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REVIEW ARTICLE

Human stem cells prevent flap necrosis in preclinical animal models: A systematic review

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ABSTRACT

Background and Aim: Adipose-derived mesenchymal stem cells (ADSCs) have been proven effective to prevent distal skin flap necrosis in preclinical models. However, to appropriately translate these findings to clinical trials, the effect of ADSC of human origin (hADSC) needs to be evaluated. We hypothesize that hADSC treatment is as effective as animal ADSC treatment at preventing distal skin flap necrosis in animal flap models.
Methods: Three databases were inquired on August 17, 2020, to evaluate the necrotic flap area after using hADSCs in animal models of ischemic flaps. No publication status or dates were considered. Studies were included if they used hADSCs, measured the surviving or necrotic skin area of flaps, used animal models, and were in English. Studies were excluded if they did not use cells of human origin. The flap survival or necrotic area, perfusion, capillary density, vascular endothelial growth factor secretion and HIF-1α expression were extracted.
Results: Ten studies met inclusion criteria. The mean absolute risk reduction (ARR) in necrotic skin area was 22.37% (95% confidence interval [CI] 16.98-27.76%, $P<0.05$) for flaps treated with animal ADSCs and 18.04% (95% CI 2.74-33.33%, $P<0.05$) for flaps treated with hADSCs. The difference between mean ARRs was not statistically significant (4.33%, 95% CI – 34.47-43.13%, $P>0.05$).
Conclusion: Human ADSCs prevent skin flap necrosis to the same degree as animal ADSCs in rodent and rabbit flap models.
Relevance for Patients: This review found that adipose-derived stem cells of human origin are equally effective at reducing the risk of surgical flap necrosis in preclinical models of small animals as autologous animal cells. The findings in this review should encourage researchers to use human adipose-derived stem cells in animal models of ischemic flaps to accelerate their translation into clinical trials and, eventually, surgical practice. The low immunogenicity of these cells should be leveraged to gain insight into the effects of the products that will be ultimately administered to patients. Furthermore, human adipose-derived stem cells' pro-angiogenic mechanism of action sets this therapy as a promising preventive measure for flap necrosis.

1. Introduction

Flaps are routinely used in plastic surgery to cover tissue defects. Although rare, irreversible ischemia leading to necrosis can occur in the distal portion of random pattern skin flaps or the random portion of pedicled and free flaps [1]. The unpredictable vascular support of random pattern skin flaps needs consideration when defining their size and shape since inappropriate length-to-width ratios may predispose to ischemia and necrosis [2,3]. Free flap loss still occurs in 5.1-7.7% of cases, with rates almost doubling

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in post-mastectomy skin flaps [4]. Furthermore, post-mastectomy skin flap treatment represents an extra expense of up to $7000 per patient [5-7]. Therefore, serious esthetic, functional, and economic repercussions can develop when skin necrosis occurs in flaps used in these sensitive areas [8,9].

Ischemic preconditioning is a process that aims to improve flap survival by exerting brief episodes of ischemia to induce tissue tolerance and lessen necrosis and apoptosis [10-12]. Ischemic preconditioning methods include surgical procedures or pharmacological therapies. Surgical procedures have the disadvantage of needing an additional surgical intervention before the final flap harvest [3,13]. On the other hand, pharmacological interventions are limited by adverse effects, high costs, the difficulty of the administration method, and the drugs' unavailability [8].

Growth factors involved in angiogenesis, such as vascular endothelial growth factor (VEGF), have also been used as preconditioning therapeutics. While systemic and viral-mediated gene therapy can expose subjects to serious side effects [14-17], other existing delivery methods are limited by their short half-life, instability, local side effects, and appropriate dosage [18]. Adipose-derived mesenchymal stem cells (ADSCs) have shown promising results in the treatment of ischemic diseases through the secretion of VEGF and other pro-angiogenic cytokines [19]. Locally transplanted ADSCs can, therefore, continuously secrete VEGF to enhance neovascularization and improve ischemia. However, most of the studies use ADSCs of animal origin.

