STEM CELLS AND DEVELOPMENT
Volume 27, Number 20, 2018

DOI: 10.1089/scd.2018.0094

COMPREHENSIVE REVIEW

Mesenchymal Stromal Cell Preconditioning: The Next Step Toward a Customized Treatment For Severe Burn

Brice Magne, Jean-Jacques Lataillade, and Marina Trouillas

Over the last century, the clinical management of severe skin burns significantly progressed with the development of burn care units, topical antimicrobials, resuscitation methods, early eschar excision surgeries, and skin grafts. Despite these considerable advances, the present treatment of severe burns remains burdensome, and patients are highly susceptible to skin engraftment failure, infections, organ dysfunction, and hypertrophic scarring. Recent researches have focused on mesenchymal stromal cell (MSC) therapy and hold great promises for tissue repair, as reported in several animal studies and clinical cases. In the present review, we will provide an up-to-date outlook of the pathophysiology of severe skin burns, clinical treatment modalities and current limitations. We will then focus on MSCs and their potential in the burn wound healing both in in vitro and in vivo studies. A specific attention will be paid to the cell preconditioning approach, as a means of improving the MSC efficacy in the treatment of major skin burns. In particular, we will debate how several preconditioning cues would modulate the MSC properties to better match up with the burn pathophysiology in the course of the cell therapy. Finally, we will discuss the clinical interest and feasibility of a MSC-based therapy in comparison to their paracrine derivatives, including microvesicles and conditioned media for the treatment of major skin burn injuries.

Keywords: major skin burn injuries and pathophysiology, mesenchymal stromal cells, cell preconditioning, clinical use

THERMAL BURNS CAN LEAD to the disruption of the cutaneous barrier and leave the body unprotected against the surrounding environment. In 2018, the World Health Organization* recorded 180,000 burn-related deaths per year with a high percentage of cases occurring in low- and middle-income countries [1,2]. Although the incidence, the severity, and the mortality rate following a burn injury have decreased [2], the management of major skin burns still remains challenging in terms of surgery and scar formation [3–5].

This review presents the limits of the classical management of severe skin burns and tackles interesting therapeutic alternatives using cell-based therapies and improved by cell preconditioning strategies.

Severe Thermal Burns

Burn pathophysiology and healing

Burn is a complex trauma whose severity depends on wound size, depth, and location. But other factors can influence the burn severity, including age, burn localization, and other related injuries. Burn is one of the most severe traumas, as it impairs the normal wound healing process and leads to irreversible functional and esthetical damages.

Burn pathophysiology. Depending on its etiology, intensity, and duration, the burn injury can trigger protein denaturation and cell membrane integrity loss at the local level, leading to a so-called coagulative necrosis [6,7]. Clinically, the burn wound can be dissected into three severity areas from the center of the wound to the periphery [8]. The center of the wound is called the coagulation zone, as the tissue is irreversibly lost. Surrounding the coagulation zone, the zone of stasis is poorly perfused and can rapidly turn into necrosis. The most external zone is referred to as the hyperemia zone, where vasodilation is governed by an acute and local inflammatory reaction. To avoid ischemia-driven burn wound progression, wound care management and fluid resuscitation are critical.

Necrotic burn tissues release large amounts of damage associated molecular patterns (DAMPs), including toxic lipid-protein complexes [9], extracellular matrix (ECM) fragments, and cytoplasmic cell content. DAMPs activate

*World Health Organization. Burns: Fact sheet. Available at: www.who.int/mediacentre/factsheets/fs365/en (Reviewed January 2018).

INSERM U1197–Institut de Recherche Biomédicale des Armées (IRBA)/Antenne Centre de Transfusion Sanguine des Armées (CTSA), Clamart, France.

the inflammasome through the Toll-like receptors (TLRs)/nuclear factor-kappa B (NFκB) pathway and initiate the systemic production of large amounts of inflammatory molecules [10–13]. Along with this systemic inflammatory response, the increased capillary permeability results in edema formation in the interstitial space and hypovolemia. Simultaneously, increased systemic vascular resistance contributes to reduce the cardiac output and leads to a so-called burn shock (Fig. 1). After about 3 days, hypermetabolism becomes established in burn patients, as a normal body response to cope with dehydration and hypovolemia-induced heat loss. Hypermetabolism is driven by catecholamines,

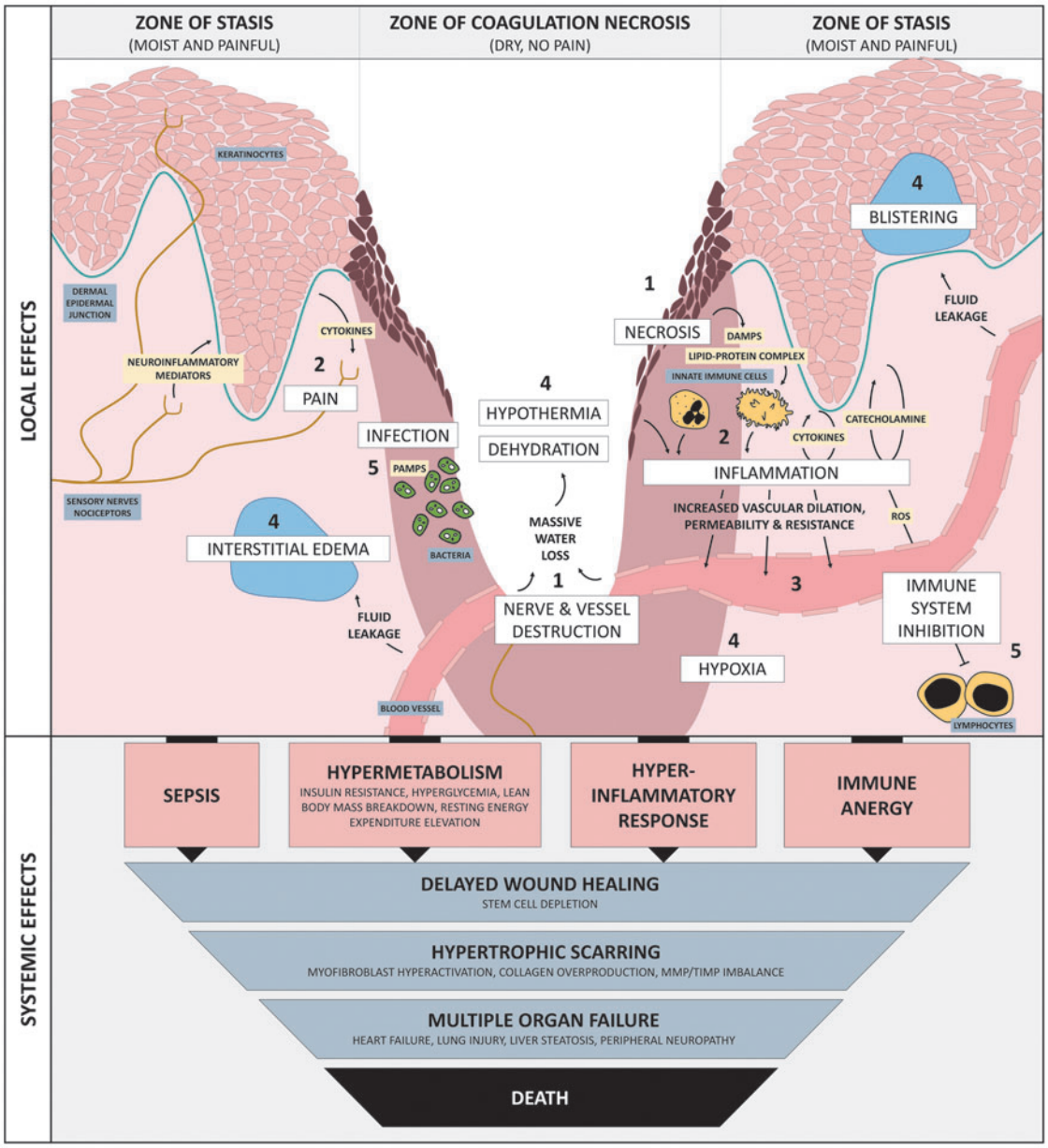

FIG. 1. Pathophysiology of full thickness burn injuries. This schematic summarizes the main biological events following a full-thickness burn injury, according to the Jackson's pathophysiological model [8]. At local level, in the zone of coagulation necrosis (1), the burn injury causes full destructions of all skin layers. Burn toxins, such as lipid–protein complexes, tissue necrosis, and ischemia, all contribute to trigger an intense and long-lasting inflammatory response that spreads to the surrounding stasis zones. Massive release of inflammatory mediators (2) (such as catecholamines, cytokines, and ROS) causes pain, as well as increased vascular dilation, permeability, and resistance (3). As a short-term result, extravascular fluids accumulate to form interstitial edemas and skin blisters or leak out of the body, contributing to patient's dehydration, hypothermia, and tissue hypoxia (4). Immune suppression is also thought to develop as a long-term consequence of chronic inflammation, leaving the body defenseless against pathogens and bacteria (5) and causing sepsis. After few days, massive systemic metabolic and inflammatory dysregulations alter the normal wound healing process leading to hypertrophic scars. Altogether, these pathological changes can lead to a lethal multiple organ failure. ROS, reactive oxygen species.

stress hormones, and inflammatory mediators and triggers glycolysis, lipolysis, and proteolysis. After a severe burn trauma, hypermetabolism and hyperinflammation usually persist for years [10], leading to lean body mass breakdown, wound healing impairment, insulin resistance, immune suppression, organ dysfunction, and hypertrophic scarring (Fig. 1).

Burn wound healing. Cutaneous injuries initiate a cascade of events that tightly regulate the wound healing, according to three distinct but overlapping phases known as inflammation, proliferation, and remodeling (Fig. 2) [14,15]. The dysregulation of one of these phases might alter the normal repair process, causing epithelialization delays and pathological scars. Accordingly, several studies from the Eming's group highlighted the key role of the inflammation phase in wound healing duration and fibrosis emergence [16,17].

Traumatic burn injuries are characterized by an extreme and persistent inflammatory response driven by catecholamines, cortisol, and inflammatory cytokines (Fig. 2) [10]. Although today the factors influencing the burn wound healing have not been clearly identified, the exaggerated inflammatory response is thought to be paramount in burn pathogenesis. Catecholamine driven inflammation is indeed known to slow down the epithelialization process and the granulation tissue formation. In particular, long-lasting activity of beta-adrenergic agonists has been shown to trigger both neutrophil persistence and keratinocyte migration impairment during wound healing [18,19]. Hyperinflammation is also thought to alter the T_H1/T_H2 ratio, thus contributing to both immune suppression and fibrogenic responses in burn patients [20–23]. According to this paradigm, serum expression levels of early interleukin 1β (IL-1β), decorin,

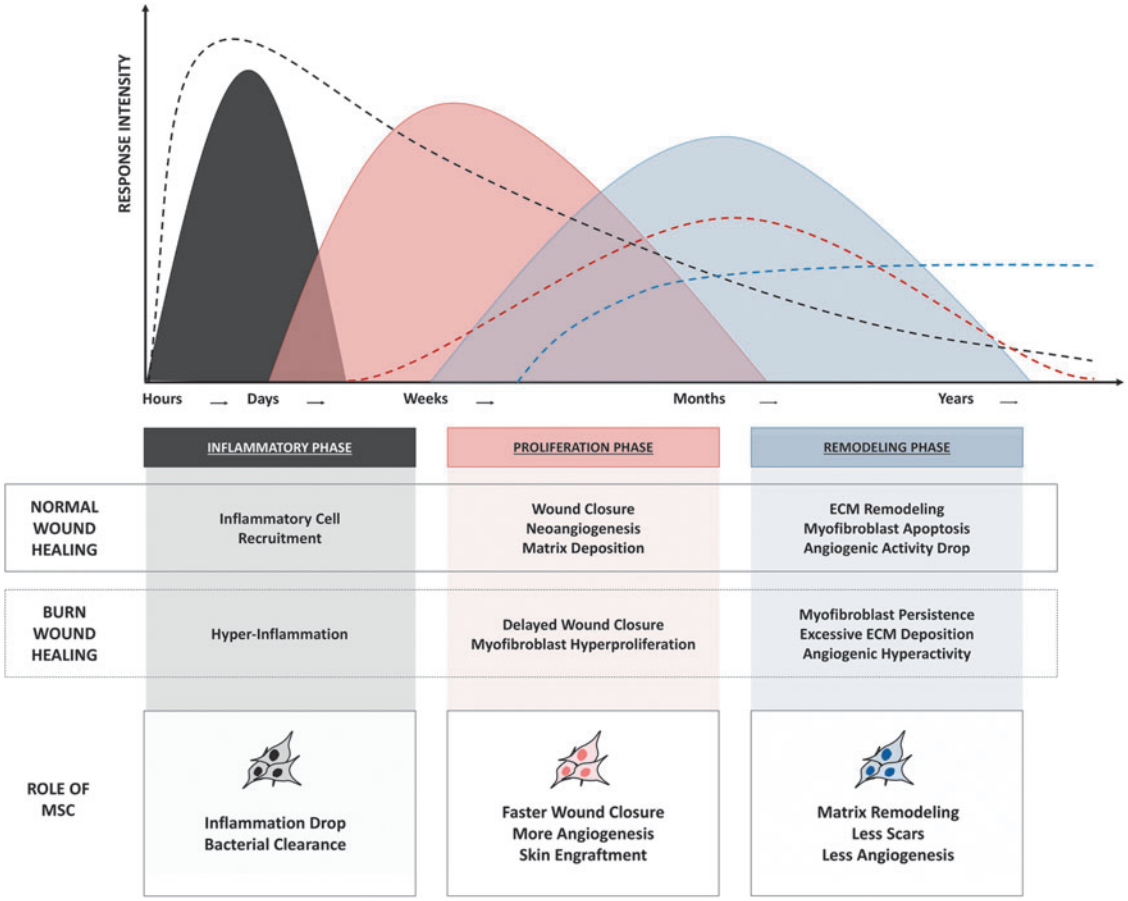

FIG. 2. Role of MSC in normal and burn wound healing. This schematic describes the three phases of wound healing both in normal and burn contexts and gives an overview of the possible therapeutic actions of MSCs. While the inflammatory phase resolves within days in a normal context, it persists for months and is more intense after burns. MSCs may help decrease inflammatory responses by secreting anti-inflammatory molecules, such as IL-1RA, TGF-β1, IDO, or TSG-6. They may also contribute to bacterial clearance through antimicrobial peptide synthesis or phagocytosis promotion. Wound closure, neoangiogenesis, and matrix deposition normally happen during the proliferation phase of wound healing. After burns however, these events are considerably slowed down and less effective. During this phase, trophic compounds secreted by MSCs may support wound closure, angiogenesis, and matrix deposition. The remodeling phase also differs between normal and burn wound healing. While myofibroblasts undergo apoptosis under normal conditions, they persist after burn, inducing excessive ECM deposition and hypercontractility. MSCs may thus aid tissue remodeling and scar mitigation through the expression of MMPs or anti-scarring molecules. ECM, extracellular matrix; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; MMPs, matrix metalloproteinases; MSC, mesenchymal stromal cell; TGF-β1, transforming growth factor beta 1; TSG-6, TNF-stimulated gene/protein 6.

and late transforming growth factor beta 1 (TGF-β1) were shown to predict the appearance of hypertrophic scars in burn patients [24].

