Primary human mucosal epithelial cells	CM was collected over a period of 16 days of culture, supplemented with or without 5% autologous serum. Exosomes were purified from filtered and CM.	Reduced wound width at 6 and 17 days of healing, in wounds treated with exosomes harvested from serum-conditioned epithelial cells.	In vitro reduced proliferation of fibroblasts, but upregulation of HGF, VEGFA, FGF2, and CTGF expression and HGF release. Moderate inhibitory effect of exosomes on Staphylococcus aureus growth. Characterization of exosome adhesion and migration into the wound, putative modulation of fibroblast proliferation and GF release, fostering wound healing.	Sjöqvist et al., 2019
Sprague Dawley rats, aged 6–8 weeks. Dorsal excisional wounds (full thickness). Topical application of velvet antler peptides in matrigel into wound margins.	Lyophilized velvet antler powder	Enzymatic digestion of velvet antler peptides to generate extract	Accelerated wound closure at 7, 14, and 21 days of healing. Increased angiogenesis, skin cell proliferation, and number of appendages, decreased collagen deposition, and COL1A2/COL3A1 ratio at 28 days of healing.	Modulation of wound remodeling and reduced scar formation through the inhibition of TGF-β1/SMAD2 signaling reducing excess α-SMA, and collagen I generation	Zhang et al., 2021c
C57BL/6 mice, female, aged 4–6 weeks. Dorsal excisional wounds (full thickness). Topical application of lipid extracts from emulsified human adipose tissue.	Primary Human adipose tissue	Adipose tissue was obtained from liposuctions, washed, concentrated, physically emulsified with PBS, and centrifuged. The liquid portion from emulsified adipose tissue (ALE) was collected and filtered.	Reduction of average wound area at 7, 11, and 14 days of healing, increased angiogenesis and adipogenesis.	Angio- and adipogenesis induction. The enrichment of proangiogenic proteins, as suggested by mass spectrometry proteomics analysis is discussed descriptively.	He et al., 2019
Balb/c mice, 18–25 g body weight. Dorsal excisional wounds (full thickness). Topical application of exosomes in hydrogel matrix.	SK-MEL-5 cells (human melanoma cell line) and B16F10 cells (mouse melanoma cell line)	For in vivo assays, exosomes were purified from serum-supplemented CM, after 48 hours of culture of PD-L1 transfected, IFNγ preconditioned, or control B16F10 cells.	Accelerated wound closure at day 10 of healing, decreased immune cell infiltration and expression of IL-6, TNF-α and Granzym B at wound side, decreased number of CD8+ cells in adjacent lymph nodes and increased migration, maturation, and proliferation of epidermal and dermal cells in mice treated with exosomes, derived from PD-L1 expressing, or IFNγ stimulated B16F10 cells	PD-L1 – PD1 mediated suppression of T-cell activation, fostering wound healing through local and systemic immunomodulatory effects, during the inflammatory phase of wound healing.	Su et al., 2019

Abbreviations: Akt, protein kinase B; Ang, angiopoietin; CM, conditioned medium; Col, collagen; CTGF, connective tissue GF; DM, diabetes mellitus; ECM, extracellular matrix; ERK, extracellular signal–regulated kinase; FGF, fibroblast GF; Gy, Gray; MMP, matrix metalloproteinase; hGF, human gingival fibroblast; MCP-1, monocyte chemoattractant protein 1; miR, microRNA; MMP, matrix metalloproteinase; pAKT, phosphorylated protein kinase B; PEP, purified (platelet) exosome product; PI3K, phosphatidylinositol 3-kinase, S.c., subcutaneous, TISSEEL, injectable surgical fibrin sealant; UCB, umbilical cord blood.

[1]In vivo wound healing model: information on species; strain; sex; age or body weight; type of wounding; and further methods of inducing concomitant pathologies, such as diabetes mellitus, as well as the route of secretome application are given, where provided in the referenced study.

[2]Method of paracrine factor purification: secretome compound used (e.g., exosomes) as well as details on the cell culture conditions from which the secretome compounds have been derived are summarized, where provided in the referenced study.

Table 6. Overview of Candidate Cell Sources of Therapeutically Applied Secretomes

Cell Source	Current Availability/Scalability[1]	Current Amount of Evidence on Efficacy and Safety in Wound Healing Trials[2]
Adipose tissue–derived mesenchymal stem/stromal cells	++	++++
Bone marrow–derived mesenchymal stem cells	+	++
Umbilical cord–derived mesenchymal stem/stromal cells	+	++
Amniotic mesenchymal stem/stromal cells	+	+
Urine-derived stem cells	+	+
Skin-derived multipotent stem/stromal cells	+	+
Epidermal stem cells	+	+
Antler stem cells	+	+
Mucosal epithelial cells	+	+
Gingival mesenchymal stem cells	+	+
Human gingival fibroblasts	+	+
PBMCs	+++	++
Thrombocytes	+++	+
Melanoma cell lines	+	+
RAW 264.7 cell line	+	+

Abbreviation: GMP, good manufacturing practice.

[1]Estimates on the current availability of the reviewed cellular sources and scalability of GMP-compliant production of the corresponding secretome are given according to the following qualitative scale: +, low abundance of primary cells and/or lack of exciting GMP infrastructure for upstream and downstream processing of secretome product; ++, in principal, continuous availability of cell source embedded in existing infrastructure but hitherto lack of routine GMP-compliant upstream and downstream processing of secretome product; and +++, highly abundant cell source and established GMP manufacturing of cell secretome–based product.

[2]Estimates on the current amount of evidence on efficacy and safety in wound healing trials are derived from the number of preclinical in vivo studies and completed human trials for each cell source reviewed in this paper: +, <5 studies; ++, 5–10 studies; +++, 10–15 studies; and ++++, >15 studies.