The architecture of human adipose tissue differs significantly from that of mice, likely affecting their functional properties [20]. *In vitro* studies have shown protein expression differences between hADSCs and animal ADSCs [21,22]. Furthermore, the differentiation capacity of hADSCs and animal ADSCs differs [23]. The evidence, therefore, implies that the results observed using ADSC of animal origin might not be entirely translatable to humans due to inherent molecular differences or differences in the mechanism of action [21,24,25].

Most preclinical studies using ADSC as a pre-operative treatment for skin flap ischemia and necrosis in animals have used either autologous or allogeneic ADSC, showing a decreased necrotic skin area in random pattern and pedicled flap models [26]. Although it is thought that the immune response in non-immunosuppressed animals might skew the outcomes when using human-derived cell products [27], a recent comparison between porcine ADSC and hADSCs showed that both cell types had an equally strong capacity to reduce the proliferation of alloreactive splenocytes in mixed lymphocyte reaction assays [28].

Based on the previous background information, this review aims to show the efficacy of hADSCs at preventing distal skin flap necrosis in animal models of random pattern, pedicled, or free flaps. We hypothesize that hADSCs treatment is as effective as animal ADSCs treatment at preventing distal skin flap necrosis by enhancing angiogenesis in response to VEGF, as measured by the percentage of necrotic skin area. Furthermore, understanding the clinical effect of hADSCs in animal models is crucial for translational medicine since the cell products that will ultimately be used in patients will be of human origin.

2. Methods

Studies were identified by searching PubMed, CINAHL, and EMBASE databases from inception to present. The search was conducted on August 17, 2020. MeSH terms for "mesenchymal stem cells," "adipose-derived mesenchymal stem cells," "bone marrow mesenchymal stem cells," and "flap" were used. Search terms were arranged as follows: (stem cell*, mesenchymal OR mesenchymal stem cell* OR bone marrow mesenchymal stem cell* OR bone marrow stromal cell* OR adipose-derived mesenchymal stem cell* OR adipose-derived mesenchymal stem cell* OR mesenchymal stem cell*, adipose-derived OR mesenchymal stem cell*, adipose-derived OR adipose tissue-derived mesenchymal stem cell* OR adipose tissue-derived mesenchymal stem cell*) AND (surgical flap OR flap, surgical OR island flaps OR pedicled flap OR flap, pedicled).

Studies were included if they (1) used hADSCs, (2) measured the survival or necrotic skin area of flaps, (3) used animal models, and (4) were in English. The primary endpoint was to show that groups treated with hADSCs had a significantly lower percentage of necrotic flap skin compared to those not receiving this therapy within 14 days of cell administration. The secondary endpoints were the following: (1) To show that groups treated with hADSCs had a significantly increased flap perfusion compared to those not receiving this therapy within 14 days of cell administration; (2) to show significantly increased VEGF secretion and HIF-1α expression in hADSCs either *in vitro* compared to control culture media or *ex vivo* compared to those groups not receiving this therapy within 14 days of cell administration; and (3) to show significantly increased capillary density in the groups treated with hADSCs compared to those not receiving this therapy within 14 days of cell administration.

No specific publication status was considered. The study selection process, along with the reasons for exclusion, is detailed in Figure 1. Eligibility assessment and data extraction were performed by one reviewer (FRA), following the PRISMA guidelines. The risk of bias of included studies was assessed using the ROBINS-I tool of the Cochrane Library for non-randomized studies. A summary and a graph were created using RevMan 5.3 (Cochrane Collaboration), which allows for bias stratification in several domains (Figures 2 and 3).

3. Results

Out of 149 studies, 10 fulfilled the inclusion criteria. Studies assessing pedicled flaps used either a long thoracic artery [29] (Supplementary Figure 1) or a superficial inferior epigastric artery pedicled flap (Supplementary Figure 2) [30]. Studies assessing random pattern skin flaps used modified McFarlane flaps in their animal models (Supplementary Figures 3 and 4). The McFarlane flap was introduced in 1965 as the first standardized surgical flap technique [31]. It was initially a cranially based flap positioned between the lower angles of the scapulae measuring 10×4 cm and yielding a length-to-width ratio of 2.5:1 [31]. Gong *et al.* [32] followed a similar surgical technique in a rabbit model. The included studies are summarized in Table 1.