In addition to the excessive inflammatory response, many other factors are believed to play a pivotal role in the development of hypertrophic scars, including myofibroblasts [25], mechanical stresses [26], and delayed wound healing [27–29]. However, whether these factors are the origins or the consequences of burn fibrogenesis still remains a matter of debate today.

Current treatments and limitations

To return to a normal wound healing process, several treatments are required to treat patients with major burn injuries [30]. They include heavy surgical procedures, intensive cares, wound dressing nursing, adapted nutrition, and sterile confinement (Table 1).

The surgical management of severe burns begins with the removal of necrotic tissues. This step is essential to clean up the wound from toxic cues and bacteria, curb local inflammation, and limit mechanical stresses. This surgery is followed by the covering of the wound bed using different kinds of grafts (ie, skin autografts, allografts, and skin substitutes) applied according to diverse surgical methods [4]. When donor sites are insufficient, alternatives to skin autografts can be used to reform the cutaneous barrier and limit the infectious ingress. In these particular cases, a two-stage procedure is carried out to restore the dermal compartment using collagenous matrices such as Integra™ [31] or cadaveric allografts [32] and the epidermal compartment using cultured epidermal autografts (CEAs) [33,34]. These alternatives provide a permanent covering of the burn wound and improve patient survival rates [35–37]. But the use of CEAs is still limited by a variable epidermal grafting efficiency [35,38–41], as a consequence of the immune and metabolic state of the patient, the immaturity of the newly formed dermal–epidermal junction (DEJ) resulting in skin blistering [42], the poor vascularization of the wound bed, and the high susceptibility to infections [30]. These obstacles therefore result in delayed wound repair and might contribute to the lack of functionality of the repaired tissue [34,43].

Given that infections are responsible in some cases of skin graft failure and sepsis, they have become a major therapeutic target. Several treatments such as topical antimicrobials or systemic antibiotics are highly effective to combat infections. Accordingly, clinicians most often agree to deliver topical antimicrobials until reaching full wound closure [44]. However, systematic practice is under debate, because of the growing bacterial drug resistance and the negative effect of certain drugs on wound closure. A challenging research area therefore seeks to develop novel antibacterial treatments for burn applications [45].

Resuscitation is one of the other major key points in the stabilization of patients with major burns. Large-volume resuscitation fluids contain crystalloids (ie, Ringer lactate)

Table 1. Global Burn Treatments and Management

Therapeutic targeting	*Strategies*	*Targeted effect*
Loss of cutaneous barrier	Wound coverage and grafting	Wound closure with auto or allogeneic skin graft, Skin substitutes
	MSC therapy	Wound closure
Burn wound conversion	Negative pressure therapy	Improving blood flow and tissue survival
	Bradykinin antagonist	Limiting vasodilatation and permeability
Infection	Escharectomy	Restraining bacterial colonization
	Topical antibiotics (silver sulfadiazine)	Protecting the wound from pathogens
	Systemic antibiotics	Protecting the whole body from pathogens
Hyperinflammation	Escharectomy	Reducing the release of inflammatory cues
	Vitamin supplementation	Limiting oxidative stress
	Fluid resuscitation (crystalloids and colloids)	Fighting hypovolemia
	Nitric oxide inhibitors (methylene blue)	Reducing vascular permeability
Hypermetabolism	Thermoregulation	Increasing room temperature to 33°C
	Enteral nutrition	Preserving intestinal mucosal integrity and decreasing stress hormone release
	Carbohydrate and fat intake monitoring	Limiting hyperglycemia and improving immune function
	Protein supplementation	Reducing lean body mass drop
	Vitamin supplementation	Improving wound healing and immune function
	Hormonal and pharmacological therapy (insulin, oxandrolone, testosterone, ketoconazole, IGF-1, propranolol)	Improving wound healing and limiting insulin resistance
Hypertrophic scarring	Surgery and grafting	Diminishing wound contraction
	Mechanical treatment (pressure therapy, splinting, physiotherapy)	Improving scar appearance
	Anti-inflammatory treatment	Limiting collagen production

The data shown in the table are extracted and summarized from previous reviews [13,30,46,48,51,126,127].
IGF-1, insulin-like growth factor-1; MSC, mesenchymal stromal cell.

and are sometimes supplemented with colloids (such as plasma or albumin). Although necessary to compensate for hypovolemia and burn shock, the excessive use of resuscitation fluids has been correlated with coagulative, pulmonary, and immunologic side effects [46]. Moreover, resuscitation fluids often induce hypernatremia, a well-known inhibitor of skin graft take [47]. Therefore, there is still a dreadful need for resuscitation fluid volume and composition optimizations.

Hyperinflammation plays a major role in burn pathophysiology. However, it is unclear whether reducing it would slow down the disease progression in humans. To investigate this possibility, several treatment modalities have been designed such as TLR antagonists, oxidative stress inhibitors, or inflammatory cytokine blockades [13,46] and are under current investigation in phase I clinical trials.

Hypermetabolism also is a major compound of the burn pathophysiology, as it disturbs the normal wound healing process and hormonal balance [48]. Anabolic agents, such as propranolol, have been evaluated in clinical trials and were shown to improve the burn wound healing [49] and reduce hypertrophic scarring [50]. However, their systematic use does not seem appropriate due to unwanted side effects.

To counteract hypertrophic scarring, the main treatments remain surgery and negative pressure therapy [51]. The direct targeting of the fibrogenic TGF-β1 signaling pathway has been early abandoned, as TGF-β1 knocked-out mice are known to develop systemic autoimmune disorders resulting in early death [52]. Other anti-scarring strategies have been investigated, such as reactive oxygen species (ROS) inhibition [53], vascular endothelial growth factor (VEGF) blockade [54], or peroxisome proliferator-activated receptor-γ activation [55,56], but none of them has led to a definitive solution.

Despite the broad range of strategies developed to treat severe burns, there still remain today many hurdles to be addressed, such as the healing time and the scar formation. However, novel cell-based therapies seem to hold great promises for the future of the burn wound management.

Mesenchymal Stromal Cell–Based Therapy

Mesenchymal stromal cells (MSCs) are fibroblastic cells that were originally found in the bone marrow [57] and later isolated from many other tissue sources [58] such as the gingiva [59], the adipose tissue [60], or the perinatal tissues [61]. MSCs are defined as plastic adherent cells, expressing specific surface markers ($CD73^+$, $CD90^+$, $CD105^+$, $CD34^-$, $CD45^-$, $CD11b^-$, $CD14^-$, $CD19^-$, $CD79a^-$, $HLA\text{-}DR^-$) and able to differentiate into osteocytes, chondrocytes, and adipocytes in vitro [62].

These cells have drawn much attention over the past decades due to their ability for tissue repair and immune tolerance. The use of MSCs in cell therapy was initially based on the hypothesis of their in situ differentiation to regenerate injured tissues. But it gradually became apparent that MSCs hold their therapeutic efficacy from their ability to modulate the surrounding cell responses through paracrine mechanisms [63]. Accordingly, MSCs have been shown to secrete a wide range of bioactive molecules, including cytokines, chemokines, growth factors, and lipid mediators [64]. In recent years, MSC-based therapies have therefore emerged as novel interesting strategies to improve the wound healing of a wide range of cutaneous diseases [65] and, more particularly, skin burn injuries [66].

MSCs in skin wound healing

MSCs have been reported to contribute to the wound healing resolution and scar mitigation by impacting on inflammation, bacterial clearance, reepithelialization, vascularization, granulation tissue formation, and ECM remodeling (Fig. 2).

The inflammation phase of wound healing can be regulated by MSCs due to their known immunomodulatory properties. Zhang et al. have indeed demonstrated that MSCs are able to modulate the inflammatory milieu and promote wound repair as a consequence of the polarization of macrophages from a pro-inflammatory to an anti-inflammatory phenotype [67]. Other studies have shown that MSCs can secrete tumor necrosis factor alpha (TNF-α)-stimulated gene/protein 6 (TSG-6), an anti-inflammatory cue known to impede TNF-α-driven inflammation and fibrosis during wound healing [68,69].

Angiogenesis and wound closure can also be controlled by MSCs to avoid chronic wound emergence. Accordingly, several studies have demonstrated that MSCs are able to stimulate acute wound closure [70] and neovascularization [71] through the paracrine release of growth factors in vivo. This paracrine action was shown to enhance keratinocyte and dermal fibroblast migration, as well as endothelial tubule formation using MSC-conditioned medium or MSC-derived microvesicles in vitro [70,72–74].

Matrix remodeling and scar mitigation can also be orchestrated by MSCs in the final stages of wound repair. The use of MSCs or their derived microvesicles/exosomes resulted in a significant reduction of α-smooth muscle actin (α-SMA), collagen expression, and a transition to a low TGF-β1/TGF-β3 ratio in acute wound animal models [69,75]. These antifibrotic activities are possibly mediated by TSG-6 [69], mi-RNAs inhibiting TGF-β2/SMAD2 pathway [75], or matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) balance [76,77].

MSCs in burn wound healing

Preclinical studies. As outlined in Table 2, MSCs have shown their beneficial effect on each stage of the burn wound healing in many animal models of burn [66].

In response to hyperinflammation, MSCs have been shown to secrete modulatory factors that limit immune cell accumulation and inflammatory cytokine production at both local [78,79] and systemic levels [80]. In particular, MSC-derived TSG-6 has been reported to reduce the burn-induced inflammation through P38 and c-Jun N-terminal kinase (JNK) signaling pathways [81]. Likewise, MiR-181c contained in MSC-secreted exosomes appeared to downregulate the TLR4-mediated inflammation in burn rats [82]. In addition to play on inflammatory pathways, MSCs administered at the burn site were shown to guide macrophage polarization toward a M2 reparative phenotype [79,83].

After burn, the proliferation phase of wound healing is significantly slowed down. Interestingly, MSCs [84] or their derived microvesicles [73,85] have been shown to counteract this effect by accelerating wound closure and neovascularization [78,86]. Accordingly, MSC administration resulted in angiopoietin *(ANG)-1/2* gene upregulation in several animal models of third degree burn [84,87].

Burn injuries often result in hypertrophic scars, because of a dysregulated matrix remodeling. MSCs were shown to mitigate this burn-induced scarring through the regulation of

Table 2. Summary of Preclinical Studies Using Mesenchymal Stromal Cell-Based Therapy to Treat Thermal Burn Injuries

Species	*MSC origin*	*Number of cells*	*Methods of MSC application*	*Results*	*References*
Mouse	BM-MSC, human, xenogeneic	500,000 cells/cm^2	Intradermal injections around the wound and topical applications in Matrigel on the wound directly after the burn	Enhancement of wound closure and angiogenesis	[84]
Rat	BM-MSC, human, xenogeneic	2,000,000 cells/rat	Injections into the muscle immediately after the burn	Decrease of systemic inflammation	[208]
	BM-MSC, allogeneic	1,000,000 cells per stasis zone (×6)	Subcutaneous injections around the wound 30 min after burn	Enhancement of tissue viability in the stasis zone and decrease of systemic inflammation	[209]
	UC-MSC, human	5,000,000 cells	Tail vein injections at day 3 after burn	Enhancement of wound closure, angiogenesis and remodeling, and decrease of inflammation	[78]
	BM-MSC, mouse, xenogeneic	5,000,000 cells/rat	Intradermal injections around the wound, 20 min after burn	Enhancement of reepithelialization and angiogenesis and decrease of inflammation	[80]
	UC-MSC, human, xenogeneic	1,130,000 cells/cm^2	Subcutaneous injections, 24 h after burn	Enhancement of wound closure and granulation tissue formation and decrease of inflammation	[210]
	UC-MSC, human, xenogeneic	5,000,000 cells/animal	Tail vein injections directly after burn	Decrease of systemic inflammation	[81]
	BM-MSC, allogenic, or autologous	40,000 cells/cm^2	***Escharectomy 2 days after burn and direct cell application with a pipette onto the wound***	Enhancement of wound closure and angiogenesis	[211]
	BM-MSC, allogeneic	20,000 cells/injection	***Escharectomy 2 days after burn and direct cell application with a pipette onto the wound***	Decrease of inflammation and increase of vessel formation at day 7	[212]
	BM-MSC, allogeneic	30,000,000 cells/cm^2	***Escharectomy 4 days after burn and cell application in fibrin gel onto the wound***	Enhancement of wound closure and absence of wound contractures, formation of hair follicle-like structures, and sebaceous glands	[88]
	ASC, allogeneic	200,000 cells/mL of fibrin	***Escharectomy 1 day after burn and cell application in fibrin gel onto the wound***	Enhancement of angiogenesis and inflammation modulation toward a M2 phenotype	[83]
	BM-MSC, allogeneic	500,000 cells/cm^2	***Escharectomy 3 days after burn and cell seeding onto a Small intestinal submucosa covering the wound***	Enhancement of wound closure and angiogenesis	[213]
Pig	BM-MSC, autologous	100,000 cells/cm^2	Application of cells in a collagen matrix onto the burn eschar	Enhancement of wound closure and angiogenesis	[214]
	BM-MSC, rabbit, xenogeneic	2,000,000 cells/cm^2	***Escharectomy directly after burn and grafting of acellular dermal matrices. On days 7 and 14 after burn, cell spraying in fibrin gel***	Enhancement of vascularization and hair follicle development	[92]
	BM-MSC, allogeneic	1,000,000 cells/cm^2	***Escharectomy and cell application in fibrin gel onto the wound***	Enhancement of wound closure	[215]
	ADRC, autologous	250,000 cells/cm^2	***Escharectomy 2 days after burn and cell spraying in fibrin gel or dermal injection***	Enhancement of wound closure and angiogenesis, reduction of inflammatory cell infiltration	[86]
	ASC, allogeneic	15,000–150,000/cm^2	***Escharectomy 4 days after burn and cell application in functionalized hydrogels***	Enhancement of angiogenesis	[94]

Highlighted in *bold* and *italic*: methods with an easiest translation to clinic.
ADRC, adipose derived regenerative cells; ASC, adipose stromal cells; BM-MSC, bone marrow mesenchymal stromal cell; UC-MSC, umbilical cord mesenchymal stromal cell.

excessive collagen deposition and fibrotic growth factor expression (ie, TGF-β1) [78,79]. In addition to their regulatory effect on matrix deposition, some investigators have even claimed that MSCs could have a favorable impact on adnexal tissue repair, especially on hair follicles and sebaceous glands [88,89].