STANDARDIZATION OF CELL SOURCING AND SECRETOME PRODUCT MANUFACTURE

Recently described potential sources of therapeutic cell secretomes vary widely in their availability and scalability (Table 6). Sourcing of primary PBMCs, thrombocytes, and ADMSC can be embedded into the infrastructure of blood banks or surgical departments, routinely performing the purification of blood cells and liposuctions, respectively. In contrast, isolation of primary MSCs from bone marrow aspirates has some major disadvantages because the availability and scalability of these cells are low. Generally, studies directly benchmarking the wound healing efficacy of secretomes derived from different cell sources are scarce. Beyond the potential influence of the cell source on the overall efficacy and safety of the secretome product, the method of sourcing has been shown to significantly influence the state of the harvested cells, which might have a major effect on the resulting secretome (Keck et al., 2014). Although it is conceivable that the viability, purity, and differentiation state of the obtained cells affect the final secretome product, the field still lacks harmonized standards for cell sourcing (upstream processing) and secretome purification (downstream processing) as well as comparability studies. To obtain secretome products, the sourced cells are commonly first cultured with or without preconditioning treatments, and the conditioned medium is harvested and subjected to further downstream processing steps. Current studies, even when using comparable cell sources, vary considerably regarding culture conditions, including media composition, cell density, and the time interval of culture before the secretome is harvested (Tables 1–5). For clinical use, both cell cultivation and downstream processing of the secretome require good manufacturing practice (GMP)-compliant production to ensure the safety, purity, efficacy, and stability of the secretome product, including pathogen clearance, purification, and stabilization steps (Figure 1). In some approaches, subfractions of the secretome are enriched before in vivo application (Tables 1–5). Currently, the EV subfraction is the most widely studied secretome subfraction (Tables 1–5). Notably, the cell source and the method of EV enrichment and purification profoundly influence the identity, purity, and efficacy of the obtained candidate therapeutic product, and the lack of techniques for rapid isolation, quantification, and identification of EVs still poses a translational challenge (Zhang et al., 2019). Moreover, some of the therapeutic effects of secretome-based therapeutics appear to be suprasummative. For example, the proangiogenic and NETosis-inhibiting effects of the whole secretome of irradiated PBMCs outperform the effect of each individual secretome subfraction (Klas et al., 2022; Wagner et al., 2018).

Conclusively, the harmonization of upstream and downstream processes still poses a major challenge for the field.

PRECONDITIONING STRATEGIES TO IMPROVE SECRETOME EFFICACY

Secretomes sourced from different cell populations often contain common biologically active paracrine factors that engage shared signaling pathways in their respective target cells and lead to similar functional outcomes in wound healing (Figure 1). Thus, preconditioning strategies that foster the release of these paracrine factors or elicit the release of additional paracrine factors might strongly enhance the regenerative potency of cell secretomes (Figure 2).

Physiologically, MSCs experience oxygen tensions far below standard cell culture conditions (partial pressure of oxygen ~160 mm Hg [=21% oxygen]) in their respective in vivo niches (e.g., partial pressure of oxygen < 32 mm Hg in the bone marrow) (Spencer et al., 2014). Concordantly, cell culture under low oxygen tension prolongs the lifespan of MSCs and suppresses differentiation (Fehrer et al., 2007), while modulating cellular metabolism and paracrine factor release (Ejtehadifar et al., 2015). In an in vivo mouse model of diabetic wound healing, incubation of adipose tissue–derived stem cells for 24 hours at 1% oxygen robustly modulated the miRNA cargo of released exosomes and markedly enhanced their beneficial effect on re-epithelialization, collagen deposition, angiogenesis, and immunomodulation (Wang et al., 2021). In other work, 3–6 hours of hypoxic preconditioning at 1% oxygen enhanced the proliferative, antiapoptotic, and angiogenic properties of exosomes released from umbilical cord–derived mesenchymal stem/stromal cells (UCMSCs) (Zhang et al., 2021), and preconditioning of bone marrow and adipose tissue–derived stem cells with the HIF-1α stabilizing hypoxia mimic deferoxamine enhanced the angiogenesis-promoting effects of their respective paracrine factors (Ding et al., 2019; De Gregorio et al., 2020). Mechanistically, hypoxia or hypoxia-mimicking preconditioning has been linked to the upregulation of GFs such as VEGF (De Gregorio et al., 2020; Wahl et al., 2016) and of multiple miRNAs with implied angiogenic, antiapoptotic, and antifibrotic effects in dermal wound healing, including miR-21-3p, miR-23a-3p, miR-31-5p, miR-125b-5p, and miR-126 (Ding et al., 2019; Zhang et al., 2021). Hypoxia, together with low pH and accumulating proinflammatory cytokines, also constitutes a feature of the early-stage wound bed (Rodrigues et al., 2019). Thus, priming stem cells with these stimuli (ex vivo) may generate the environment a stem cell would putatively encounter during wound healing (in vivo). For example, pretreatment of umbilical MSCs with IFN-γ and TNF-α potently upregulated VEGFC expression and enhanced the effect of their secretome on re-epithelialization, angiogenesis, and collagen constriction in vivo (Zhu et al., 2020). Furthermore, the ex vivo pretreatment of B16F10 cells with IFN-γ upregulated PD-L1 in their secreted exosomes, which abrogated T-cell–driven inflammation through the PD-L1/PD1 axis and yielded improved wound healing in an in vivo mouse wounding model (Su et al., 2019).

Programmed cell death of immune cells marks another key event during tissue damage and subsequent physiological wound healing (Wu and Chen, 2014). Accumulating evidence has implicated paracrine factors of apoptotic and necroptotic leukocytes as tissue messengers that modulate the survival, growth, and metabolism of surrounding cells (Beer et al., 2016; Medina et al., 2020). Indeed, ionizing radiation–induced cell death of PBMCs ex vivo modulates the synthesis and release of phospholipid species, cytokines, and GFs (e.g., IL-8, monocyte chemoattractant protein 1, osteopontin, and VEGF) as well as EV cargo (e.g., enrichment of miR-21, miR-31, and miR-125), potentiating the cytoprotective, trophic, and immunomodulatory properties of the final secretome product in dermal wound healing, among other organ systems (Beer et al., 2016; Hacker et al., 2020; Simader et al., 2019; Wagner et al., 2018).