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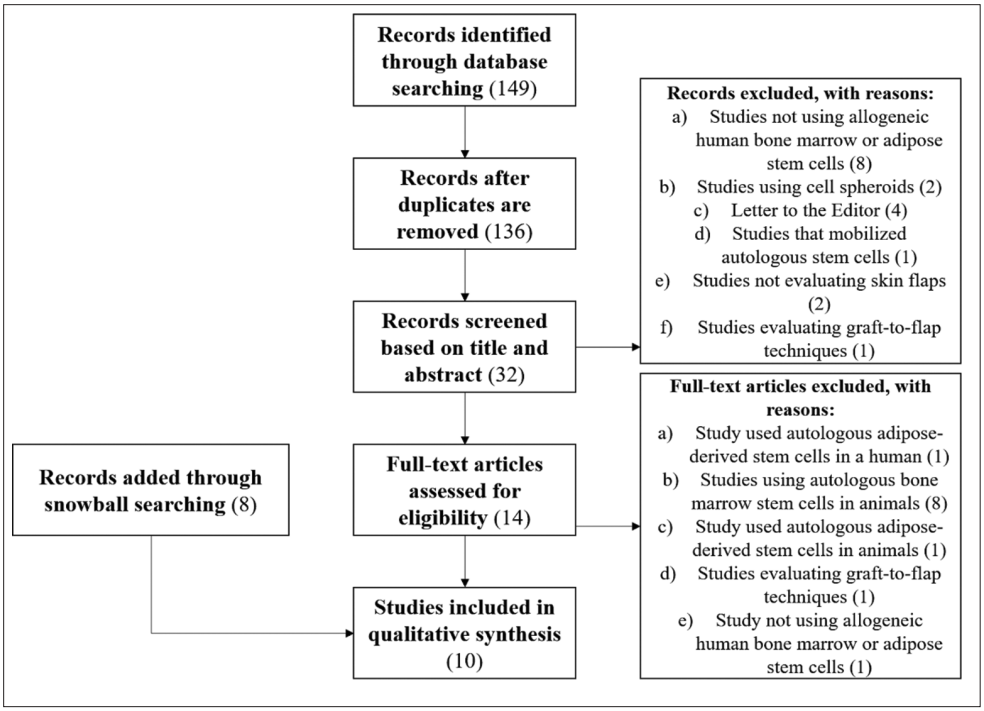

Figure 1. Study selection flowchart. Flowchart describing the study selection process according to PRISMA guidelines.

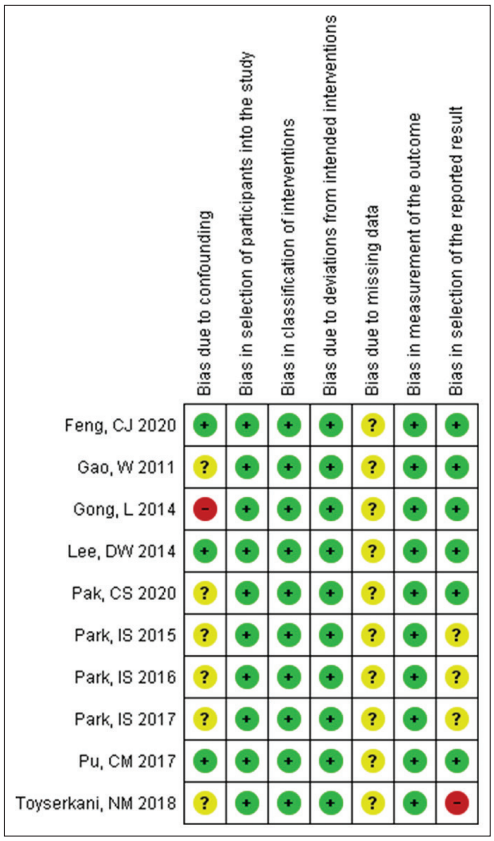

Figure 2. Risk of bias summary. Risk of bias summary created with RevMan 5.3 following the ROBINS-I guidelines of the Cochrane Library. Green indicates low risk of bias, yellow indicates unclear risk of bias, and red indicates high risk of bias.