Regarding infection and sepsis, MSCs can exert a bactericidal activity through direct and indirect mechanisms. They can secrete antimicrobial peptides and proteins such as LL-37 [90] or stimulate macrophage phagocytosis through phosphoinositide 3-kinase activation and prostaglandin E2 (PGE_2) production [91].

Limitations of the current preclinical models. Despite major beneficial outcomes, MSC treatment strategies used in preclinical burn models remain difficult to apply in clinical practice. As reported in Table 2, the burn eschar is rarely removed in preclinical studies, while it is the standard practice in the clinics. Likewise, skin grafts remain poorly used in research studies, while they are essential for patients. Recently, a few studies have although come closer to the clinical practice, using both MSCs and dermal grafts to treat burns [92]. This combination was shown to reduce fibrosis and improve skin engraftment and angiogenesis in early time post-treatment [93,94]. Another concern is the time at which MSCs are administered. Research studies often use them immediately after burn induction, while this scenario appears clinically barely possible, especially in case of autologous treatments. Early treatment could yet be of real interest to limit burn-induced inflammation and necrosis spreading. However, this option implies that allogeneic MSCs or MSC-derived products are already banked and readily available. To reach this goal, time, dose, and mode of MSC administration will still have to be optimized to significantly improve the clinical treatment of burns [95].

Clinical case reports

Current clinical case studies. In 2005, Rasulov et al. were the first to report the successful use of MSCs and skin grafts to treat a 45-year-old woman suffering from a 30% total body surface area (TBSA) full-thickness burn [96]. Since then, only a few other similar cases have been reported in the literature (Table 3). MSC therapy was generally used in combination with a surgical treatment allowing the removal of the eschar and the application of an autograft. In these clinical studies, the MSC therapy was strikingly shown to attenuate pain [96] and scarring [97,98] and promote skin engraftment [96,99] through granulation tissue formation

Table 3. Summary of the Clinical Cases Involving Mesenchymal Stromal Cell-Based Therapy to Treat Severe Thermal Burn Injuries

%TBSA[a]	Number of patients, gender, age	MSC therapy				References
		Source	Dose	Treatment protocol	Results	
30	1, Female, 45 years old	BM-MSC, allogeneic	20,000–30,000 cells/cm^2	Topical application of cells after escharectomy and 4 days later application of autografts combined with cells (on autograft and donor site)	Rapid formation of granulation tissue, neoangiogenesis, and better graft take	[96]
30	1, Male, 26 years old	BM-MSC, allogeneic	10,000 cells/cm^2	Cell spraying in fibrin matrix after escharectomy, 35 days later application of an expanded autologous skin graft with cells	Better granulation, tissue formation, reepithelialization from the wound margins, and angiogenesis	[99]
60 and 40	2, Female, 22 years old, male, 41 years old	BM-MSC, autologous	Not mentioned	Covering of the wound with a cell loaded collagen sponge	Wound closure achieved between 2 and 4 weeks, better scar appearance	[97]
From 10 to 25	60, Female and male, from 15 to 50 years old	BM-MSC, autologous and UC-MSC allogeneic	100,000 cells/cm^2	Topical or subcutaneous injections of cells at day 2 and 10 after escharectomy	Better wound healing, hospitalization time shortage	[98]
80	1, Male, 19 years old	BM-MSC, autologous	2,100,000 cells/mL	Subcutaneous cell injections after scar excision and autologous skin grafting	Less skin graft contraction, less scar complications	[100]

[a]%TBSA only refers to the extent of the third-degree burns and might not encompass the entire burn surface area of the patient.
TBSA, total body surface area.

[96] and angiogenesis [99]. A recent study involving 60 patients reported a better wound healing rate in groups treated with both skin autografts and MSCs compared to skin autografts alone [98]. However, conversely to preclinical data, only a single study outlined the regulatory effect of MSCs on inflammatory cell infiltration in burn patients [97]. MSCs were also used at a late time point during the remodeling phase of the burn wound healing in an attempt to treat hypertrophic scars and reduce skin contraction [100].

MSC source, dose, and timing of administration. Although no study has clearly identified the best tissue source of MSCs, the bone marrow remains so far the preferred harvest location in burn clinical studies (Table 3). Yet, current clinical data indicate that other cell sources could be of equal efficacy such as umbilical cord tissues [98]. Regarding the applied cell doses, no consensus has been found yet, and great variabilities between cell administration protocols can be noticed from a study to another. Of interest, no dose has been reported to trigger deleterious effects, and the average applied dose in the current clinical reports is around 20,000 MSCs/cm^2.

To our knowledge, the MSC-based therapy has never been used in the acute phase of the burn wound healing. Instead, most clinical cases reported the use of MSCs during the proliferation phase of the burn wound healing and more rarely during its remodeling phase [100]. Moreover, the application of MSCs in clinics has always been performed in combination with a skin graft. However, it is currently unknown whether the MSC-based therapy is more likely to work in concert with cultured epithelial autografts, allograft cell sheets, or keratinocyte cell suspensions compared to other current treatments [41,101,102].

Toward a clinical use of MSC-based therapies

Cell-based therapies: autologous or allogeneic source? Although several clinical studies have reported no adverse effects in the use of autologous and allogeneic MSC (Table 3), it is still hard to conclude on the best donor source option, due to the very few number of patients involved in these trials. Autologous cells are advantageous in terms of immune tolerance. However, as they come from a pathological environment they may hold a substandard therapeutic efficacy. Moreover, their host well-acceptance may possibly trigger a higher risk of tumorigenesis, especially after a cultivation step in vitro [103]. In terms of clinical production, autologous cell sources are more expansive due to patient-dependent production times and lack of immediate availability.

On the contrary, the use of allogeneic cells is supported by their intrinsic ability to bypass the immune surveillance due to low major histocompatibility complex (MHC) and costimulatory molecule expression levels [104,105]. However, studies have shown that bank cryopreserved MSCs exhibit less efficient immunomodulatory properties after thawing [106–108], although a functional recovery is observed after 1 day in vitro culture [106]. Moreover, pathological inflammatory contexts are likely to induce the MHC expression in allogeneic MSCs, making them a possible target of the host immune system [109]. Thus, intramuscular injections of allogeneic MSCs were shown to stimulate anti-donor IgG formation [110]. In addition, these cells may not present tumorigenic risks as they can rapidly be eliminated from the body.

From a clinical point of view, there is no clear picture regarding the use of allogeneic MSCs. Both disappointing and encouraging results were indeed reported in phase-III clinical trials [111]. These discrepancies obviously highlight the key role of cell preparation and administration protocols that vary a lot from a trial to another. Recently, the success of a phase III clinical trial involving allogeneic MSC for the treatment of Fistular Crohn's disease was indeed partly due to a local high-dose administration of cultured-amplified MSC after thawing [112].

In the context of early burn wound management, the use of banked, cryopreserved allogeneic MSC is the only possible option. However, when considering the treatment of later burn phases, the use of autologous MSC becomes an alternative, as production times are no longer a constrain. At present, the autologous cell source may hold the best efficiency compared to the allogeneic source [113]. But further preclinical and clinical studies are needed to clarify the impact of the immune response on MSC efficacy in the context of severe burn.

Cell-derived product therapy. Due to the fact that MSCs mainly operate through their paracrine secretions, derivative secretory products, such as conditioned media or microvesicles, have drawn much attention in the recent years [114]. The main advantage of using these secretory products is to avoid the use of cells that may induce a risk of tumorigenesis, immunogenicity, embolus formation, and pathogen transmission. Moreover, these products might be easier to bring to the market, as they can be framed under the current pharmaceutical regulations. However, secretory product standardization might be difficult to set in place, due to the variability observed among various cell sources and donors. Accordingly, MSC-derived secretomes originating from unrelated tissue sources were recently shown to contain different profiles of neurotrophic and neurogenic factors [115]. In other studies, MSC secretory products originating from different donors were reported to bear disparate immunomodulatory effects [108,116]. At last, cell-derived MSC products are known to be less efficient than MSCs themselves, especially in inflammatory disease models [117].

Cell preconditioning. Cell-based and cell-derived product therapies both face critical issues that limit their wide clinical use. While cell-based therapies have to cope with interdonor variability and cell exhaustion, cell-derived products lack effectiveness. Preconditioning, also known as priming or licensing, is a strategy used to improve the response of a tissue or cell population to a particular environment. This approach was first reported in the literature in 1986 by Reimer et al. who successfully improved the myocardium resilience to ischemia by exposing it to repeated short cycles of hypoxia [118,119].

In the context of cell therapy, the preconditioning approach can help prepare MSCs to a pathological environment by stimulating protective and survival pathways. Preconditioning can also help design customized cell therapies through the promotion of specific signaling pathways, targeting tissue homeostasis dysfunction or disease [120]. In fact, a recent report showed that the reduced immunomodulatory functions of MSCs induced by a long-term exposure to palmitate, a known factor found in type 2 diabetes environment, could be rescued by a short preconditioning of these cells [121]. Our laboratory has thus showed that the immune suppression property of MSC-derived products is strongly improved using priming method [122]. Interestingly, other investigators have also shown that the preconditioning approach could also serve to minimize interdonor variability [109].

MSC Preconditioning Approach in Severe Burn Cell Therapy

As described by Caplan and Correa in 2011, wound healing is a biological process in which the preconditioning paradigm is at stake. Upon injury, resident and recruited cells get primed by mechanical and biological stressors that initiate the wound repair process [123]. Similarly, the burn wound gives rise to different priming stressors at early, middle, and late stages, altering cell behaviors at both local and systemic levels. Therefore, the treatment of burns must be adapted to the stage of the disease to reach high efficacy. In this section, we will review the preconditioning modalities that may potentiate the MSC therapy at each stage of the burn trauma. Hence, we will sequentially dissect the burn pathophysiology and give an overview of the priming modalities susceptible to improve MSC therapeutic efficacy (summary in Fig. 3 and Table 4).

MSC preconditioning strategy to tackle early-stage burn responses

Early pathophysiological responses to major burns are characterized by infections, hyperinflammatory responses, and hypermetabolism. These features prevent the burn wound from healing spontaneously and appear to be governed by an accumulation of ROS/reactive nitrogen species (RNS) [124,125], stress hormones, including catecholamines [126–128], and free fatty acids (FFAs) [48]. In this section, we will emphasize the potential of mild inflammatory primings to augment MSC therapeutic effects during early burns.

Fighting wound infections. To prevent postburn infections, mild inflammatory primings are interesting strategies, as they promote direct and indirect MSC antimicrobial activities. To our current knowledge, MSCs are able to synthesize four different antimicrobial peptides, including LL-37, beta-defensin 2, hepcidin, and lipocalin-2 [90]. In an ex vivo model of lung injury, lipopolysaccharide (LPS) was reported to promote the MSC-derived synthesis of LL-37 [129]. Recent observations in our laboratory also showed that low-dose IL-1β primed MSCs could secrete high levels of fibroblast growth factor (FGF)-7 (unpublished data), a factor known to promote peripheral blood mononuclear cell (PBMC) survival and phagocytic activity [130].

Mitigating the hyperinflammatory response. After traumatic burn injuries, DAMP, pathogen-associated molecular pattern (PAMP), and stress hormones activate the JNK signaling pathway through G-protein coupled receptors and initiate an inflammatory cascade [127]. To attenuate this pathological process, inflammation-related cues have been the most commonly used priming agents [131–134]. Mild inflammatory licensings are indeed known to make MSCs secrete a

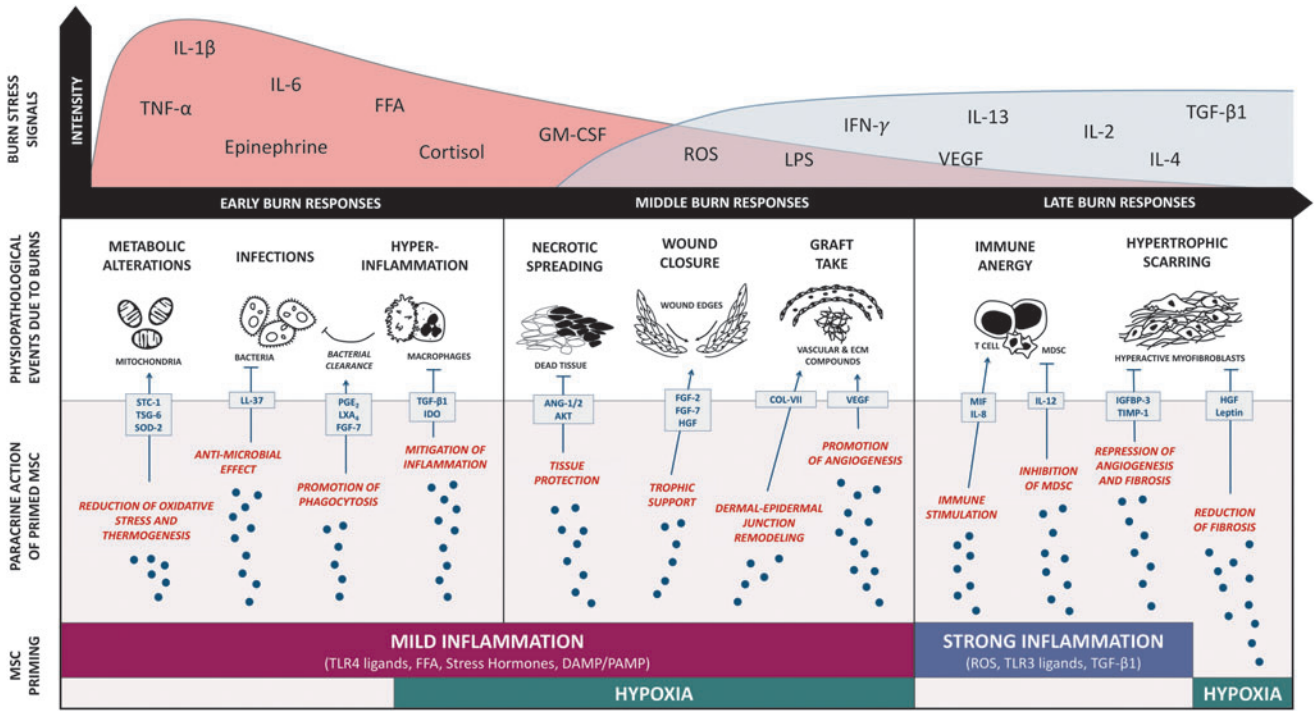

FIG. 3. Adapting the preconditioning to the burn pathophysiology. This schematic presents, on the upper part, the main pathological stressors involved in the burn pathophysiology. During early burn responses, IL-1β, TNF-α, IL-6, Cortisol, FFA, or epinephrine is massively released at local and systemic levels. In middle and late burn responses, a stress signal switch occurs with an augmentation of TGF-β1, IL-2, IL-4, IL-13, and VEGF, all of which are implicated in immune suppression and hypertrophic scar formation. The lower part of this schematic shows the effect of several preconditioning modalities on MSC actions (in *red*) and paracrine activity (in *blue*). As shown, mild inflammatory primings can improve MSC therapy during the early and middle stages of the burn pathophysiology. Hypoxia is rather to be used at the middle stages. At last, strong immunomodulatory priming can serve during the late stages. Interestingly, burn stress signals that are expressed during the course of the burn pathophysiology often correspond to the preconditioning agents used to improve MSC therapy. FFA, free fatty acid; IL, interleukin; TGF-β1, transforming growth factor beta 1; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