Other preconditioning strategies include the ex vivo treatment of MSCs with the peroxisome proliferator–activated receptor modulator pioglitazone, bone marrow concentrate, or serum from neonatal mice, resulting in the potentiation of the angiogenic and antifibrotic properties of their paracrine factors (Hu et al., 2021; Qiu et al., 2020). However, the transfer of preconditioning agents to the final therapeutic product could prove disadvantageous because of undesirable in vivo effects from the preconditioning stimulus. Therefore, ionized radiation or hypoxia currently represents the most promising preconditioning strategies. The purification of individual secretome fractions, most commonly exosomes, after preconditioning might mitigate these disadvantages but would be difficult to scale industrially under GMP conditions.

SECRETOME-BASED WOUND HEALING TREATMENTS IN HUMANS: CLINICAL TRIALS

Several clinical trials of the safety and efficacy of stem cell–based therapy in wound healing and skin regeneration have been conducted (for a review, see the study by Bian et al. [2022]), but reports on the therapeutic use of cell secretomes to improve wound healing in humans remain scarce.

Most available clinical trials have used topical application of cell secretomes as adjuvant therapy in cosmetic interventions, such as after ablative carbon dioxide fractional laser (AFL) treatment (Abdel-Maguid et al., 2021; Kim et al., 2020; Zhou et al., 2016). Zimber et al. (2012) applied conditioned media derived from neonatal cells to lesional skin after laser resurfacing and observed modest beneficial effects in the reduction of microcrusts and erythema and amelioration of epidermal water loss. However, the authors did not report the lineage of these neonatal cells, significantly limiting the reproducibility of their findings. In 23 patients undergoing AFL treatment, Kim et al. (2020) observed a short-term reduction in microcrust area and erythema in skin areas cotreated with human UCMSC-conditioned medium containing serum and cream compared with skin areas cotreated with UCMSC-conditioned media containing cream alone. Their study did not include a negative control condition, precluding a comprehensive assessment of treatment efficacy. Recently, a comparative study of 33 patients undergoing AFL treatment of atrophic acne scars showed no statistically significant beneficial effects of human amniotic ADMSC-conditioned medium (Abdel-Maguid et al., 2021). In contrast, similar trials showed multiple beneficial effects from ADMSC-conditioned medium application after AFL treatment, such as increased skin hydration, elasticity, and melanin index and decreased erythema and transepidermal water loss (Zhou et al., 2016, 2013).

Of note, almost no reports describe the effect of secretomes on chronic wound healing in humans. Prakoeswa et al. (2018) enrolled 66 patients with Hansen's disease suffering from chronic lower extremity ulcers. The authors compared the efficacy of topically applied ADMSC-conditioned medium with or without additional vitamin C or E, reporting safety, tolerability, and a reduction in mean ulcer size in all

groups, with superior ulcer size reduction in the ADMSC-conditioned medium + vitamin E group. This study also lacked a negative control condition.

In MARSYAS I, Simader et al. (2017) reported positive phase I trial data on the safety and tolerability of a topically applied PBMC secretome in full-thickness skin wounds of healthy volunteers, which led to the initiation of the MARSYAS II study. Data on this randomized, double-blind, placebo-controlled, multicenter phase II trial assessing the safety and efficacy of the secretome of stressed PBMCs in diabetic foot ulcers (NCT04277598) (Gugerell et al., 2021) are expected in summer 2023. Likewise, a phase I, open-label trial is currently exploring the safety and efficacy of topically applied platelet-derived EVs in patients needing skin graft transplantation (NCT04664738). Furthermore, patients are currently being enrolled in the SER-VES-HEAL trial (NCT04652531), aimed at testing the safety and efficacy of autologous serum-derived EVs in the treatment of therapy-refractive venous ulcers.

FROM BENCH TO BEDSIDE: CHALLENGES AND PERSPECTIVES

Undoubtedly, a considerable gap persists between the therapeutic potential of secretome-based wound healing in preclinical settings and its translation to clinical application. Several aspects beyond efficacy must be considered. Generally, all secretome-based therapies lack the distinct disadvantages observed in cell transplantation-based approaches. Along with limited survival and nonspecific ectopic tissue entrapment (Eggenhofer et al., 2012) and transdifferentiating failure (Murry et al., 2004), transplanted stem cells bear the danger of tumorigenicity through malignant transformation or benign teratoma formation (Lee et al., 2013). Previous reports have documented the homing and engraftment of MSCs into tumor stromal tissue, contributing to tumor growth and metastasis (Shinagawa et al., 2010; Suzuki et al., 2011). However, the risk of developing tumors after transplantation of MSC is debated in the context of cutaneous wound healing. For example, Li et al. (2019a) have not observed in vivo tumor formation after intramuscular application of human AMSCs over a 5-month observation period. In fact, at the wound site, the transplanted stem cells were short lived and gradually died over a 21-day period. Other recent toxicology work showed no tumorigenic effects of transplanted MSCs in mice models (Tayebi et al., 2022), and several studies have indicated positive safety and efficacy profiles of therapeutically applied MSCs in human wound healing trials (Dama et al., 2023; Nalisa et al., 2022). Notably, several GFs commonly found in a variety of secretomes, such as VEGF, bear protumorigenic potential (Goel and Mercurio, 2013) and thus might potentially foster the growth of pre-existing malignantly transformed cells.

The therapeutic use of cellular paracrine factors instead of transplantation of the source cells offers further advantages. In contrast to transplanted cells, secretome-based biologicals can undergo extensive pathogen clearance, such as methylene blue/light- and gamma irradiation–based viral clearance (Gugerell et al., 2020). They can also be generated from non–stem cell sources, such as peripheral immune cells (Hacker et al., 2020; Li et al., 2019b; Mildner et al., 2013) or thrombocytes (Shi et al., 2021), circumventing ethical concerns connected with using primary stem cells. Beyond ethical considerations, using blood cell populations as a secretome source allows for easier scaling, thanks to their abundance and the pre-existing infrastructure and expertise of blood banks and transfusion units, simplifying harmonization with regulatory demands. Moreover, cell secretomes derived from the biological material of multiple donors can be pooled, countering the problem of biological variability inherent to the transplantation of one batch of primary cells at a time. Notably, recent advances have presented GMP-compliant cell banking approaches in which master and working cell banks are established, mitigating this problem (Pakzad et al., 2022).