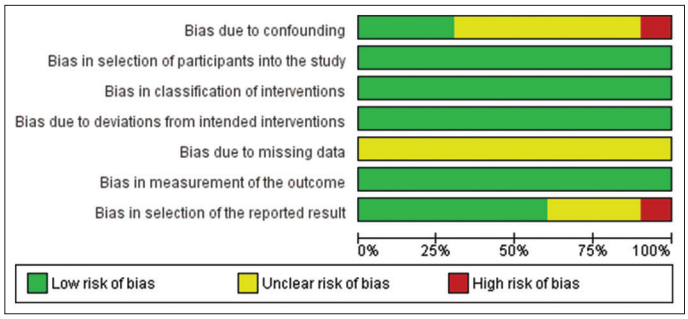

Figure 3. Risk of bias graph. Risk of bias graph created with RevMan 5.3 following the ROBINS-I guidelines of the Cochrane Library. Green indicates low risk of bias, yellow indicates unclear risk of bias, and red indicates high risk of bias.

3.1. Necrotic flap area

Eight out of 10 included studies found a decreased necrotic flap area when using hADSCs (Table 2) [29,30,32-37]. However, only five studies reported a statistically significant difference between their hADSC-treated groups and the untreated control groups [29,30,32-34]. Three studies graphically showed decreased necrotic flap area, but did not provide a statistical comparison [35-37]. Two studies did not find a decreased necrotic flap area compared to the untreated control groups [38,39].

3.2. Flap perfusion

Six out of 10 studies measured flap perfusion after hADSC treatment (Table 2) [30,33-37]. However, only five reported the results of these measurements [33-37]. Furthermore, only two of these five studies reported significantly higher perfusion in the

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Table 1. Summary of experimental studies using adipose tissue-derived mesenchymal stem cells in flap survival improvement.

Author	Cell type	Provider	Model	Additional treatment	Flap characteristics	Number of cells transplanted	Cell transplantation site	Time for flap assessment	Results
Gao *et al.*, 2011. China	hADSCs	Harvested	BALB/c-nu/nu male STZ-induced diabetic mice aged 7-8 weeks weighing 20-25 g	N/A	1×3 cm random pattern, caudally based dorsal flap including superficial fascia, panniculus carnosus, subcutaneous tissue, and skin	1×10^7 in 0.1 mL of serum-free LG DMEM	10 injections on the flap's longitudinal axis	7 days after surgical procedure	Local injection of hADSCs could improve ischemic random skin flap viability by enhancing neovascularization in STZ-induced diabetic mice
Lee *et al.*, 2014. Korea	hADSCs	Harvested	Sprague Dawley rats weighing 300-400 g	N/A	3×8 cm cranially based dorsal flap including the panniculus carnosus. A silicone sheet was inserted to separate the flap from the wound bed	1×10^7 suspended in 1 ml PBS	Tail vein injection; subcutaneous injection in even distribution; collagen sponge application; fibrin glue application	Flaps' status was followed for 2 weeks after procedures	The use of a collagen sponge for hADSC delivery was the best method to increase flap viability
Gong *et al.*, 2014. China	hADSCs	Harvested	New Zealand white rabbits	N/A	2 longitudinally parallel 6×2 cm cranially based flaps, 2 cm apart, including the subdermal vascular plexus. A sterile silicone sheet of 3 cm×7 cm× 0.1 mm was inserted to separate flap from wound bed	4×10^5	5 transfer sites each 1 cm separated from each other along the central axis of the right flap	7 days after surgical procedure	hADSCs enhance random pattern flap viability, probably by increased secretion of angiogenesis promoting growth factors, without serious immune rejection of stem cells
Park *et al.*, 2015. Korea	hADSCs	CEFO (Seoul, Korea)	BALB/c male mice aged 7 weeks	LLLT (flap)	4×2 cm cranially based dorsal flap (base 1 cm caudal to occipital neckline). A 0.13 mm thick silicone sheet was inserted to separate flap from the wound bed	1.5×10^6 in 0.3 mL of TCP mixed with PBS	3 intramuscular injections along the skin flap	Flaps' status was followed for 2 weeks after procedures	hADSC transplantation with LLLT treatment accelerates angiogenesis through endothelial cell differentiation and growth factor secretion, enhancing the functional recovery of skin flap area
Park *et al.*, 2016. Korea	hADSCs and hADSCs spheroids	CEFO (Seoul, Korea)	BALB/c male mice aged 7 weeks	LLLT (flap)	4×2 cm cranially based dorsal flap (base 1 cm caudal to occipital neckline). A 0.13 mm-thick silicone sheet was inserted to separate flap from the wound bed	Monolayer hADSCs 15×10^5, spheroid hADSCs 10 masses both in 300 µL of PBS	6 intramuscular injections along the skin flap	Flaps' status was followed for 2 weeks after procedures	LLLT enhances hADSC survival in flaps, stimulating growth factor secretion