Table 4. Summary of Preclinical Studies Using Preconditioned Mesenchymal Stromal Cells to Treat Thermal Burn Injuries

Target		*Cell-based solution*			
Burn phase	*Event*	*Experimental setting*	*Priming*	*Results*	*Ref.*
Early stage	Infections	*Escherichia coli* and *Staphylococcus aureus*	LPS	⇗ LL-37, bacterial clearance	[129]
		Cecal ligation and puncture (mouse)[a]	CO	⇗ Phagocytosis, efferocytosis	[187]
	Metabolic alteration	Lung epithelial cell line	Low pH-induced apoptotic signals	⇗ STC-1 ⇗ cell survival	[152]
		Scald burn injury (rat)[a]	TNF-α+LPS	⇗ TSG-6 ⇗ body temperature	[81]
	Hyperinflammation	PMA activated PBMC	TNF-α	⇗ SOD2 ⇗ IFIT1 ⇗ MX1 ⇗ IL-10	[216]
		LPS activated T cells	SP	⇗ TGF-β1 ⇘ IL-2	[140]
		LPS activated macrophages	IL-1β	⇗ M2 polarization	[142]
		DSS-induced colitis (mouse)[a]	IL-1β	⇗ Treg ⇗ $CD206^+$ cells	[217]
Middle stage	Tissue injury	HUVEC on Matrigel	Hypoxia	⇗ ANG-1/ANG-2 ⇗ tube formation	[163]
		Hind limb ischemia (mouse)[a]	Hypoxia	⇗ Tissue perfusion	[156]
	Delayed wound closure	Epithelial cell lines	TNF-α+IL-1β	⇗ FGF-2, HGF ⇗ wound closure	[158]
		Full-thickness excisional wounds (mouse)	AA	⇗ MT3-MMP ⇗ wound closure	[162]
		Full-thickness excisional wounds (mouse)[a]	S100A8/A9	⇗ IL-32 ⇗ MMP-27 ⇗ C3a ⇗ wound closure	[160]
	Graft take impairment	Recessive epidermolysis bullosa (mouse)[a]	TGF-β1+TNF-α	⇗ COL-7 ⇘ skin blistering	[166]
		Full-thickness excisional wounds (mouse)[a]	Hypoxia	⇗ Graft viability ⇗ angiogenesis	[93]
Late stage	Immune anergy	LPS and IFN-γ activated B cells	IFN-γ+LPS	⇗ COX-2 ⇘ IL-10	[180]
		IL-12 and IL-18 activated NK cells	IL-12+IL-18	⇗ NK activation ⇗ IFN-γ	[218]
		PMN	LPS+poly(I:C)	⇗ PMN survival ⇗ IL-6 ⇗ GM-CSF	[186]
	Hypertrophic scars	Hypoxic fibroblasts	Hypoxia	⇘ α-SMA ⇘ pSMAD	[194]
		Scald burn injury (mouse)[a]	Hypoxia	⇘ Collagens ⇗ HIF-1α	[195]
		Ear wound injury (rabbit)[a]	H_2O_2	⇘ Scar formation ⇘ TGF-β1	[68]
		Bleomycin-induced fibrosis (mouse)[a]	Hypoxia	⇘ ECM proteins ⇗ HGF	[196]

[a]*In vivo* studies.

AA, arachidonic acid; ANG, angiopoietin; α-SMA, α-smooth muscle actin; COL, collagen; C3a, complement component 3a; CO, carbon monoxide; COX-2, cyclooxygenase 2; DSS, dextran sulfate sodium; ECM, extracellular matrix; FGF-2, fibroblast growth factor; GM-CSF, granulocyte macrophage-colony stimulating factor; HIF-1α, hypoxia-inducible factor 1-alpha; IFIT1, interferon induced protein with tetratricopeptide repeats 1; IFN-γ, interferon-γ; IL, interleukin; HGF, hepatocyte growth factor; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; MT3-MMP, membrane-type3 matrix metalloproteinase; MX1, interferon-induced GTP-binding protein; NK, natural killer; PBMC, peripheral blood mononuclear cell; PMA, phorbol myristate acetate; PMN, polymorphonuclear neutrophils; SOD2, superoxide dismutase 2; STC-1, stanniocalcin-1; TGF-β1, transforming growth factor beta 1; TNF-α, tumor necrosis factor alpha; TSG-6, TNF-stimulated gene/protein 6.

wide range of anti-inflammatory molecules such as TGF-β1, IDO (indoleamine 2,3-dioxygenase), TSG-6, hepatocyte growth factor (HGF), galectins, and semaphorins [131,135–138]. Recently, a study showed that TNF-α and LPS primed MSCs could alleviate the inflammatory reaction in a scald burn rat model, as highlighted by reduced systemic levels of TNF-α, IL-1β, and IL-6 [81]. In another study, the neurogenic and pro-inflammatory Substance P, known to be released in high amounts after burn [139], was shown to induce the secretion of TGF-β1 from primed MSCs and improve the ability of these cells to suppress $CD4^+$ T cell activities [140]. Inflammatory primings can also have an indirect impact on immune cell functions. Accordingly, inflammation-licensed MSCs are able to synthesize high levels of PGE_2 and Lipoxin A4, two lipid inducers of efferocytosis and phagocytosis [117,141]. In another study, Song et al. successfully polarized macrophages toward a M2-like phenotype using IL-1β stimulated MSCs and observed a diminution of TNF-α synthesis and an increase in IL-10 release in response to LPS [142].

Apart from inflammatory cytokines, stress hormones can also promote antioxidative and anti-inflammatory secretions in MSCs. In a recent study, epinephrine was shown to activate MSC immunomodulation when cocultured with LPS-treated macrophages by regulating key inflammatory cytokines, including TNF-α, IL-1β, IL-1RA, and IL-10 [143]. This effect was confirmed in a rat model of lung injury, where primed MSCs reduced inflammation, edema formation, and hemorrhage. Exendin-4, a glucagon-like peptide-1 homolog, was shown to trigger the synthesis and the activity of antioxidative enzymes such as glutathione, glutathione peroxidase, and superoxide dismutase in hydrogen peroxide-intoxicated MSC [144]. In the same study, Exendin-4 stimulation reduced the production of malondialdehyde, a lipid peroxidation end product found in burns.

Other studies have also reported a better ability of MSCs to mitigate inflammatory processes after a hypoxic or ROS preconditioning. Accordingly, mild hypoxia [145] and hydrogen peroxide [68] licensed MSCs were able to suppress the proliferation of activated PBMCs, in a TSG-6 dependent mechanism [68]. In accordance with these in vitro observations, the use of a conditioned medium from hypoxic MSCs was reported to reduce the amount of inflammatory and oxidative stress mediators, including IL-17, interferon-γ (IFN-γ), inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and JNK in an experimental autoimmune encephalomyelitis mouse model [146].

Suppressing hypermetabolism. To cope with hypothermia, a catabolic switch is often observed in burn patients, which in turn stimulates the process of thermogenesis. This metabolic change leads to excessive glycolysis, proteolysis, and lipolysis and contributes to lean body mass breakdown [128]. As a consequence, elevated plasma levels of triglycerides and FFAs are found in both animals and humans with major burns [48,127,147]. Saturated and mono-unsaturated FFAs are known to support TLR4 inflammation [148], contribute to insulin resistance [48] and, therefore, impair the normal wound healing process. Inflammation-licensed MSCs have been shown to bridle metabolic dysregulations. In particular, TNF-α and LPS preconditioned MSCs were able to minimize the body temperature drop early after injury in a scald burn rat model [81]. MSCs were also shown to contribute to insulin sensitization through the blockade of the NLRP3 (NOD-like receptor protein 3) and the IRS-1 (insulin receptor substrate 1) [149] by secreting STC-1 (stanniocalcin-1) [150], an antioxidative and anti-inflammatory protein, known to be upregulated after a 3D-spheroid culture [151] or an apoptotic signal [152] preconditioning of MSCs.

MSC preconditioning strategy to combat middle-stage burn responses

As discussed earlier in this review, the treatment of deep and extensive burns requires a surgical act that includes burn eschar excision to impede necrosis progression and skin grafting to recreate a functional barrier. However, due to the dysregulated metabolic and inflammatory state of burn patients, skin donor sites and burn wounds struggle to heal rapidly. As a result, comorbidities and pathological scars develop due to delayed reepithelialization [153]. Herein, we will provide an overview of the licensing modalities that could drive MSCs toward pro-healing and reparative activities to improve the middle-stage burn wound healing. In particular, we will emphasize the contribution of both the hypoxic and inflammatory primings in MSC-mediated wound closure, angiogenesis, and tissue protection.

Fighting tissue viability loss, ischemic injury, and necrosis. Retardation in burn eschar removal is known to promote the extension of the coagulation necrosis to the surrounding stasis zone [154]. This pathological progression therefore results in greater tissue viability loss, ischemic injuries, and necrosis. Hypoxia is an interesting priming to combat these tissue alterations as it stimulates the MSC synthesis of pro-survival factors such as VEGF, insulin-like growth factor-1 (IGF-1), FGF-2, and HGF [70,155]. Several animal models of reperfusion injury or wound healing have underlined the benefit of hypoxia-preconditioned MSCs in terms of tissue survival and neovascularization using direct administration of cells [156], conditioned media [70], or microvesicles [157]. From a mechanistic point of view, hypoxia would activate pro-survival and pro-angiogenic pathways in MSCs, including Akt and c-Met [156].

As for hypoxia, inflammatory licensings can also activate pro-survival and trophic factors in MSCs. Under low stimulation levels of IL-1β and TNF-α stimulation, for example, MSCs can release high levels of VEGF and HGF and activate the pro-survival extracellular signal-regulated kinase (ERK)1/2 signaling pathway in airway epithelial cells [158]. Priming MSC with IL-1β, TNF-α, and NO could also enhance secretion of pro-angiogenic mediators and promote tissue survival in model of radiation-induced intestinal injury [159].

Improving donor site healing. Slow- or nonhealing skin donor sites are major curbs in the management of severe burns, as they lead to increased occurrence of infections and prolonged hospitalization times. In an attempt to improve the repair of these donor sites, the MSC preconditioning strategy can serve to trigger the secretion of MSC-derived trophic factors accelerating local wound closure.

Inflammatory-primed MSCs are known to accelerate cutaneous wound closure. In a recent study, Broekman et al. successfully improved the proliferation and migration of epithelial cells through an epidermal growth factor receptor (EGFR) dependent mechanism using IL-1β and TNF-α

licensed MSCs [158]. Licensing MSCs with S100A8/A9, a calcium-binding DAMP protein known to activate the TLR4 signaling, were also shown to accelerate wound closure in a full-thickness skin excisional murine model through IL-32, MMP-27, and complement component 3a (C3a) upregulation [160]. In other studies, hypoxia caused MSCs to release pro-migratory factors stimulating the migration of epithelial cells in vitro [70,156] and accelerating the closure of excisional wounds in vivo [145]. Lipid mediators such as all-trans retinoic acid [161] and arachidonic acid (AA) [162] have also been reported to promote MSC-driven tissue repair and acceleration of wound closure in excisional skin wound mouse models. In particular, the AA priming of MSCs was shown to regulate fibronectin degradation and wound closure through the activation of the mTORC2 (mechanistic target of rapamycin complex 2) pathway and the expression of MT3 (membrane-type-3)-MMP [162].

Promoting skin engraftment. Although many studies have reported the beneficial effect of primed MSCs on wound closure, very little is known about their ability to help skin graft take in large tissue defects. Based on clinical observations, both functional blood supply and mature DEJ would be needed to prevent skin graft viability loss and blistering. Therefore, MSC licensings are expected to trigger pro-angiogenic and DEJ remodeling responses to positively impact on skin graft take.

Hypoxia-preconditioned MSCs were shown to secrete high protein levels of VEGF and ANG-1 and induced better endothelial tubule formation in vitro [163,164]. Once injected with endothelial cells, hypoxic MSCs were reported to promote graft viability and vascularization and to reduce contraction in a model of full-thickness skin excision [93]. In a very different setting, exosomes from hypoxic MSCs were shown to promote fat graft survival through enhanced angiogenesis and reduced inflammation [165].

Inflammation-related primings may also influence MSC-mediated DEJ remodeling. Both TGF-β1 and TNF-α licensed MSCs improved type-VII collagen deposition and mitigated skin blistering in a model of recessive epidermolysis bullosa [166]. Other studies reported that IFN-γ [131] and IL-1β [138] preconditioned MSCs secrete high levels of TGF-β1, a growth factor known to play pivotal roles in DEJ formation [167–170].

MSC preconditioning strategy to bypass late-stage burn responses

After the hyperinflammatory reaction, the pathophysiology of severe burns evolves toward immune suppression and hypertrophic scarring [154,171]. As previously mentioned in this review, this pathological switch partly ensues from a systemic dysregulated expression of the TLR downstream mediators [13], which include IL-10, IL-4, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1β, IL-2, and IFN-γ [10,172]. The expression levels of several growth factors, such as TGF-β1 and VEGF, are also increased in the late stages of the burn trauma, leading to hypertrophic scars [173,174]. In this complex pathological system, the use of licensed MSCs is expected to reverse the immune suppression and mitigate the scarring response. Herein, a specific attention will be drawn to hypoxic and strong inflammatory MSC preconditionings.

Reactivating the immune system. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous cell population initially discovered in tumors and later described in burns [175]. These cells play significant roles in acquired immune system suppression and angiogenesis promotion [176,177]. The mobilization of MDSC is driven by VEGF and GM-CSF and leads to the release of Arginase, RNS, and ROS, all of which are implicated in inflammatory processes, bacterial clearance activity, and immune dysfunction [177]. Cancer research studies have shown that immune system reactivation could be accomplished by suppressing MDSC activities either through induced differentiation into mature myeloid cells or mobilization and expansion arrest [177]. Although the role of MDSC on postburn immune suppression has not been studied in depth like in cancer, one might assume that inhibiting these cells in a late burn context could improve immune reactivity. To our knowledge, the impact of the MSC preconditioning on MDSCs has scarcely been studied. What we know at best is that tumor-educated MSCs can promote MDSC growth and activity [178]. But an emerging body of evidences is now suggesting that under specific priming conditions MSCs could repress MDSC activities and reactivate the immune system.