Despite these advantages, several hurdles must be cleared before candidates can enter clinical trials (summarized in Figure 1), which might explain the low numbers of such trials. Secretome-based products can be pharmaceutically categorized as biological medicinal products, although regulatory guidelines devised for the development of advanced therapy medicinal products bear partial relevance to the development of secretome-based therapeutics (Beer et al., 2016; Lener et al., 2015). Several demands must be met to satisfy ethical as well as international and regional regulatory standards for the advancement of secretome-based therapeutics into clinical trials. Although regulatory guidelines vary considerably across the Asia-Pacific region (Lim, 2018), GMP requirements for the manufacturing of an authorized drug are largely harmonized in the European Union through Eudralex Volume 4 (EudraLex, 2015) and the Federal Food, Drug, and Cosmetic Act, chapter V in the United States (U.S. Food & Drug Administration, 2018), whereas International Council for Harmonization (ICH) guidelines (International Council for Harmonization, 2023) pose an internationally relevant regulatory framework.

The aims of GMP are safety, identity, strength, purity, and quality to ensure the production of a safe drug with consistent quality throughout the entirety of the manufacturing process. Other internationally harmonized requirements for a safe drug, derived from the ICH framework (International Council for Harmonization, 2023), are tests for safety pharmacology (ICH S7A), toxicokinetics and pharmacokinetics (ICH S3A and S3B), carcinogenicity studies (ICH S1A-S1C), nonclinical safety studies (ICH M3(R2)), or viral safety studies (ICH Q5A) (for a review, see the studies Beer et al. [2016], Kingham et al. [2013], and Lener et al. [2015]). Particularly, the establishment of potency assays and their linkage to specific mechanisms of action pose a challenge because secretome-based therapeutics are definitionally compositions of different biologically active substances that often exert therapeutic effects through pleiotropic and suprasummative mechanisms.

CONCLUSION

The last decade has seen a substantial increase in studies of secretome-based wound healing therapies, generating an emerging understanding of the mechanisms of action underlying their beneficial properties. Although the cellular sources and methods of secretome purification and application are highly diverse, many therapeutic secretomes share remarkable similarities in composition, along with targeted

cells and molecular signaling cascades through the phases of wound healing. Moreover, all secretome-based therapies share the same technical and regulatory hurdles to clear on the way to clinical application. The scope of potential indications for secretome-based therapeutics extends the treatment of chronic wounds because the tissue-regenerative properties of these candidates have been documented in almost all organ systems (Beer et al., 2016; Moghadasi et al., 2021). Yet, the road from bench to bedside for systemically applied biologicals is especially complicated, particularly for secretome-based approaches, which in many aspects represent their own pharmaceutical category. Successful application of secretome-based therapeutics in dermal wound healing not only might alleviate unmet needs in chronic wound healing but also serve as a stepping stone to novel tissue regenerative approaches for other diseases.

ORCIDs
Daniel Bormann: http://orcid.org/0000-0002-5662-0087
Hednrik Jan Ankersmit: http://orcid.org/0000-0002-8761-3517
Michael Mildner: http://orcid.org/0000-0002-6892-925X
Alfred Gugerell: http://orcid.org/0000-0002-5178-9967

CONFLICT OF INTEREST
The authors are employees of Aposcience AG.

ACKNOWLEDGMENTS
The study was financed by the Austrian Research Promotion Agency, the Vienna Business Agency, and Aposcience AG.

AUTHOR CONTRIBUTIONS
Conceptualization: DB, AG, MM, HJA; Funding Acquisition: HJA, MM; Visualization: DB, MM; Writing – Original Draft Preparation: DB, MM; Writing – Review and Editing: DB, AG, HJA, MM

REFERENCES
Abdel-Maguid EM, Awad SM, Hassan YS, El-Mokhtar MA, El-Deek HE, Mekkawy MM. Efficacy of stem cell-conditioned medium vs. platelet-rich plasma as an adjuvant to ablative fractional CO2 laser resurfacing for atrophic post-acne scars: a split-face clinical trial. J Dermatolog Treat 2021;32:242–9.

Ahangar P, Mills SJ, Smith LE, Gronthos S, Cowin AJ. Human gingival fibroblast secretome accelerates wound healing through anti-inflammatory and pro-angiogenic mechanisms. NPJ Regen Med 2020a;5:24.

Ahangar P, Mills SJ, Smith LE, Strudwick XL, Ting AE, Vaes B, et al. Human multipotent adult progenitor cell-conditioned medium improves wound healing through modulating inflammation and angiogenesis in mice. Stem Cell Res Ther 2020b;11:299.

An YH, Kim DH, Lee EJ, Lee D, Park MJ, Ko J, et al. High-efficient production of adipose-derived stem cell (ADSC) secretome through maturation process and its non-scarring wound healing applications. Front Bioeng Biotechnol 2021;9:681501.

Baraniak PR, McDevitt TC. Stem cell paracrine actions and tissue regeneration. Regen Med 2010;5:121–43.

Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen 2008;16:585–601.

Beer L, Mildner M, Gyöngyösi M, Ankersmit HJ. Peripheral blood mononuclear cell secretome for tissue repair. Apoptosis 2016;21:1336–53.

Bian D, Wu Y, Song G, Azizi R, Zamani A. The application of mesenchymal stromal cells (MSCs) and their derivative exosome in skin wound healing: a comprehensive review. Stem Cell Res Ther 2022;13:24.

Bjarnsholt T, Kirketerp-Møller K, Jensen PØ, Madsen KG, Phipps R, Krogfelt K, et al. Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen 2008;16:2–10.

Chan AML, Sampasivam Y, Lokanathan Y. Biodistribution of mesenchymal stem cells (MSCs) in animal models and implied role of exosomes following systemic delivery of MSCs: a systematic review. Am J Transl Res 2022;14:2147–61.