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Table 1. *(Continued)*

Author	Cell type	Provider	Model	Additional treatment	Flap characteristics	Number of cells transplanted	Cell transplantation site	Time for flap assessment	Results
Park *et al.*, 2017. Korea	hADSCs and hADSCs spheroids	CEFO (Seoul, Korea)	BALB/c male mice aged 7 weeks	LLLT (hADSCs)	4×2 cm cranially based dorsal flap (base 1 cm caudal to occipital neckline). A 0.13 mm-thick silicone sheet was inserted to separate flap from the wound bed	Monolayer hADSCs 15×10^5, spheroid hADSCs 10 masses 1.2-1.5 mm in diameter both in 100 µL of PBS	Four intradermal injections at the border of the skin flap	Flaps' status was followed for 2 weeks after procedures	hADSCs spheroids accelerate tissue regeneration through endothelial cell differentiation and growth factor secretion
Pu *et al.*, 2017. Taiwan	hADSCs	Harvested	C57BL/6J and C57BL/6J-derived *IL6*$^{-/-}$ male mice weighing 25-30 g	N/A	4×1 cm pectoral skin flap based over the right long thoracic vessels (flap pedicle was clamped for 3 h)	1×10^6 in 0.12 mL of saline	Three injections in the proximal, middle, and distal parts of the flap were applied to the subcutaneous layer between the flap and the wound bed	5 days after surgical procedure	hADSCs, hADSC-CM, and hADSC-Exo enhance skin flap survival after I/R injury through IL-6-mediated angiogenesis
Toyserkani *et al.*, 2018. Denmark	hADSCs	Harvested	Outbred male Sprague Dawley rats weighing 300 g	N/A	2×7 cm caudally based dorsal flap including a triangular area (total surface area 15 cm^2)	5×10^6 in 0.3 mL of PBS	Three subcutaneous injections of 0.1 mL each at 3, 3.5, and 4 cm from the base of the flap	7 days after surgical procedure	hSVF increases skin flap survival when compared with controls, while hASDCs do not. However, differences in flap survival area were not significantly different between hSVF and hADSCs
Feng *et al.*, 2020. Taiwan	hADSCs	Harvested	BALB/CAnN. Cg-*Foxn1*^{nu}/CrlNarl nude mice aged 8 weeks weighing 20 g	N/A	Unipedicled 3×3 cm left SIEA flap from the xiphoid to the pubis and from the posterior to the anterior axillary line	Low-dose group received 1×10^3, medium-dose group received 1×10^4, and high-dose group received 1×10^5 (cells were suspended in 0.2 mL of PBS in all cases)	30-gauge needle was inserted in the right femoral artery	7 days after surgical procedure	Intra-arterial injection of hADSCs increases the survival of the random part of axial skin flaps
Pak *et al.*, 2020	hADSCs	Harvested	Sprague Dawley rats weighing 250±10 g	rIPC	3×9 cm caudally based dorsal flap including the panniculus carnosus	5×10^5 suspended in 100 µL of PBS	Hypodermal injection at three sites of the skin flap boundary	Flaps' status was followed for 2 weeks after procedures	rIPC+hADSCs treatment reduces skin flap necrosis and increased neovascularization

hADSC: Human adipose-derived stem cells; N/A: Not available; PU: Perfusion unit; CEFO: Cell engineering for origin; LLLT: Low-level light treatment; LG DMEM: Low-glucose Dulbecco's modified Eagle's medium; PBS: Phosphate-buffered saline; hADSC-CM: Human adipose-derived stem cell culture media; hADSC-Exo: Human adipose-derived stem cell exosomes; I/R: Ischemia-reperfusion; hSVF: Human stromal vascular fraction; SIEA: Superficial inferior epigastric artery; rIPC: Remote ischemia preconditioning

hADSC-treated groups than the untreated control groups [33,34]. Although three studies graphically showed elevated flap perfusion in the hADSC-treated groups, they did not provide a statistical comparison to the untreated control groups [35-37].