TLR agonists are known to regulate MSC immunomodulatory activities. They can thus trigger antagonistic inflammatory responses in MSCs, depending on the priming dose or on the presence of other priming agents. Lately, our team showed that MSCs can rescue LPS-intoxicated T cell survival, after a priming with both LPS and pamidronate, but not with LPS alone [179]. High-dose LPS and IFN-γ preconditioned MSCs were recently shown to prevent LPS-activated B cells from secreting IL-10, a well-known repressor of T_H1 cell subset activation [180]. The LPS priming also triggered MSC derived synthesis of IL-8 and macrophage migration inhibitory factor, two promoting factors of polymorphonuclear neutrophil survival, migration, and function [181].

In addition to their direct contribution to MSC-driven immune stimulation, TLR agonists are likely to influence interactions between MSCs and MDSC. Accordingly, the preconditioning of MSCs with CpG oligodeoxynucleotide [182] or poly(I:C) [183] was shown to induce the production of IL-12, a putative MDSC differentiation inducer, along with vitamin A metabolites and vitamin D3 [184]. TLR agonists therefore constitute privileged priming cues to direct MSC immunomodulatory functions. However, because their mechanisms of action still remain poorly understood, conflicting data have emerged in the literature [185,186], highlighting the complexity of these priming cues and the need for further investigations especially regarding the dose.

Analogously to TLR agonists, ROS primings can modify MSC immunomodulatory properties depending on the other priming cues expressed in the local milieu. Recently, Tsoyi et al. showed that carbon monoxide (CO) and AA primed MSC release high levels of pro-inflammatory prostaglandins, while CO and DHA (docosahexaenoic acid) primed MSC synthesize anti-inflammatory resolvins [187]. As a consequence of lipid catabolism and thermogenesis, circulating saturated FAs, such as AA, are found in excess in the early stage of the burn pathophysiology and persist over time [147]. CO-primed MSC might therefore be noxious if used at the onset of the burn pathology, as they could sustain

or worsen the hyperinflammatory response. If used later, however, they might help reactivate the immune system. These interesting data therefore demonstrate the importance of carefully choosing a preconditioning that is adapted to the stage of disease pathophysiology.

Aside from TLR agonists and ROS, other factors have been reported to modulate the effect of MSC on the immune system. For example, Zhou et al. highlighted the significance of the MSC-to-T cell ratio as it can trigger both stimulation and inhibition of T cell expansion in vitro. Accordingly, MSCs can stimulate the proliferation of activated CD4 and CD8 T cell subsets at low MSC-to-T cell ratios, while they are inhibitory at higher ratios [188].

Harnessing the development of hypertrophic scars. While hypertrophic scars are common postburn sequelae, their pathogenesis remains largely unknown. Clinical and experimental observations have led to the hypothesis that hypertrophic scars would stem from aberrant outgrowth and survival of myofibroblasts, imbalanced ECM deposition [189–191], and overactivated TGF-β signaling [191]. MDSCs have also been designated as putative targets in scarring processes. In addition to secrete pro-fibrotic ROS and TGF-β1, these cells are known to suppress the immune system activity through MyD88 [192], a regulator protein found in excess in hypertrophic scars [13]. Given the actual need for an antiscarring treatment and the scarcity of candidates for it, the MSC therapy has surfaced as a possible solution. Here again, the success of such a therapy must be considered within the scope of the licensing approach, since MSCs can both be pro- and antifibrotic, depending on their microenvironment [193].

Hypoxia is known to trigger antifibrotic mechanisms in MSCs, involving both leptins [194] and HIF-1α (hypoxia-inducible factor 1-alpha) [195], through collagen and α-SMA downregulation. In particular, hypoxic MSCs were shown to decrease the expression level of connective tissue growth factor, a downstream mediator of fibrosis, and increase the expression of HGF, a well-known TGF-β1 signaling inhibitor [196,197].

Recently, a study highlighted the central role of the tumor-suppressive gene p53 in the antiscarring effect of MSC [198]. In particular, p53-silenced MSCs were shown to promote fibroblast proliferation through iNOS production and myofibroblast conversion. The tumor-suppressive gene p53 is known to be involved in growth arrest, apoptosis, and antiangiogenesis, as it triggers the synthesis of key molecules such as insulin-like growth factor-binding protein 3 (IGFBP-3) and 14-3-3σ [199]. To improve MSC antiscarring effects, the activation of the tumor-suppressive gene p53 could therefore be an interesting option. This activation can be achieved through the use of stress signal primings, including radiation, hypoxia, or deoxyribonucleic acid damage [199].

Excessive vascularization is known to contribute to scar formation [173]. Promoting MSC antiangiogenic responses using well-defined licensing cues could therefore be a way to mitigate the emergence of hypertrophic scars after burns. As previously mentioned, the priming effect of inflammatory signals on MSCs can have an impact on angiogenesis but it appears to be highly dose dependent. As a result, the inflammatory priming of MSCs has both been shown to support the release of pro-angiogenic mediators at low doses [159] and suppress the endothelial cell migration, tubule formation, and in vivo vasculoprotection at higher doses [200–202]. These conflicting data can be explained by the release of TIMP-1, a well-known angiogenesis antagonist, differentially expressed under inflammatory licensing conditions. TIMP-1 is indeed overexpressed in MSCs primed with elevated doses of pro-inflammatory mediators [200], while it remains at a baseline expression level in low dose priming conditions [137].

Interestingly, TGF-β1-primed MSCs have been also recently shown to release high protein levels of IGFBP-3 [203], a growth factor known to block VEGF and MMP-9 pathways in in vitro and in vivo models of angiogenesis and vessel sprouting [204,205]. Therefore, the TGF-β1 licensing of MSCs could be used to block MDSC activity, as it relies on JAK2 (janus kinase 2)/STAT3 (signal transducer and activator of transcription 3) and VEGF signaling [184].

Toward the Clinical Use of MSC Preconditionings for Burn Treatment

Presently, there are only five reported clinical cases where MSC-based therapies were used to treat major burns (Table 3). In the majority of them, MSCs were applied during the middle stage of the burn disease progression, when reepithelialization, tissue survival, and skin graft take needed to be promoted. As reported in this review, hypoxic and mild inflammatory preconditionings are therefore appealing candidates to improve the MSC therapy at this specific pathological stage. However, these priming modalities might also be of interest to treat early burn stages to help mitigate hypermetabolism, hyperinflammation, and wound infections.

Although preconditioning approaches open up new therapeutic avenues in the field of cell therapy, a particular attention has to be drawn on preconditioning development and pathological context of use. In this review, we indeed reported that distinct priming doses and environments could promote antagonistic therapeutic outcomes. In that way, we specifically highlighted inflammatory and hypoxic/ROS preconditionings, but this might apply for many other licensing modalities. Therefore, there still is a need for thorough and comprehensive studies ensuring the benefit of a specific priming protocol for a chosen disease or pathological event.

As reported in this review, a standardized priming dose and priming duration need to be found for the clinical translation of the preconditioning approach. At the cell therapy level, other questions will also have to be answered such as the dose and the administration route of primed MSC or primed MSC-derived products. Preclinical studies will therefore be required to explore the best therapeutical options. Although many questions remain to be answered, the clinical preconditioning approach can be attainable. Indeed, in terms of safety, Guess et al. reported no sign of toxicity, tumorigenesis, or biodistribution of IFN-γ-primed MSC [206]. Few clinical studies have already been performed to evaluate the safety and efficacy of primed MSC. Hypoxic allogeneic MSCs were, for example, shown to improve nonischemic cardiomyopathy, as highlighted by a strengthening of the left ventricular ejection fraction in patients [207].

Conclusion and Perspectives

Because of a complex pathophysiology, the treatment of deep and extensive burns remains a challenge today.

However, the MSC therapy holds great promises in terms of safety, adaptability, and feasibility. Depending on the pathological target and the stage of the disease progression, the MSC potency can further be improved using well-characterized preconditioning protocols. Now that preclinical studies have demonstrated the real interest of this approach to improve the burn wound healing, the proof of safety and efficacy remains to be done in major burn patients.

Author Disclosure Statement

No competing financial interests exist.

References

1. Peck MD. (2011). Epidemiology of burns throughout the world. Part I: distribution and risk factors. Burns 37: 1087–1100.
2. Smolle C, J Cambiaso-Daniel, AA Forbes, P Wurzer, G Hundeshagen, LK Branski, F Huss and LP Kamolz. (2017). Recent trends in burn epidemiology worldwide: a systematic review. Burns 43:249–257.
3. Wolf SE, RG Tompkins and DN Herndon. (2014). On the horizon: research priorities in burns for the next decade. Surg Clin North Am 94:917–930.
4. Girard D, B Laverdet, V Buhé, M Trouillas, K Ghazi, MM Alexaline, C Egles, L Misery, B Coulomb, et al. (2017). Biotechnological management of skin burn injuries: challenges and perspectives in wound healing and sensory recovery. Tissue Eng Part B Rev 23:59–82.
5. Zuo KJ, A Medina and EE Tredget. (2017). Important developments in burn care. Plast Reconstr Surg 139: 120e–138e.
6. Arturson G. (1996). Pathophysiology of the burn wound and pharmacological treatment. The Rudi Hermans Lecture, 1995. Burns 22:255–274.
7. Keck M, DH Herndon, LP Kamolz, M Frey and MG Jeschke. (2009). Pathophysiology of burns. Wien Med Wochenschr 159:327–336.
8. Jackson DM. (1953). The diagnosis of the depth of burning. Br J Surg 40:588–596.
9. Allgöwer M, GA Schoenenberger and BG Sparkes. (2008). Pernicious effectors in burns. Burns 34 (Suppl 1):S1–S55.
10. Jeschke MG, GG Gauglitz, GA Kulp, CC Finnerty, FN Williams, R Kraft, OE Suman, RP Mlcak and DN Herndon. (2011). Long-term persistance of the pathophysiologic response to severe burn injury. PLoS One 6:e21245.
11. Manson J, C Thiemermann and K Brohi. (2012). Trauma alarmins as activators of damage-induced inflammation. Br J Surg 99 (Suppl 1):12–20.
12. Islam MN, BA Bradley and R Ceredig. (2016). Sterile post-traumatic immunosuppression. Clin Transl Immunol 5:e77.
13. D'Arpa P and KP Leung. (2017). Toll-like receptor signaling in burn wound healing and scarring. Adv Wound Care (New Rochelle) 6:330–343.
14. Eming SA, P Martin and M Tomic-Canic. (2014). Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med 6:265sr6.
15. Singer AJ and RA Clark. (1999). Cutaneous wound healing. N Engl J Med 341:738–746.
16. Eming SA, M Hammerschmidt, T Krieg and A Roers. (2009). Interrelation of immunity and tissue repair or regeneration. Semin Cell Dev Biol 20:517–527.
17. Eming SA, TA Wynn and P Martin. (2017). Inflammation and metabolism in tissue repair and regeneration. Science 356:1026–1030.
18. Pullar CE, CG Manabat-Hidalgo, RS Bolaji and RR Isseroff. (2008). Beta-adrenergic receptor modulation of wound repair. Pharmacol Res 58:158–164.
19. Kim MH, F Gorouhi, S Ramirez, JL Granick, BA Byrne, AM Soulika, SI Simon and RR Rivkah Isseroff. (2014). Catecholamine stress alters neutrophil trafficking and impairs wound healing by beta2-adrenergic receptor-mediated upregulation of IL-6. J Invest Dermatol 134:809–817.
20. Tredget EE, L Yang, M Delehanty, H Shankowsky and PG Scott. (2006). Polarized Th2 cytokine production in patients with hypertrophic scar following thermal injury. J Interferon Cytokine Res 26:179–189.
21. Rani M, Q Zhang and MG Schwacha. (2014). Burn wound gammadelta T-cells support a Th2 and Th17 immune response. J Burn Care Res 35:46–53.
22. Jewo PI and IO Fadeyibi. (2015). Progress in burns research: a review of advances in burn pathophysiology. Ann Burns Fire Disasters 28:105–115.
23. Kim A, T Lang, M Xue, A Wijewardana, C Jackson and J Vandervord. (2017). The role of Th-17 cells and gammadelta T-cells in modulating the systemic inflammatory response to severe burn injury. Int J Mol Sci 18:E758.
24. Kwan PO, J Ding and EE Tredget. (2016). Serum decorin, IL-1beta, and TGF-beta predict hypertrophic scarring postburn. J Burn Care Res 37:356–366.
25. Gauglitz GG, HC Korting, T Pavicic, T Ruzicka and MG Jeschke. (2011). Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies. Mol Med 17:113–125.
26. Barnes LA, CD Marshall, T Leavitt, MS Hu, AL Moore, JG Gonzalez, MT Longaker and GC Gurtner. (2018). Mechanical forces in cutaneous wound healing: emerging therapies to minimize scar formation. Adv Wound Care (New Rochelle) 7:47–56.
27. Cubison TC, SA Pape and N Parkhouse. (2006). Evidence for the link between healing time and the development of hypertrophic scars (HTS) in paediatric burns due to scald injury. Burns 32:992–999.
28. Zhu Z, J Ding and EE Tredget. (2016). The molecular basis of hypertrophic scars. Burns Trauma 4:2.
29. Lonie S, P Baker and RP Teixeira. (2017). Healing time and incidence of hypertrophic scarring in paediatric scalds. Burns 43:509–513.
30. Rowan MP, LC Cancio, EA Elster, DM Burmeister, LF Rose, S Natesan, RK Chan, RJ Christy and KK Chung. (2015). Burn wound healing and treatment: review and advancements. Crit Care 19:243.
31. Burke JF, IV Yannas, WC Quinby, CC Bondoc and WK Jung. (1981). Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. Ann Surg 194:413–428.
32. Cuono C, R Langdon and J McGuire. (1986). Use of cultured epidermal autografts and dermal allografts as skin replacement after burn injury. Lancet 1:1123–1124.
33. Rheinwald JG and H Green. (1975). Formation of a keratinizing epithelium in culture by a cloned cell line derived from a teratoma. Cell 6:317–330.
34. Chua AW, YC Khoo, BK Tan, KC Tan, CL Foo and SJ Chong. (2016). Skin tissue engineering advances in severe burns: review and therapeutic applications. Burns Trauma 4:3.