Charitos IA, Ballini A, Cantore S, Boccellino M, Di Domenico M, Borsani E, et al. Stem cells: A historical review about biological, religious, and ethical issues. Stem Cells Int 2021;2021:9978837.

Chen CY, Rao SS, Ren L, Hu XK, Tan YJ, Hu Y, et al. Exosomal DMBT1 from human urine-derived stem cells facilitates diabetic wound repair by promoting angiogenesis. Theranostics 2018a;8:1607–23.

Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature 2018b;560:382–6.

Cheng R, Ma JX. Angiogenesis in diabetes and obesity. Rev Endocr Metab Disord 2015;16:67–75.

Copic D, Direder M, Schossleitner K, Laggner M, Klas K, Bormann D, et al. Paracrine factors of stressed peripheral blood mononuclear cells activate proangiogenic and anti-proteolytic processes in whole blood cells and protect the endothelial barrier. Pharmaceutics 2022;14:1600.

Dama G, Du J, Zhu X, Liu Y, Lin J. Bone marrow-derived mesenchymal stem cells: A promising therapeutic option for the treatment of diabetic foot ulcers. Diabetes Res Clin Pract 2023;195:110201.

Davies LC, Heldring N, Kadri N, Le Blanc K. Mesenchymal stromal cell secretion of programmed Death-1 ligands regulates T cell mediated immunosuppression. Stem Cells 2017;35:766–76.

De Gregorio C, Contador D, Díaz D, Cárcamo C, Santapau D, Lobos-Gonzalez L, et al. Human adipose-derived mesenchymal stem cell-conditioned medium ameliorates polyneuropathy and foot ulceration in diabetic BKS db/db mice. Stem Cell Res Ther 2020;11:168.

Ding J, Wang X, Chen B, Zhang J, Xu J. Exosomes derived from human bone marrow mesenchymal stem cells stimulated by deferoxamine accelerate cutaneous wound healing by promoting angiogenesis. BioMed Res Int 2019;2019:9742765.

DiPietro LA. Angiogenesis and wound repair: when enough is enough. J Leukoc Biol 2016;100:979–84.

Direder M, Weiss T, Copic D, Vorstandlechner V, Laggner M, Pfisterer K, et al. Schwann cells contribute to keloid formation. Matrix Biol 2022;108:55–76.

Duan M, Zhang Y, Zhang H, Meng Y, Qian M, Zhang G. Epidermal stem cell-derived exosomes promote skin regeneration by downregulating transforming growth factor-β1 in wound healing. Stem Cell Res Ther 2020;11:452.

Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol 2012;3:297.

Ejtehadifar M, Shamsasenjan K, Movassaghpour A, Akbarzadehlaleh P, Dehdilani N, Abbasi P, et al. The effect of hypoxia on mesenchymal stem cell biology. Adv Pharm Bull 2015;5:141–9.

Ellis S, Lin EJ, Tartar D. Immunology of wound healing. Curr Dermatol Rep 2018;7:350–8.

EudraLex CE. Good Manufacturing Practice (GMP) guidelines. https://health.ec.europa.eu/medicinal-products/eudralex/eudralex-volume-4_en; 2015. (accessed January 26, 2023).

Fang S, Xu C, Zhang Y, Xue C, Yang C, Bi H, 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;5:1425–39.

Fehrer C, Brunauer R, Laschober G, Unterluggauer H, Reitinger S, Kloss F, et al. Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. Aging Cell 2007;6:745–57.

Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. Adv Wound Care (New Rochelle) 2015;4:560–82.

Goel HL, Mercurio AM. VEGF targets the tumour cell. Nat Rev Cancer 2013;13:871–82.

Gugerell A, Gouya-Lechner G, Hofbauer H, Laggner M, Trautinger F, Almer G, et al. Safety and clinical efficacy of the secretome of stressed peripheral blood mononuclear cells in patients with diabetic foot ulcer-study protocol of the randomized, placebo-controlled, double-blind, multicenter, international phase II clinical trial MARSYAS II. Trials 2021;22:10.

Gugerell A, Sorgenfrey D, Laggner M, Raimann J, Peterbauer A, Bormann D, et al. Viral safety of APOSECTM: a novel peripheral blood mononuclear

cell derived-biological for regenerative medicine. Blood Transfus 2020;18:30–9.

Gushiken LFS, Beserra FP, Bastos JK, Jackson CJ, Pellizzon CH. Cutaneous wound healing: an update from physiopathology to current therapies. Life (Basel) 2021;11:665.

Hacker S, Mittermayr R, Nickl S, Haider T, Lebherz-Eichinger D, Beer L, et al. Paracrine factors from irradiated peripheral blood mononuclear cells improve skin regeneration and angiogenesis in a porcine burn model. Sci Rep 2016;6:25168.

Hacker S, Mittermayr R, Traxler D, Keibl C, Resch A, Salminger S, et al. The secretome of stressed peripheral blood mononuclear cells increases tissue survival in a rodent epigastric flap model. Bioeng Transl Med 2020;6:e10186.

Harman RM, Yang S, He MK, Van de Walle GR. Antimicrobial peptides secreted by equine mesenchymal stromal cells inhibit the growth of bacteria commonly found in skin wounds. Stem Cell Res Ther 2017;8:157.

He Y, Xia J, Chen H, Wang L, Deng C, Lu F. Human adipose liquid extract induces angiogenesis and adipogenesis: a novel cell-free therapeutic agent. Stem Cell Res Ther 2019;10:252.

Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. Pharmacol Rev 2004;56:549–80.

Holzinger C, Zuckermann A, Kopp C, Schöllhammer A, Imhof M, Zwölfer W, et al. Treatment of non-healing skin ulcers with autologous activated mononuclear cells. Eur J Vasc Surg 1994;8:351–6.

Hu CH, Tseng YW, Chiou CY, Lan KC, Chou CH, Tai CS, et al. Bone marrow concentrate-induced mesenchymal stem cell conditioned medium facilitates wound healing and prevents hypertrophic scar formation in a rabbit ear model. Stem Cell Res Ther 2019;10:275.