3.3. VEGF levels and HIF-1α expression

Six out of 10 studies measured either VEGF, HIF-1α, or both (Table 2) [30,32,33,35-37]. All these studies found increased

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Table 2. Percentage of surviving and necrotic areas of hADSC-treated flaps reported in the included studies.

Author	Necrotic/surviving flap area[†]	Perfusion[Δ]	VEGF and HIF-1α levels	Capillary density
Gao *et al.*, 2011. China	The surviving area was significantly higher in hADSC-treated group (83.2 ± 5.3%) compared to culture media-treated (47 ± 4.5%) and control groups (43.7 ± 4.5%) [a]	Perfusion units were significantly higher in the hADSC-treated group (863.26 ± 76.52) compared to media-treated (382.52 ± 125.64) and control groups (356.31 ± 93.91) [a]	VEGF levels and HIF-1α expression were significantly higher in the hADSC-treated group compared to media-treated and control groups (no specific values were provided) [a]	The number of capillaries and CD31 + cells was significantly higher in the hADSC-treated group compared to media-treated and controls (no specific value was provided) [a]
Lee *et al.*, 2014. Korea	The surviving area was significantly higher in the groups receiving hADSCs by SQ injection (53.2 ± 5.8%) and CS seeding (54.9 ± 5.4%) compared to the control group (39.2 ± 4.3%) [b]	The ratio[#] was significantly higher in the groups receiving hADSC by IV injection (1.71 ± 0.41), SQ injection (1.79 ± 0.30), and SC seeding (1.81 ± 0.31) compared to the control group[b]	N/A	The number of capillaries was significantly higher in the groups receiving hADSCs by IV injection (16.9 ± 2.8) and CS seeding (17.9 ± 2.1) compared to the control group[b]
Gong *et al.*, 2014. China	The surviving area was significantly higher in hADSC-treated group (59.7 ± 0.030%) compared to control group (46.4 ± 0.038%) [b]	N/A	VEGF levels were significantly higher in hADSC media supernatant (928.56 ± 105.24 pg/10^6 cells) compared to those of DMEM without cells (21.05 ± 1.21 pg/10^6 cells) [a]	The number of capillaries was significantly higher in the hADSC-treated group (9 ± 1.5) compared to the control group (5 ± 1) [a]
Park *et al.*, 2015. Korea	The necrotic area was lower in the hADSC-treated group compared to the control group[d]	Perfusion units were higher in the hADSC-treated group compared to the control group[d]	Protein levels and expression were higher in the hADSC-treated group compared to the control group[d] (no specific values were provided)	The number of arterioles per mm^2 was significantly higher in the hADSC alone group compared to the control group[d] (no specific value was provided)
Park *et al.*, 2016. Korea	The necrotic area was lower in the hADSC-treated group compared to the control group[d]	Perfusion units were higher in the hADSC-treated group compared to the control group[d]	Protein levels and expression were lower in the hADSC monolayer-treated group compared to the hADSC spheroid-treated group[d] (no specific values were provided)	The number of $CD31^+$ vessel-like structures per mm^2 in the monolayer hADSC-treated group was not compared to an untreated group (no specific value was provided)
Park *et al.*, 2017. Korea	The necrotic area was lower in the hADSC-treated group compared to the control group[d]	Perfusion units were higher in the hADSC-treated group compared to the control group[d]	Protein levels were lower in the hADSC monolayer-treated group compared to the hADSC spheroid-treated group[d] (no specific values were provided)	The number of $CD31^+$ vessel-like structures per mm^2 in the monolayer hADSC-treated group was not compared to an untreated group (no specific value was provided)
Pu *et al.*, 2017. Taiwan	The surviving area was significantly higher in the group treated with hADSCs compared to the one not receiving treatment[b]	N/A	N/A	The number of microvessels was significantly higher in the hADSC-treated group (16.3 ± 1.9) compared to the untreated group (5.8 ± 1.4)[b]
Toyserkani *et al.*, 2018. Denmark	The surviving area was not significantly different in the hADSC-treated group (50.4% [SD 9.1%]) compared to the control group (45.7% [SD 9.5%])	N/A	N/A	The number of $CD31^+$ vessels was significantly increased in the hADSC-treated group (12.22 ± 2.52) compared to the control group (8.36 ± 2.47)[a]
Feng *et al.*, 2020. Taiwan	The necrotic area was significantly lower in all hADSC-treated groups, especially in the medium-dose hADSC-treated group (20.71 ± 2.42%) compared to the control group (52.62 ± 3.71%) [c]	N/A	VEGF levels in the flap were significantly higher in the high-dose hADSC-treated group (0.56 ± 0.05 pg/mg) compared to the control group (0.33 ± 0.02 pg/mg) [a]	Vessel density was significantly higher in the medium-dose hADSC-treated group (6.58 ± 0.56) compared to the control group (3.67 ± 0.82)[b]
Pak *et al.*, 2020	There were no significant differences in the necrotic area observed in the hADSC-treated group (36.64 ± 3.38%) and the control group (40.60 ± 3.27%).	N/A	N/A	The number of vWF^+ vessels was significantly higher in the hADSC-treated group (4.44 ± 0.85) compared to the control group (0.44 ± 0.24)[a] The number of $CD31^+$ vessels was significantly higher in the hADSC-treated group compared to the control group[b] (no specific values were provided)