35. Cirodde A, T Leclerc, P Jault, P Duhamel, JJ Lataillade and L Bargues. (2011). Cultured epithelial autografts in massive burns: a single-center retrospective study with 63 patients. Burns 37:964–972.
36. Kym D, H Yim, J Yoon, HT Yang, YS Cho, J Hur, W Chun and JH Kim. (2015). The application of cultured epithelial autografts improves survival in burns. Wound Repair Regen 23:340–344.
37. Chua AWC, YC Khoo, TTH Truong, E Woo, BK Tan and SJ Chong. (2018). From skin allograft coverage to allograft-micrograft sandwich method: a retrospective review of severe burn patients who received conjunctive application of cultured epithelial autografts. Burns 44:1302–1307.
38. Atiyeh BS and M Costagliola. (2007). Cultured epithelial autograft (CEA) in burn treatment: three decades later. Burns 33:405–413.
39. Neveux Y, JM Rives, C Le Breton, E Gentilhomme, P Saint-Blancar and H Carsin. (1995). Clinical interest of cutaneous models reproduced in vitro for severe burn treatment: histopathological and ultrastructural study. Cell Biol Toxicol 11:173–178.
40. Sood R, D Roggy, M Zieger, J Balledux, S Chaudhari, DJ Koumanis, HS Mir, A Cohen, C Knipe, K Gabehart and JJ Coleman. (2010). Cultured epithelial autografts for coverage of large burn wounds in eighty-eight patients: the Indiana University experience. J Burn Care Res 31:559–568.
41. Auxenfans C, V Menet, Z Catherine, H Shipkov, P Lacroix, M Bertin-Maghit, O Damour and F Braye. (2015). Cultured autologous keratinocytes in the treatment of large and deep burns: a retrospective study over 15 years. Burns 41:71–79.
42. Takeda A, K Kadoya, N Shioya, E Uchinuma, M Tsunenaga, S Amano, T Nishiyama and RE Burgeson. (1999). Pretreatment of human keratinocyte sheets with laminin 5 improves their grafting efficiency. J Invest Dermatol 113: 38–42.
43. Fang T, WC Lineaweaver, FC Sailes, C Kisner and F Zhang. (2014). Clinical application of cultured epithelial autografts on acellular dermal matrices in the treatment of extended burn injuries. Ann Plast Surg 73:509–515.
44. Dale EL and CS Hultman. (2017). Patient safety in burn care: application of evidence-based medicine to improve outcomes. Clin Plast Surg 44:611–618.
45. Wang Y, J Beekman, J Hew, S Jackson, AC Issler-Fisher, R Parungao, SS Lajevardi, Z Li and PKM Maitz. (2017). Burn injury: challenges and advances in burn wound healing, infection, pain and scarring. Adv Drug Deliv Rev 123:3–17.
46. Farina JA Jr., MJ Rosique and RG Rosique. (2013). Curbing inflammation in burn patients. Int J Inflam 2013: 715645.
47. Namdar T, PL Stollwerck, FH Stang, W Eisenbeiss, F Siemers, P Mailander and T Lange. (2011). Impact of hypernatremia on burn wound healing: results of an exploratory, retrospective study. Ostomy Wound Manage 57:30–34.
48. Auger C, O Samadi and MG Jeschke. (2017). The biochemical alterations underlying post-burn hypermetabolism. Biochim Biophys Acta 1863:2633–2644.
49. Ali A, DN Herndon, A Mamachen, S Hasan, CR Andersen, RJ Grogans, JL Brewer, JO Lee, J Heffernan, OE Suman and CC Finnerty. (2015). Propranolol attenuates hemorrhage and accelerates wound healing in severely burned adults. Crit Care 19:217.
50. El Ayadi A, A Prasai, Y Wang, DN Herndon and CC Finnerty. (2018). Beta-adrenergic receptor trafficking, degradation, and cell-surface expression are altered in dermal fibroblasts from hypertrophic scars. J Invest Dermatol 138:1645–1655.
51. Kwan PO and EE Tredget. (2017). Biological principles of scar and contracture. Hand Clin 33:277–292.
52. Dang H, AG Geiser, JJ Letterio, T Nakabayashi, L Kong, G Fernandes and N Talal. (1995). SLE-like autoantibodies and Sjogren's syndrome-like lymphoproliferation in TGF-beta knockout mice. J Immunol 155:3205–3212.
53. Pietra D, A Borghini and AM Bianucci. (2015). In vitro studies of antifibrotic and cytoprotective effects elicited by proto-berberine alkaloids in human dermal fibroblasts. Pharmacol Rep 67:1081–1089.
54. Wilgus TA, AM Ferreira, TM Oberyszyn, VK Bergdall and LA Dipietro. (2008). Regulation of scar formation by vascular endothelial growth factor. Lab Invest 88: 579–590.
55. Wu M, DS Melichian, E Chang, M Warner-Blankenship, AK Ghosh and J Varga. (2009). Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. Am J Pathol 174:519–533.
56. Jeon KI, RP Phipps, PJ Sime and KR Huxlin. (2015). Inhibitory effects of PPARgamma ligands on TGF-beta1-induced CTGF expression in cat corneal fibroblasts. Exp Eye Res 138:52–58.
57. Friedenstein AJ, JF Gorskaja and NN Kulagina. (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 4:267–274.
58. Macrin D, JP Joseph, AA Pillai and A Devi. (2017). Eminent sources of adult mesenchymal stem cells and their therapeutic imminence. Stem Cell Rev 13:741–756.
59. Fournier BP, FC Ferre, L Couty, JJ Lataillade, M Gourven, A Naveau, B Coulomb, A Lafont and B Gogly. (2010). Multipotent progenitor cells in gingival connective tissue. Tissue Eng Part A 16:2891–2899.
60. Naderi N, EJ Combellack, M Griffin, T Sedaghati, M Javed, MW Findlay, CG Wallace, A Mosahebi, PE Butler, AM Seifalian and IS Whitaker. (2017). The regenerative role of adipose-derived stem cells (ADSC) in plastic and reconstructive surgery. Int Wound J 14:112–124.
61. Peltzer J, F Montespan, C Thepenier, L Boutin, G Uzan, N Rouas-Freiss and JJ Lataillade. (2015). Heterogeneous functions of perinatal mesenchymal stromal cells require a preselection before their banking for clinical use. Stem Cells Dev 24:329–344.
62. Dominici M, K Le Blanc, I Mueller, I Slaper-Cortenbach, F Marini, D Krause, R Deans, A Keating, Dj Prockop and E Horwitz. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315–317.
63. Gnecchi M, P Danieli, G Malpasso and MC Ciuffreda. (2016). Paracrine mechanisms of mesenchymal stem cells in tissue repair. Methods Mol Biol 1416:123–146.
64. Meirelles Lda S, AM Fontes, DT Covas and AI Caplan. (2009). Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 20:419–427.

65. Kim KH, G Blasco-Morente, N Cuende and S Arias-Santiago. (2017). Mesenchymal stromal cells: properties and role in management of cutaneous diseases. J Eur Acad Dermatol Venereol 31:414–423.
66. Rodgers K and SS Jadhav. (2018). The application of mesenchymal stem cells to treat thermal and radiation burns. Adv Drug Deliv Rev 123:75–81.
67. Zhang QZ, WR Su, SH Shi, P Wilder-Smith, AP Xiang, A Wong, AL Nguyen, CW Kwon and AD Le. (2010). Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing. Stem Cells 28:1856–1868.
68. Liu S, L Jiang, H Li, H Shi, H Luo, Y Zhang, C Yu and Y Jin. (2014). Mesenchymal stem cells prevent hypertrophic scar formation via inflammatory regulation when undergoing apoptosis. J Invest Dermatol 134:2648–2657.
69. Qi Y, D Jiang, A Sindrilaru, A Stegemann, S Schatz, N Treiber, M Rojewski, H Schrezenmeier, S Vander Beken, et al. (2014). TSG-6 released from intradermally injected mesenchymal stem cells accelerates wound healing and reduces tissue fibrosis in murine full-thickness skin wounds. J Invest Dermatol 134:526–537.
70. Chen L, Y Xu, J Zhao, Z Zhang, R Yang, J Xie, X Liu and S Qi. (2014). Conditioned medium from hypoxic bone marrow-derived mesenchymal stem cells enhances wound healing in mice. PLoS One 9:e96161.
71. Santos JM, SP Camões, E Filipe, M Cipriano, RN Barcia, M Filipe, M Teixeira, S Simões, M Gaspar, et al. (2015). Three-dimensional spheroid cell culture of umbilical cord tissue-derived mesenchymal stromal cells leads to enhanced paracrine induction of wound healing. Stem Cell Res Ther 6:90.
72. Walter MN, KT Wright, HR Fuller, S MacNeil and WE Johnson. (2010). Mesenchymal stem cell-conditioned medium accelerates skin wound healing: an in vitro study of fibroblast and keratinocyte scratch assays. Exp Cell Res 316:1271–1281.
73. Zhang B, M Wang, A Gong, X Zhang, X Wu, Y Zhu, H Shi, L Wu, W Zhu, H Qian and W Xu. (2015). HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. Stem Cells 33:2158–2168.
74. Shabbir A, A Coz, L Rodriguez, M Salgado and E Badiavas. (2015). Mesenchymal stem cell exosomes induce the proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro. Stem Cells Dev 24:1635–1647.
75. Fang S, C Xu, Y Zhang, C Xue, C Yang, H Bi, X Qian, M Wu, K Ji, et al. (2016). Umbilical cord-derived mesenchymal stem cell-derived exosomal microRNAs suppress myofibroblast differentiation by inhibiting the transforming growth factor-beta/SMAD2 pathway during wound healing. Stem Cells Transl Med 5:1425–1439.
76. Li M, F Luan, Y Zhao, H Hao, J Liu, L Dong, X Fu and W Han. (2017). Mesenchymal stem cell-conditioned medium accelerates wound healing with fewer scars. Int Wound J 14:64–73.
77. Zhao B, Y Zhang, S Han, W Zhang, Q Zhou, H Guan, J Liu, J Shi, L Su and D Hu. (2017). Exosomes derived from human amniotic epithelial cells accelerate wound healing and inhibit scar formation. J Mol Histol 48:121–132.
78. Liu L, Y Yu, Y Hou, J Chai, H Duan, W Chu, H Zhang, Q Hu and J Du. (2014). Human umbilical cord mesenchymal stem cells transplantation promotes cutaneous wound healing of severe burned rats. PLoS One 9:e88348.
79. Linard C, F Tissedre, E Busson, V Holler, T Leclerc, C Strup-Perrot, L Couty, B L'Homme, M Benderitter, et al. (2015). Therapeutic potential of gingival fibroblasts for cutaneous radiation syndrome: comparison to bone marrow-mesenchymal stem cell grafts. Stem Cells Dev 24:1182–1193.
80. Caliari-Oliveira C, JN Yaochite, LNZ Ramalho, PVB Palma, D Carlos, Fde Q Cunha, DA De Souza, MA Frade, DT Covas, et al. (2016). Xenogeneic mesenchymal stromal cells improve wound healing and modulate the immune response in an extensive burn model. Cell Transplant 25:201–215.
81. Liu L, H Song, H Duan, J Chai, J Yang, X Li, Y Yu, X Zhang, X Hu, et al. (2016). TSG-6 secreted by human umbilical cord-MSCs attenuates severe burn-induced excessive inflammation via inhibiting activations of P38 and JNK signaling. Sci Rep 6:30121.
82. Li X, L Liu, J Yang, Y Yu, J Chai, L Wang, L Ma and H Yin. (2016). Exosome derived from human umbilical cord mesenchymal stem cell mediates miR-181c attenuating burn-induced excessive inflammation. EBioMedicine 8: 72–82.
83. Chung E, VY Rybalko, PL Hsieh, SL Leal, MA Samano, AN Willauer, RS Stowers, S Natesan, DO Zamora, RJ Christy and LJ Suggs. (2016). Fibrin-based stem cell containing scaffold improves the dynamics of burn wound healing. Wound Repair Regen 24:810–819.
84. Xue L, YB Xu, JL Xie, JM Tang, B Shu, L Chen, SH Qi and XS Liu. (2013). Effects of human bone marrow mesenchymal stem cells on burn injury healing in a mouse model. Int J Clin Exp Pathol 6:1327–1336.
85. Zhang B, X Wu, X Zhang, Y Sun, Y Yan, H Shi, Y Zhu, L Wu, Z Pan, et al. (2015). Human umbilical cord mesenchymal stem cell exosomes enhance angiogenesis through the Wnt4/beta-catenin pathway. Stem Cells Transl Med 4: 513–522.
86. Foubert P, AD Gonzalez, S Teodosescu, F Berard, M Doyle-Eisele, K Yekkala, M Tenenhaus and JK Fraser. (2016). Adipose-derived regenerative cell therapy for burn wound healing: a comparison of two delivery methods. Adv Wound Care (New Rochelle) 5:288–298.
87. Ahmeda HH, LA Rashed, S Mahfouz, RE Hussein, M Alkaffasa, S Mostafab and A Abusree. (2017). Can mesenchymal stem cells pretreated with platelet rich plasma modulate tissue remodeling in a rat burn? Biochem Cell Biol 95:537–548.
88. Yang Y, W Zhang, Y Li, G Fang and K Zhang. (2014). Scalded skin of rat treated by using fibrin glue combined with allogeneic bone marrow mesenchymal stem cells. Ann Dermatol 26:289–295.
89. Muhammad G, J Xu, JWM Bulte, A Jablonska, P Walczak and M Janowski. (2017). Transplanted adipose-derived stem cells can be short-lived yet accelerate healing of acid-burn skin wounds: a multimodal imaging study. Sci Rep 7:4644.
90. Alcayaga-Miranda F, J Cuenca and M Khoury. (2017). Antimicrobial Activity of mesenchymal stem cells: current status and new perspectives of antimicrobial peptide-based therapies. Front Immunol 8:339.
91. Rabani R, A Volchuk, M Jerkic, L Ormesher, L Garces-Ramirez, J Canton, C Masterson, S Gagnon, KC Tatham, et al. (2018). Mesenchymal stem cells enhance NOX2 dependent ROS production and bacterial killing in macrophages during sepsis. Eur Respir J 51:1702021.