Hu Y, Rao SS, Wang ZX, Cao J, Tan YJ, Luo J, et al. Exosomes from human umbilical cord blood accelerate cutaneous wound healing through miR-21-3p-mediated promotion of angiogenesis and fibroblast function. Theranostics 2018;8:169–84.

Hu Y, Tao R, Chen L, Xiong Y, Xue H, Hu L, et al. Exosomes derived from pioglitazone-pretreated MSCs accelerate diabetic wound healing through enhancing angiogenesis. J Nanobiotechnology 2021;19:150.

International Council for Harmonization. Index of ICH Guidelines. https://www.ich.org/page/search-index-ich-guidelines; 2023. (accessed January 26, 2023).

Irons RF, Cahill KW, Rattigan DA, Marcotte JH, Fromer MW, Chang S, et al. Acceleration of diabetic wound healing with adipose-derived stem cells, endothelial-differentiated stem cells, and topical conditioned medium therapy in a swine model. J Vasc Surg 2018;68:115S–25S.

Jiang T, Wang Z, Sun J. Human bone marrow mesenchymal stem cell-derived exosomes stimulate cutaneous wound healing mediates through TGF-β/Smad signaling pathway. Stem Cell Res Ther 2020;11:198.

Kanno E, Kawakami K, Ritsu M, Ishii K, Tanno H, Toriyabe S, et al. Wound healing in skin promoted by inoculation with Pseudomonas aeruginosa PAO1: the critical role of tumor necrosis factor-α secreted from infiltrating neutrophils. Wound Repair Regen 2011;19:608–21.

Karar J, Maity A. PI3K/AKT/mTOR pathway in angiogenesis. Front Mol Neurosci 2011;4:51.

Kasiri MM, Beer L, Nemec L, Gruber F, Pietkiewicz S, Haider T, et al. Dying blood mononuclear cell secretome exerts antimicrobial activity. Eur J Clin Invest 2016;46:853–63.

Keck M, Kober J, Riedl O, Kitzinger HB, Wolf S, Stulnig TM, et al. Power assisted liposuction to obtain adipose-derived stem cells: impact on viability and differentiation to adipocytes in comparison to manual aspiration. J Plast Reconstr Aesthet Surg 2014;67:e1–8.

Kim J, Kim B, Kim S, Lee YI, Kim J, Lee JH. The effect of human umbilical cord blood-derived mesenchymal stem cell media containing serum on recovery after laser treatment: A double-blinded, randomized, split-face controlled study. J Cosmet Dermatol 2020;19:651–6.

Kingham R, Klasa G, Carver KH. Key regulatory guidelines for the development of biologics in the United States and Europe. Hoboken, NJ: Wiley Online Library; 2013. p. 1–36.

Klas K, Ondracek AS, Hofbauer TM, Mangold A, Pfisterer K, Laggner M, et al. The effect of paracrine factors released by irradiated peripheral blood mononuclear cells on neutrophil extracellular trap formation. Antioxidants (Basel) 2022;11:1559.

Krishnaswamy VR, Mintz D, Sagi I. Matrix metalloproteinases: the sculptors of chronic cutaneous wounds. Biochim Biophys Acta Mol Cell Res 2017;1864:2220–7.

Krzyszczyk P, Schloss R, Palmer A, Berthiaume F. The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. Front Physiol 2018;9:419.

Laggner M, Acosta GS, Kitzmüller C, Copic D, Gruber F, Altenburger LM, et al. The secretome of irradiated peripheral blood mononuclear cells attenuates activation of mast cells and basophils. EBiomedicine 2022;81:104093.

Laggner M, Copic D, Nemec L, Vorstandlechner V, Gugerell A, Gruber F, et al. Therapeutic potential of lipids obtained from γ-irradiated PBMCs in dendritic cell-mediated skin inflammation. EBiomedicine 2020;55:102774.

Lee AS, Tang C, Rao MS, Weissman IL, Wu JC. Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. Nat Med 2013;19:998–1004.

Lener T, Gimona M, Aigner L, Börger V, Buzas E, Camussi G, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles 2015;4:30087.

Li C, An Y, Sun Y, Yang F, Xu Q, Wang Z. Adipose mesenchymal stem cell-derived exosomes promote wound healing through the WNT/β-catenin signaling pathway in dermal fibroblasts. Stem Cell Rev Rep 2022;18:2059–73.

Li JY, Ren KK, Zhang WJ, Xiao L, Wu HY, Liu QY, et al. Human amniotic mesenchymal stem cells and their paracrine factors promote wound healing by inhibiting heat stress-induced skin cell apoptosis and enhancing their proliferation through activating PI3K/AKT signaling pathway. Stem Cell Res Ther 2019a;10:247.

Li M, Wang T, Tian H, Wei G, Zhao L, Shi Y. Macrophage-derived exosomes accelerate wound healing through their anti-inflammation effects in a diabetic rat model. Artif Cells Nanomed Biotechnol 2019b;47:3793–803.

Li X, Wang Y, Shi L, Li B, Li J, Wei Z, et al. Magnetic targeting enhances the cutaneous wound healing effects of human mesenchymal stem cell-derived iron oxide exosomes. J Nanobiotechnology 2020;18:113.

Lichtenauer M, Mildner M, Hoetzenecker K, Zimmermann M, Podesser BK, Sipos W, et al. Secretome of apoptotic peripheral blood cells (APOSEC) confers cytoprotection to cardiomyocytes and inhibits tissue remodelling after acute myocardial infarction: a preclinical study. Basic Res Cardiol 2011;106:1283–97.

Lim JCW. Strengthening health products regulatory systems to enhance access to quality health products in the Asia-Pacific. Ther Innov Regul Sci 2018;52:751–4.

Lin CW, Hung CM, Chen WJ, Chen JC, Huang WY, Lu CS, et al. New horizons of macrophage immunomodulation in the healing of diabetic foot ulcers. Pharmaceutics 2022;14:2065.

Liu J, Yan Z, Yang F, Huang Y, Yu Y, Zhou L, et al. 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;17:305–17.

Md Fadilah NI, Mohd Abdul Kader Jailani MS, Badrul Hisham MAI, Sunthar Raj N, Shamsuddin SA, Ng MH, et al. Cell secretomes for wound healing and tissue regeneration: next generation acellular based tissue engineered products. J Tissue Eng 2022;13:20417314221114273.

Medina CB, Mehrotra P, Arandjelovic S, Perry JSA, Guo Y, Morioka S, et al. Metabolites released from apoptotic cells act as tissue messengers. Nature 2020;580:130–5.

Mildner M, Hacker S, Haider T, Gschwandtner M, Werba G, Barresi C, et al. Secretome of peripheral blood mononuclear cells enhances wound healing. PLoS One 2013;8:e60103.

Moghadasi S, Elveny M, Rahman HS, Suksatan W, Jalil AT, Abdelbasset WK, et al. A paradigm shift in cell-free approach: the emerging role of MSCs-derived exosomes in regenerative medicine. J Transl Med 2021;19:302.

Moore AL, Marshall CD, Barnes LA, Murphy MP, Ransom RC, Longaker MT. Scarless wound healing: transitioning from fetal research to regenerative healing7. Wiley Interdiscip Rev Dev Biol; 2018. https://doi.org/10.1002/wdev.309.

Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004;428:664–8.

Nalisa DL, Moneruzzaman M, Changwe GJ, Mobet Y, Li LP, Ma YJ, et al. Stem cell therapy for diabetic foot ulcers: theory and practice. J Diabetes Res 2022;2022:6028743.

Olsson M, Järbrink K, Divakar U, Bajpai R, Upton Z, Schmidtchen A, et al. The humanistic and economic burden of chronic wounds: A systematic review. Wound Repair Regen 2019;27:114–25.

Pakzad M, Hassani SN, Abbasi F, Hajizadeh-Saffar E, Taghiyar L, Fallah N, et al. A roadmap for the production of a GMP-compatible cell bank of allogeneic bone marrow-derived clonal mesenchymal stromal cells for cell therapy applications. Stem Cell Rev Rep 2022;18:2279–95.

Panahipour L, Kochergina E, Laggner M, Zimmermann M, Mildner M, Ankersmit HJ, et al. Role for lipids secreted by irradiated peripheral blood mononuclear cells in inflammatory resolution in vitro. Int J Mol Sci 2020;21:4694.

Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, et al. Epithelialization in wound healing: A comprehensive review. Adv Wound Care (New Rochelle) 2014;3:445–64.

Penn JW, Grobbelaar AO, Rolfe KJ. The role of the TGF-β family in wound healing, burns and scarring: a review. Int J Burns Trauma 2012;2:18–28.

Prakoeswa CRS, Natallya FR, Harnindya D, Thohiroh A, Oktaviyanti RN, Pratiwi KD, et al. The efficacy of topical human amniotic membrane-mesenchymal stem cell-conditioned medium (hAMMSC-CM) and a mixture of topical hAMMSC-CM + vitamin C and hAMMSC-CM + vitamin E on chronic plantar ulcers in leprosy:a randomized control trial. J Dermatolog Treat 2018;29:835–40.

Prasai A, Jay JW, Jupiter D, Wolf SE, El Ayadi A. Role of exosomes in dermal wound healing: a systematic review. J Invest Dermatol 2022;142(3):662–78.e8.

Qian L, Pi L, Fang BR, Meng XX. Adipose mesenchymal stem cell-derived exosomes accelerate skin wound healing via the lncRNA H19/miR-19b/SOX9 axis. Lab Investig 2021;101:1254–66.

Qiu X, Liu J, Zheng C, Su Y, Bao L, Zhu B, et al. Exosomes released from educated mesenchymal stem cells accelerate cutaneous wound healing via promoting angiogenesis. Cell Prolif 2020;53:e12830.

Rangel-Huerta E, Maldonado E. Transit-amplifying cells in the fast lane from stem cells towards differentiation. Stem Cells Int 2017;2017:7602951.

Ren S, Chen J, Duscher D, Liu Y, Guo G, Kang Y, 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;10:47.

Robert AW, Azevedo Gomes F, Rode MP, Marques da Silva M, Veleirinho MBDR, Maraschin M, et al. The skin regeneration potential of a pro-angiogenic secretome from human skin-derived multipotent stromal cells. J Tissue Eng 2019;10:2041731419833391.

Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: A cellular perspective. Physiol Rev 2019;99:665–706.

Rong X, Chu W, Zhang H, Wang Y, Qi X, Zhang G, et al. Antler stem cell-conditioned medium stimulates regenerative wound healing in rats. Stem Cell Res Ther 2019;10:326.

Sen CK. Human wounds and its burden: an updated compendium of estimates. Adv Wound Care (New Rochelle) 2019;8:39–48.

Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, et al. Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair Regen 2009;17:763–71.

Shi A, Li J, Qiu X, Sabbah M, Boroumand S, Huang TC, et al. TGF-β loaded exosome enhances ischemic wound healing in vitro and in vivo. Theranostics 2021;11:6616–31.

Shi Q, Qian Z, Liu D, Sun J, Wang X, Liu H, et al. GMSC-derived exosomes combined with a chitosan/silk hydrogel sponge accelerates wound healing in a diabetic rat skin defect model. Front Physiol 2017;8:904.

Shiekh PA, 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.

Shinagawa K, Kitadai Y, Tanaka M, Sumida T, Kodama M, Higashi Y, et al. Mesenchymal stem cells enhance growth and metastasis of colon cancer. Int J Cancer 2010;127:2323–33.

Simader E, Beer L, Laggner M, Vorstandlechner V, Gugerell A, Erb M, et al. Tissue-regenerative potential of the secretome of γ-irradiated peripheral blood mononuclear cells is mediated via TNFRSF1B-induced necroptosis. Cell Death Dis 2019;10:729.

Simader E, Traxler D, Kasiri MM, Hofbauer H, Wolzt M, Glogner C, et al. Safety and tolerability of topically administered autologous, apoptotic PBMC secretome (APOSEC) in dermal wounds: a randomized Phase 1 trial (MARSYAS I). Sci Rep 2017;7:6216.