92. Mansilla E, R Spretz, G Larsen, L Nuñez, H Drago, F Sturla, GH Marin, G Roque, K Martire, et al. (2010). Outstanding survival and regeneration process by the use of intelligent acellular dermal matrices and mesenchymal stem cells in a burn pig model. Transplant Proc 42:4275–4278.
93. Chen L, Q Xing, Q Zhai, M Tahtinen, F Zhou, L Chen, Y Xu, S Qi and F Zhao. (2017). Pre-vascularization enhances therapeutic effects of human mesenchymal stem cell sheets in full thickness skin wound repair. Theranostics 7:117–131.
94. Burmeister DM, R Stone, N Wrice, A Laborde, SC Becerra, S Natesan and RJ Christy. (2018). Delivery of allogeneic adipose stem cells in polyethylene glycol-fibrin hydrogels as an adjunct to meshed autografts after sharp debridement of deep partial thickness burns. Stem Cells Transl Med 7:360–372.
95. Ozturk S and H Karagoz. (2015). Experimental stem cell therapies on burn wound: do source, dose, timing and method matter? Burns 41:1133–1139.
96. Rasulov MF, AV Vasilchenkov, NA Onishchenko, ME Krasheninnikov, VI Kravchenko, TL Gorshenin, RE Pidtsan and IV Potapov. (2005). First experience of the use bone marrow mesenchymal stem cells for the treatment of a patient with deep skin burns. Bull Exp Biol Med 139:141–144.
97. Yoshikawa T, H Mitsuno, I Nonaka, Y Sen, K Kawanishi, Y Inada, Y Takakura, K Okuchi and A Nonomura. (2008). Wound therapy by marrow mesenchymal cell transplantation. Plast Reconstr Surg 121:860–877.
98. Abo-Elkheir W, F Hamza, AM Elmofty, A Emam, M Abdl-Moktader, S Elsherefy and H Gabr. (2017). Role of cord blood and bone marrow mesenchymal stem cells in recent deep burn: a case-control prospective study. Am J Stem Cells 6:23–35.
99. Mansilla E, GH Marín, M Berges, S Scafatti, J Rivas, A Núñez, M Menvielle, R Lamonega, C Gardiner, et al. (2015). Cadaveric bone marrow mesenchymal stem cells: first experience treating a patient with large severe burns. Burns Trauma 3:17.
100. Xu Y, S Huang and X Fu. (2012). Autologous transplantation of bone marrow-derived mesenchymal stem cells: a promising therapeutic strategy for prevention of skin-graft contraction. Clin Exp Dermatol 37:497–500.
101. Auxenfans C, J Fradette, C Lequeux, L Germain, B Kinikoglu, N Bechetoille, F Braye, FA Auger and O Damour. (2009). Evolution of three dimensional skin equivalent models reconstructed in vitro by tissue engineering. Eur J Dermatol 19:107–113.
102. Ter Horst B, G Chouhan, NS Moiemen and LM Grover. (2018). Advances in keratinocyte delivery in burn wound care. Adv Drug Deliv Rev 123:18–32.
103. Barkholt L, E Flory, V Jekerle, S Lucas-Samuel, P Ahnert, L Bisset, D Büscher, W Fibbe, A Foussat, et al. (2013). Risk of tumorigenicity in mesenchymal stromal cell-based therapies—bridging scientific observations and regulatory viewpoints. Cytotherapy 15:753–759.
104. Aggarwal S and MF Pittenger. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105:1815–1822.
105. Krampera M, S Glennie, J Dyson, D Scott, R Laylor, E Simpson and F Dazzi. (2003). Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101: 3722–3729.
106. François M, IB Copland, S Yuan, R Romieu-Mourez, EK Waller and J Galipeau. (2012). Cryopreserved mesenchymal stromal cells display impaired immunosuppressive properties as a result of heat-shock response and impaired interferon-gamma licensing. Cytotherapy 14:147–152.
107. Moll G, JJ Alm, LC Davies, L von Bahr, N Heldring, L Stenbeck-Funke, OA Hamad, R Hinsch, L Ignatowicz, et al. (2014). Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? Stem Cells 32:2430–2442.
108. Ketterl N, G Brachtl, C Schuh, K Bieback, K Schallmoser, A Reinisch and D Strunk. (2015). A robust potency assay highlights significant donor variation of human mesenchymal stem/progenitor cell immune modulatory capacity and extended radio-resistance. Stem Cell Res Ther 6:236.
109. Menard C, L Pacelli, G Bassi, J Dulong, F Bifari, I Bezier, J Zanoncello, M Ricciardi, M Latour, et al. (2013). Clinical-grade mesenchymal stromal cells produced under various good manufacturing practice processes differ in their immunomodulatory properties: standardization of immune quality controls. Stem Cells Dev 22:1789–1801.
110. Alagesan S, C Sanz-Nogués, X Chen, M Creane, T Ritter, R Ceredig, T O'Brien and MD Griffin. (2018). Anti-donor antibody induction following intramuscular injections of allogeneic mesenchymal stromal cells. Immunol Cell Biol 96:536–548.
111. Galipeau J and L Sensébé. (2018). Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. Cell Stem Cell 22:824–833.
112. Panés J, D García-Olmo, G Van Assche, JF Colombel, W Reinisch, DC Baumgart, A Dignass, M Nachury, M Ferrante, et al. (2018). Long-term efficacy and safety of stem cell therapy (Cx601) for complex perianal fistulas in patients with Crohn's disease. Gastroenterology 154:1334–1342.e4.
113. Berglund AK, LA Fortier, DF Antczak and LV Schnabel. (2017). Immunoprivileged no more: measuring the immunogenicity of allogeneic adult mesenchymal stem cells. Stem Cell Res Ther 8:288.
114. Vizoso FJ, N Eiro, S Cid, J Schneider and R Perez-Fernandez. (2017). Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. Int J Mol Sci 18:E1852.
115. Pires AO, B Mendes-Pinheiro, FG Teixeira, SI Anjo, S Ribeiro-Samy, ED Gomes, SC Serra, NA Silva, B Manadas, N Sousa and AJ Salgado. (2016). Unveiling the differences of secretome of human bone marrow mesenchymal stem cells, adipose tissue-derived stem cells, and human umbilical cord perivascular cells: a proteomic analysis. Stem Cells Dev 25:1073–1083.
116. Paladino FV, LR Sardinha, CA Piccinato and AC Goldberg. (2017). Intrinsic variability present in Wharton's jelly mesenchymal stem cells and T cell responses may impact cell therapy. Stem Cells Int 2017:8492797.
117. Németh K, A Leelahavanichkul, PS Yuen, B Mayer, A Parmelee, K Doi, PG Robey, K Leelahavanichkul, BH Koller, et al. (2009). Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 15:42–49.
118. Reimer KA, CE Murry, I Yamasawa, ML Hill and RB Jennings. (1986). Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. Am J Physiol Heart Circ Physiol 251:H1306–H1315.

119. Murry CE, RB Jennings and KA Reimer. (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74:1124–1136.
120. Hu C and L Li. (2018). Preconditioning influences mesenchymal stem cell properties in vitro and in vivo. J Cell Mol Med 22:1428–1442.
121. Boland L, AJ Burand, AJ Brown, D Boyt, VA Lira and JA Ankrum. (2018). IFN-γ and TNF-α pre-licensing protects mesenchymal stromal cells from the pro-inflammatory effects of palmitate. Mol Ther 26:860–873.
122. Giuliani M, A Bennaceur-Griscelli, A Nanbakhsh, N Oudrhiri, S Chouaib, B Azzarone, A Durrbach and JJ Lataillade. (2014). TLR ligands stimulation protects MSC from NK killing. Stem Cells 32:290–300.
123. Caplan AI and D Correa. (2011). The MSC: an injury drugstore. Cell Stem Cell 9:11–15.
124. Szczesny B, A Brunyánszki, A Ahmad, G Oláh, C Porter, T Toliver-Kinsky, L Sidossis, DN Herndon and C Szabo. (2015). Time-dependent and organ-specific changes in mitochondrial function, mitochondrial DNA integrity, oxidative stress and mononuclear cell infiltration in a mouse model of burn injury. PLoS One 10:e0143730.
125. Latha B and M Babu. (2001). The involvement of free radicals in burn injury: a review. Burns 27:309–317.
126. Williams FN and DN Herndon. (2017). Metabolic and endocrine considerations after burn injury. Clin Plast Surg 44:541–553.
127. Kaddoura I, G Abu-Sittah, A Ibrahim, R Karamanoukian and N Papazian. (2017). Burn injury: review of pathophysiology and therapeutic modalities in major burns. Ann Burns Fire Disasters 30:95–102.
128. Clark A, J Imran, T Madni and SE Wolf. (2017). Nutrition and metabolism in burn patients. Burns Trauma 5:11.
129. Krasnodembskaya A, Y Song, X Fang, N Gupta, V Serikov, JW Lee and MA Matthay. (2010). Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. Stem Cells 28:2229–2238.
130. Lee JW, A Krasnodembskaya, DH McKenna, Y Song, J Abbott and MA Matthay. (2013). Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria. Am J Respir Crit Care Med 187:751–760.
131. Ryan JM, F Barry, JM Murphy and BP Mahon. (2007). Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol 149:353–363.
132. Zhang Q, S Shi, Y Liu, J Uyanne, Y Shi, S Shi and AD Le. (2009). Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 183:7787–7798.
133. Urrutia M, S Fernández, M González, R Vilches, P Rojas, M Vásquez, M Kurte, AM Vega-Letter, F Carrión, et al. (2016). Overexpression of glutamate decarboxylase in mesenchymal stem cells enhances their immunosuppressive properties and increases GABA and nitric oxide levels. PLoS One 11:e0163735.
134. Hegyi B, G Kudlik, E Monostori and F Uher. (2012). Activated T-cells and pro-inflammatory cytokines differentially regulate prostaglandin E2 secretion by mesenchymal stem cells. Biochem Biophys Res Commun 419:215–220.
135. Choi H, RH Lee, N Bazhanov, JY Oh and DJ Prockop. (2011). Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-kappaB signaling in resident macrophages. Blood 118:330–338.
136. Fayyad-Kazan M, M Najar, H Fayyad-Kazan, G Raicevic and L Lagneaux. (2017). Identification and evaluation of new immunoregulatory genes in mesenchymal stromal cells of different origins: comparison of normal and inflammatory conditions. Med Sci Monit Basic Res 23:87–96.
137. Hengartner NE, J Fiedler, H Schrezenmeier, M Huber-Lang and RE Brenner. (2015). Crucial role of IL1beta and C3a in the in vitro-response of multipotent mesenchymal stromal cells to inflammatory mediators of polytrauma. PLoS One 10:e0116772.
138. Andreeva E, P Bobyleva, A Gornostaeva and L Buravkova. (2017). Interaction of multipotent mesenchymal stromal and immune cells: bidirectional effects. Cytotherapy 19:1152–1166.
139. Dunnick CA, NS Gibran and DM Heimbach. (1996). Substance P has a role in neurogenic mediation of human burn wound healing. J Burn Care Rehabil 17:390–396.
140. Jin Y, HS Hong and Y Son. (2015). Substance P enhances mesenchymal stem cells-mediated immune modulation. Cytokine 71:145–153.
141. Fang X, J Abbott, L Cheng, JK Colby, JW Lee, BD Levy and MA Matthay. (2015). Human mesenchymal stem (stromal) cells promote the resolution of acute lung injury in part through lipoxin A4. J Immunol 195:875–881.
142. Song Y, H Dou, X Li, X Zhao, Y Li, D Liu, J Ji, F Liu, L Ding, Y Ni and Y Hou. (2017). Exosomal miR-146a contributes to the enhanced therapeutic efficacy of interleukin-1beta-primed mesenchymal stem cells against sepsis. Stem Cells 35:1208–1221.
143. Wu X, Z Wang, M Qian, L Wang, C Bai and X Wang. (2014). Adrenaline stimulates the proliferation and migration of mesenchymal stem cells towards the LPS-induced lung injury. J Cell Mol Med 18:1612–1622.
144. Zhou H, D Li, C Shi, T Xin, J Yang, Y Zhou, S Hu, F Tian, J Wang and Y Chen. (2015). Effects of Exendin-4 on bone marrow mesenchymal stem cell proliferation, migration and apoptosis in vitro. Sci Rep 5:12898.
145. Jiang CM, J Liu, JY Zhao, L Xiao, S An, YC Gou, HX Quan, Q Cheng, YL Zhang, et al. (2015). Effects of hypoxia on the immunomodulatory properties of human gingiva–derived mesenchymal stem cells. J Dent Res 94:69–77.
146. Giacoppo S, SR Thangavelu, F Diomede, P Bramanti, P Conti, O Trubiani and E Mazzon. (2017). Anti-inflammatory effects of hypoxic-preconditioned human periodontal ligament cells secretome in an experimental model of multiple sclerosis: a key role of IL-37. FASEB J 31:1–17.
147. Cetinkale O and Z Yazici. (1997). Early post-burn fatty acid profile in burn patients. Burns 23:392–399.
148. Shi H, MV Kokoeva, K Inouye, I Tzameli, H Yin and JS Flier. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116:3015–3025.
149. Sun X, H Hao, Q Han, X Song, J Liu, L Dong, W Han and Y Mu. (2017). Human umbilical cord-derived mesenchymal stem cells ameliorate insulin resistance by suppressing NLRP3 inflammasome-mediated inflammation in type 2 diabetes rats. Stem Cell Res Ther 8:241.
150. Oh JY, JH Ko, HJ Lee, JM Yu, H Choi, MK Kim, WR Wee and DJ Prockop. (2014). Mesenchymal stem/stromal cells inhibit the NLRP3 inflammasome by decreasing mitochondrial reactive oxygen species. Stem Cells 32: 1553–1563.

151. Bartosh TJ, JH Ylöstalo, A Mohammadipoor, N Bazhanov, K Coble, K Claypool, RH Lee, H Choi and DJ Prockop. (2010). Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties. Proc Natl Acad Sci U S A 107:13724–13729.
152. Block GJ, S Ohkouchi, F Fung, J Frenkel, C Gregory, R Pochampally, G DiMattia, DE Sullivan and DJ Prockop. (2009). Multipotent stromal cells are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1. Stem Cells 27:670–681.
153. Boyce ST and GD Warden. (2002). Principles and practices for treatment of cutaneous wounds with cultured skin substitutes. Am J Surg 183:445–456.
154. Rose LF and RK Chan. (2016). The burn wound microenvironment. Adv Wound Care (New Rochelle) 5: 106–118.
155. Gnecchi M, H He, N Noiseux, OD Liang, L Zhang, F Morello, H Mu, LG Melo, RE Pratt, JS Ingwall and VJ Dzau. (2006). Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J 20: 661–669.
156. Rosová I, M Dao, B Capoccia, D Link and JA Nolta. (2008). Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells 26:2173–2182.
157. Zhang HC, XB Liu, S Huang, XY Bi, HX Wang, LX Xie, YQ Wang, XF Cao, J Lv, et al. (2012). Microvesicles derived from human umbilical cord mesenchymal stem cells stimulated by hypoxia promote angiogenesis both in vitro and in vivo. Stem Cells Dev 21:3289–3297.
158. Broekman W, GD Amatngalim, Y de Mooij-Eijk, J Oostendorp, H Roelofs, C Taube, J Stolk and PS Hiemstra. (2016). TNF-alpha and IL-1beta-activated human mesenchymal stromal cells increase airway epithelial wound healing in vitro via activation of the epidermal growth factor receptor. Respir Res 17:3.
159. Chen H, XH Min, QY Wang, FW Leung, L Shi, Y Zhou, T Yu, CM Wang, G An, WH Sha and QK Chen. (2015). Preactivation of mesenchymal stem cells with TNF-alpha, IL-1beta and nitric oxide enhances its paracrine effects on radiation-induced intestinal injury. Sci Rep 5:8718.
160. Basu A, S Munir, MA Mulaw, K Singh, D Crisan, A Sindrilaru, N Treiber, M Wlaschek, M Huber-Lang, F Gebhard and K Scharffetter-Kochanek. (2018). A novel S100A8/A9 induced fingerprint of mesenchymal stem cells associated with enhanced wound healing. Sci Rep 8: 6205.
161. Pourjafar M, M Saidijam, K Mansouri, H Ghasemibasir, F Karimi Dermani and R Najafi. (2017). All-trans retinoic acid preconditioning enhances proliferation, angiogenesis and migration of mesenchymal stem cell in vitro and enhances wound repair in vivo. Cell Prolif 50; DOI: 10.1111/cpr.12315.
162. Oh SY, SJ Lee, YH Jung, HJ Lee and HJ Han. (2015). Arachidonic acid promotes skin wound healing through induction of human MSC migration by MT3-MMP-mediated fibronectin degradation. Cell Death Dis 6:e1750.
163. Wu Y, L Chen, PG Scott and EE Tredget. (2007). Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells 25:2648–2659.
164. Bader AM, K Klose, K Bieback, D Korinth, M Schneider, M Seifert, YH Choi, A Kurtz, V Falk and C Stamm. (2015). Hypoxic preconditioning increases survival and pro-angiogenic capacity of human cord blood mesenchymal stromal cells in vitro. PLoS One 10:e0138477.
165. Han YD, Y Bai, XL Yan, J Ren, Q Zeng, XD Li, XT Pei and Y Han. (2018). Co-transplantation of exosomes derived from hypoxia-preconditioned adipose mesenchymal stem cells promotes neovascularization and graft survival in fat grafting. Biochem Biophys Res Commun 497:305–312.
166. Perdoni C, JA McGrath and J Tolar. (2014). Preconditioning of mesenchymal stem cells for improved transplantation efficacy in recessive dystrophic epidermolysis bullosa. Stem Cell Res Ther 5:121.
167. Naso M, J Uitto and JF Klement. (2003). Transcriptional control of the mouse Col7a1 gene in keratinocytes: basal and transforming growth factor-b regulated expression. J Invest Dermatol 121:1469–1478.
168. König A and L Bruckner-Tuderman. (1992). Transforming growth factor-β stimulates collagen VII expression by cutaneous cells in vitro. J Cell Biol 117:679–685.
169. Vollberg TM, MD George and AM Jetten. (1991). Induction of extracellular matrix gene expression in normal human keratinocytes by transforming growth factor beta is altered by cellular differentiation. Exp Cell Res 193: 93–100.
170. Feru J, E Delobbe, L Ramont, B Brassart, C Terryn, A Dupont-Deshorgue, C Garbar, JC Monboisse, FX Maquart and S Brassart-Pasco. (2016). Aging decreases collagen IV expression in vivo in the dermo-epidermal junction and in vitro in dermal fibroblasts: possible involvement of TGF-β1. Eur J Dermatol 26:350–360.
171. Çakir B and BC Yegen. (2004). Systemic responses to burn injury. Turk J Med Sci 34:215–226.
172. Finnerty CC, MG Jeschke, DN Herndon, R Gamelli, NS Gibran, M Klein, G Silver, B Arnoldo, D Remick, RG Tompkins; Investigators of the Inflammation and the Host Response Glue Grant. (2008). Temporal cytokine profiles in severely burned patients: a comparison of adults and children. Mol Med 14:553–560.
173. DiPietro LA. (2016). Angiogenesis and wound repair: when enough is enough. J Leukoc Biol 100:979–984.
174. Bijlard E, L Uiterwaal, CA Kouwenberg, MA Mureau, SE Hovius and FJPM Huygen. (2017). A systematic review on the prevalence, etiology, and pathophysiology of intrinsic pain in dermal scar tissue. Pain Phys 20:1–13.
175. Noel JG, X Guo, D Wells-Byrum, S Schwemberger, CC Caldwell and CK Ogle. (2005). Effect of thermal injury on splenic myelopoiesis. Shock 23:115–122.
176. Cuenca AG, MJ Delano, KM Kelly-Scumpia, C Moreno, PO Scumpia, DM Laface, PG Heyworth, PA Efron and LL Moldawer. (2011). A paradoxical role for myeloid-derived suppressor cells in sepsis and trauma. Mol Med 17:281–292.
177. Gabrilovich DI and S Nagaraj. (2009). Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9:162–174.
178. Giallongo C, D Tibullo, NL Parrinello, P La Cava, M Di Rosa, V Bramanti, C Di Raimondo, C Conticello, A Chiarenza, et al. (2016). Granulocyte-like myeloid derived suppressor cells (G-MDSC) are increased in multiple myeloma and are driven by dysfunctional mesenchymal stem cells (MSC). Oncotarget 7:85764–85775.
179. Le Burel S, C Thepenier, L Boutin, JJ Lataillade and J Peltzer. (2017). Effect of mesenchymal stromal cells on T cells in a septic context: immunosuppression or immunostimulation? Stem Cells Dev 26:1477–1489.

180. Hermankova B, A Zajicova, E Javorkova, M Chudickova, P Trosan, M Hajkova, M Krulova and V Holan. (2016). Suppression of IL-10 production by activated B cells via a cell contact-dependent cyclooxygenase-2 pathway upregulated in IFN-gamma-treated mesenchymal stem cells. Immunobiology 221:129–136.
181. Brandau S, M Jakob, H Hemeda, K Bruderek, S Janeschik, F Bootz and S Lang. (2010). Tissue-resident mesenchymal stem cells attract peripheral blood neutrophils and enhance their inflammatory activity in response to microbial challenge. J Leukoc Biol 88:1005–1015.
182. Tomchuck SL, KJ Zwezdaryk, SB Coffelt, RS Waterman, ES Danka and AB Scandurro. (2008). Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses. Stem Cells 26:99–107.
183. Waterman RS, SL Henkle and AM Betancourt. (2012). Mesenchymal stem cell 1 (MSC1)-based therapy attenuates tumor growth whereas MSC2-treatment promotes tumor growth and metastasis. PLoS One 7:e45590.
184. Wesolowski R, J Markowitz and WE Carson. (2013). Myeloid derived suppressor cells—a new therapeutic target in the treatment of cancer. J Immunother Cancer 1:10.
185. Betancourt AM. (2013). New cell-based therapy paradigm: induction of bone marrow-derived multipotent mesenchymal stromal cells into pro-inflammatory MSC1 and anti-inflammatory MSC2 phenotypes. Adv Biochem Eng Biotechnol 130:163–197.
186. Cassatella MA, F Mosna, A Micheletti, V Lisi, N Tamassia, C Cont, F Calzetti, M Pelletier, G Pizzolo and M Krampera. (2011). Toll-like receptor-3-activated human mesenchymal stromal cells significantly prolong the survival and function of neutrophils. Stem Cells 29:1001–1011.
187. Tsoyi K, SR Hall, J Dalli, RA Colas, S Ghanta, B Ith, A Coronata, LE Fredenburgh, RM Baron, et al. (2016). Carbon monoxide improves efficacy of mesenchymal stromal cells during sepsis by production of specialized proresolving lipid mediators. Crit Care Med 44:e1236–e1245.
188. Zhou Y, A Day, S Haykal, A Keating and TK Waddell. (2013). Mesenchymal stromal cells augment CD4+ and CD8+ T-cell proliferation through a CCL2 pathway. Cytotherapy 15:1195–1207.
189. Jinnin M. (2010). Mechanisms of skin fibrosis in systemic sclerosis. J Dermatol 37:11–25.
190. Yates CC, R Bodnar and A Wells. (2011). Matrix control of scarring. Cell Mol Life Sci 68:1871–1881.
191. Penn JW, AO Grobbelaar and KJ Rolfe. (2012). The role of the TGF-β family in wound healing, burns and scarring: a review. Int J Burns Trauma 2:18–28.
192. Hong EH, SY Chang, BR Lee, YS Kim, JM Lee, CY Kang, MN Kweon and HJ Ko. (2013). Blockade of Myd88 signaling induces antitumor effects by skewing the immunosuppressive function of myeloid-derived suppressor cells. Int J Cancer 132:2839–2848.
193. El Agha E, R Kramann, RK Schneider, X Li, W Seeger, BD Humphreys and S Bellusci. (2017). Mesenchymal stem cells in fibrotic disease. Cell Stem Cell 21:166–177.
194. Chen P, R Wu, W Zhu, Z Jiang, Y Xu, H Chen, Z Zhang, H Chen, L Zhang, et al. (2014). Hypoxia preconditioned mesenchymal stem cells prevent cardiac fibroblast activation and collagen production via leptin. PLoS One 9: e103587.
195. Du L, R Lv, X Yang, S Cheng, J Xu and T Ma. (2016). Hypoxia enhances the protective effects of placenta-derived mesenchymal stem cells against scar formation through hypoxia-inducible factor-1alpha. Biotechnol Lett 38:931–939.
196. Lan YW, KB Choo, CM Chen, TH Hung, YB Chen, CH Hsieh, HP Kuo and KY Chong. (2015). Hypoxia-preconditioned mesenchymal stem cells attenuate bleomycin-induced pulmonary fibrosis. Stem Cell Res Ther 6:97.
197. Ueno T, A Nakashima, S Doi, T Kawamoto, K Honda, Y Yokoyama, T Doi, Y Higashi, N Yorioka, et al. (2013). Mesenchymal stem cells ameliorate experimental peritoneal fibrosis by suppressing inflammation and inhibiting TGF-β1 signaling. Kidney Int 84:297–307.
198. Liu YL, W-H Liu, J Sun, T-J Hou, Y-M Liu, H-R Liu, Y-H Luo, N-N Zhao, Y Tang and F-M Deng. (2014). Mesenchymal stem cell-mediated suppression of hypertrophic scarring is p53 dependent in a rabbit ear model. Stem Cell Res Ther 5:136.
199. Bargonetti J and JJ Manfredi. (2002). Multiple roles of the tumor suppressor p53. Curr Opin Oncol 14:86–91.
200. Zanotti L, R Angioni, B Calì, C Soldani, C Ploia, F Moalli, M Gargesha, G D'Amico, S Elliman, et al. (2016). Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1. Leukemia 30:1143–1154.
201. Andreeva ER, OO Udartseva, OV Zhidkova, SV Buravkov, MI Ezdakova and LB Buravkova. (2018). IFN-gamma priming of adipose-derived stromal cells at ''physiological'' hypoxia. J Cell Physiol 233:1535–1547.
202. Kavanagh DP, S Suresh, PN Newsome, J Frampton and N Kalia. (2015). Pretreatment of mesenchymal stem cells manipulates their vasculoprotective potential while not altering their homing within the injured gut. Stem Cells 33:2785–2797.
203. Rodríguez TM, A Saldías, M Irigo, JV Zamora, MJ Perone and RA Dewey. (2015). Effect of TGF-beta1 stimulation on the secretome of human adipose-derived mesenchymal stromal cells. Stem Cells Transl Med 4:894–898.
204. Liu B, KW Lee, M Anzo, B Zhang, X Zi, Y Tao, L Shiry, M Pollak, S Lin and P Cohen. (2007). Insulin-like growth factor-binding protein-3 inhibition of prostate cancer growth involves suppression of angiogenesis. Oncogene 26:1811–1819.
205. Benini S, M Zuntini, MC Manara, P Cohen, G Nicoletti, P Nanni, Y Oh, P Picci and K Scotlandi. (2006). Insulin-like growth factor binding protein 3 as an anticancer molecule in Ewing's sarcoma. Int J Cancer 119:1039–1046.
206. Guess AJ, B Daneault, R Wang, H Bradbury, KMD La Perle, J Fitch, SL Hedrick, E Hamelberg, C Astbury, et al. (2017). Safety profile of good manufacturing practice manufactured interferon gamma primed mesenchymal stem/stromal cells for clinical trials. Stem Cells Transl Med 6:1868–1879.
207. Butler J, SE Epstein, SJ Greene, AA Quyyumi, S Sikora, RJ Kim, AS Anderson, JE Wilcox, NI Tankovich, et al. (2017). Intravenous allogeneic mesenchymal stem cells for nonischemic cardiomyopathy: safety and efficacy results of a phase II—a randomized trial. Circ Res 120:332–340.
208. Yagi H, A Soto-Gutierrez, Y Kitagawa, AW Tilles, RG Tompkins and ML Yarmush. (2010). Bone marrow mesenchymal stromal cells attenuate organ injury induced by LPS and burn. Cell Transplant 19:823–830.

209. Öksüz S, E Ülkür, O Öncül, GT Köse, Z Küçükodaci and M Urhan. (2013). The effect of subcutaneous mesenchymal stem cell injection on statis zone and apoptosis in an experimental burn model. Plast Reconstr Surg 131:463–471.
210. Zhang J, X La, L Fan, P Li, Y Yu, Y Huang, J Ding and Y Xing. (2015). Immunosuppressive effects of mesenchymal stem cell transplantation in rat burn models. Int J Clin Exp Pathol 8:5129–5136.
211. Shumakov VI, NA Onishchenko, MF Rasulov, ME Krasheninnikov and VA Zaidenov. (2003). Mesenchymal bone marrow stem cells more effectively stimulate regeneration of deep burn wounds than embryonic fibroblasts. Bull Exp Biol Med 136:192–195.
212. Rasulov MF, VT Vasilenko, VA Zaidenov and NA Onishchenko. (2006). Cell transplantation inhibits inflammatory reaction and stimulates repair processes in burn wound. Bull Exp Biol Med 142:112–115.
213. Guo X, B Xia, XB Lu, ZJ Zhang, Z Li, WL Li, AB Xiong, L Deng, MY Tan and YC Huang. (2016). Grafting of mesenchymal stem cell-seeded small intestinal submucosa to repair the deep partial-thickness burns. Connect Tissue Res 57:388–397.
214. Liu P, Z Deng, S Han, T Liu, N Wen, W Lu, X Geng, S Huang and Y Jin. (2008). Tissue-engineered skin containing mesenchymal stem cells improves burn wounds. Artif Organs 32:925–931.
215. Clover AJ, AH Kumar, M Isakson, D Whelan, A Stocca, BM Gleeson and NM Caplice. (2015). Allogeneic mesenchymal stem cells, but not culture modified monocytes, improve burn wound healing. Burns 41:548–557.
216. Miettinen JA, M Pietilä, RJ Salonen, S Ohlmeier, K Ylitalo, HV Huikuri and P Lehenkari. (2011). Tumor necrosis factor alpha promotes the expression of immunosuppressive proteins and enhances the cell growth in a human bone marrow-derived stem cell culture. Exp Cell Res 317:791–801.
217. Fan H, G Zhao, L Liu, F Liu, W Gong, X Liu, L Yang, J Wang and Y Hou. (2012). Pre-treatment with IL-1beta enhances the efficacy of MSC transplantation in DSS-induced colitis. Cell Mol Immunol 9:473–481.
218. Thomas H, M Jäger, K Mauel, S Brandau, S Lask and SB Flohé. (2014). Interaction with mesenchymal stem cells provokes natural killer cells for enhanced IL-12/IL-18-induced interferon-gamma secretion. Mediators Inflamm 2014:143463.

Address correspondence to:
Dr. Marina Trouillas
INSERM U1197–Institut de Recherche Biomédicale des Armées (IRBA)/Antenne Centre de Transfusion Sanguine des Armées (CTSA)
1 rue du lieutenant Raoul Batany
92141 Clamart
France

E-mail: trouillasmarina@gmail.com

Received for publication May 7, 2018
Accepted after revision July 23, 2018
Prepublished on Liebert Instant Online July 23, 2018