Sjöqvist S, Ishikawa T, Shimura D, Kasai Y, Imafuku A, Bou-Ghannam S, et al. Exosomes derived from clinical-grade oral mucosal epithelial cell sheets promote wound healing. J Extracell Vesicles 2019;8:1565264.

Spencer JA, Ferraro F, Roussakis E, Klein A, Wu J, Runnels JM, et al. Direct measurement of local oxygen concentration in the bone marrow of live animals. Nature 2014;508:269–73.

Su D, Tsai HI, Xu Z, Yan F, Wu Y, Xiao Y, et al. Exosomal PD-L1 functions as an immunosuppressant to promote wound healing. J Extracell Vesicles 2019;9:1709262.

Sugimoto MA, Sousa LP, Pinho V, Perretti M, Teixeira MM. Resolution of inflammation: what controls its onset? Front Immunol 2016;7:160.

Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, et al. Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med 2011;17:579–87.

Tayebi B, Babaahmadi M, Pakzad M, Hajinasrollah M, Mostafaei F, Jahangiri S, et al. Standard toxicity study of clinical-grade allogeneic human bone marrow-derived clonal mesenchymal stromal cells. Stem Cell Res Ther 2022;13:213.

Thiam HR, Wong SL, Wagner DD, Waterman CM. Cellular mechanisms of NETosis. Annu Rev Cell Dev Biol 2020;36:191–218.

Tjalsma H, Antelmann H, Jongbloed JD, Braun PG, Darmon E, Dorenbos R, et al. Proteomics of protein secretion by Bacillus subtilis: separating the "secrets" of the secretome. Microbiol Mol Biol Rev 2004;68:207–33.

U.S. Food & Drug Administration. FD&C Act Chapter V: Drugs and Devices. https://www.fda.gov/regulatory-information/federal-food-drug-and-cosmetic-act-fdc-act/fdc-act-chapter-v-drugs-and-devices; 2018. (accessed January 26, 2023).

Wagner T, Traxler D, Simader E, Beer L, Narzt MS, Gruber F, et al. Different pro-angiogenic potential of γ-irradiated PBMC-derived secretome and its subfractions. Sci Rep 2018;8:18016.

Wahl EA, Schenck TL, Machens HG, Balmayor ER. VEGF released by deferoxamine preconditioned mesenchymal stem cells seeded on collagen-GAG substrates enhances neovascularization. Sci Rep 2016;6:36879.

Wang C, Wang M, Xu T, Zhang X, Lin C, Gao W, et al. Engineering bioactive self-healing antibacterial exosomes hydrogel for promoting chronic diabetic wound healing and complete skin regeneration. Theranostics 2019;9:65–76.

Wang J, Wu H, Peng Y, Zhao Y, Qin Y, Zhang Y, et al. Hypoxia adipose stem cell-derived exosomes promote high-quality healing of diabetic wound involves activation of PI3K/Akt pathways. J Nanobiotechnology 2021;19:202.

Witherel CE, Sao K, Brisson BK, Han B, Volk SW, Petrie RJ, et al. Regulation of extracellular matrix assembly and structure by hybrid M1/M2 macrophages. Biomaterials 2021;269:120667.

Wong SL, Demers M, Martinod K, Gallant M, Wang Y, Goldfine AB, et al. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. Nat Med 2015;21:815–9.

Wu YS, Chen SN. Apoptotic cell: linkage of inflammation and wound healing. Front Pharmacol 2014;5:1.

Xiao S, Xiao C, Miao Y, Wang J, Chen R, Fan Z, et al. Human acellular amniotic membrane incorporating exosomes from adipose-derived mesenchymal stem cells promotes diabetic wound healing. Stem Cell Res Ther 2021;12:255.

Zhang G, Wang D, Ren J, Sun H, Li J, Wang S, et al. Velvet antler peptides reduce scarring via inhibiting the TGF-β signaling pathway during wound healing. Front Med (Lausanne) 2021c;8:799789.

Zhang XF, Wang T, Wang ZX, Huang KP, Zhang YW, Wang GL, et al. Hypoxic ucMSC-secreted exosomal miR-125b promotes endothelial cell survival

and migration during wound healing by targeting TP53INP1. Mol Ther Nucleic Acids 2021d;26:347–59.

Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci 2019;9:19.

Zhang Y, Pan Y, Liu Y, Li X, Tang L, Duan M, et al. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulate regenerative wound healing via transforming growth factor-β receptor inhibition. Stem Cell Res Ther 2021a;12:434.

Zhang Y, Yan J, Liu Y, Chen Z, Li X, Tang L, et al. Human amniotic fluid stem cell-derived exosomes as a novel cell-free therapy for cutaneous regeneration. Front Cell Dev Biol 2021b;9:685873.

Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in chronic wounds. Int J Mol Sci 2016;17:2085.

Zhou BR, Xu Y, Guo SL, Xu Y, Wang Y, Zhu F, et al. The effect of conditioned media of adipose-derived stem cells on wound healing after ablative fractional carbon dioxide laser resurfacing. BioMed Res Int 2013;2013:519126.

Zhou BR, Zhang T, Bin Jameel AA, Xu Y, Xu Y, Guo SL, et al. The efficacy of conditioned media of adipose-derived stem cells combined with ablative carbon dioxide fractional resurfacing for atrophic acne scars and skin rejuvenation. J Cosmet Laser Ther 2016;18:138–48.

Zhu M, Chu Y, Shang Q, Zheng Z, Li Y, Cao L, et al. Mesenchymal stromal cells pretreated with pro-inflammatory cytokines promote skin wound healing through VEGFC-mediated angiogenesis. Stem Cells Transl Med 2020;9:1218–32.

Zhu S, Yu Y, Ren Y, Xu L, Wang H, Ling X, et al. The emerging roles of neutrophil extracellular traps in wound healing. Cell Death Dis 2021;12:984.

Zimber MP, Mansbridge JN, Taylor M, Stockton T, Hubka M, Baumgartner M, et al. Human cell-conditioned media produced under embryonic-like conditions result in improved healing time after laser resurfacing. Aesthetic Plast Surg 2012;36:431–7.