[†]Every study evaluated the measured outcome by visually identifying the area of interest (survival/necrosis area) and analyzing it digitally using different image analysis software. The percentages of surviving flap area are relative to the total flap surface area. [Δ]Perfusion units are arbitrary units and are therefore not comparable among studies. [#]Ratio of post-operative PU to pre-operative PU. [a]$P < 0.01$. [b]$P < 0.05$. [c]$P < 0.001$. [d]Unknown if statistically significant. hADSC: Human adipose-derived stem cell; IV: Intravenous; SQ: Subcutaneous; CS: Collagen scaffold; SD: Standard deviation; PU: Perfusion units; VEGF: Vascular endothelial growth factor; HIF-1α: Hypoxia-inducible factor-1α; CD: Cluster of differentiation; vWF: von Willebrand factor

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VEGF levels or HIF-1α expression. However, only two studies provided specific values to their measurements [30,32]. Although three studies graphically showed elevated levels and expression of VEGF or HIF-1α in either the culture supernatant or the *ex vivo* analysis of the flaps, they did not provide a statistical comparison of the hADSC-treated groups and the untreated control groups [35-37].

3.4. Capillary density

All the studies measured the number of capillaries in the flaps using different techniques (Table 2) [29,30,32-39]. Eight studies found a statistically significant increase in the number of capillaries in the hADSC-treated groups than the untreated control groups [29,30,32-35,38,39]. Two studies did not provide a statistical comparison between the hADSC-treated group and an untreated control group [36,37].

3.5. Comparison between hADSCs and animal ADSCs

In addition to extracting the necrotic or surviving skin areas observed in animal models of random pattern and pedicled skin flaps treated with hADSCs, we extracted these data from studies that used ADSCs of animal origin to prevent skin flap necrosis (Table 3). If the studies provided the surviving skin areas, these values were subtracted from the total area to obtain the necrotic skin area. The studies that did not provide specific values for these data were not included in the calculation. Therefore, based on the available data, the use of animal ADSCs was associated with a decrease in skin necrotic area of 22.37% compared to the control group (absolute risk reduction [ARR]: 22.37%; 95% confidence interval [CI] 16.98%-27.76%). On the other hand, the use of hADSCs was associated with a decrease in skin necrotic area of 18.04% compared to the control group (ARR: 18.04%; 95% CI 2.74-33.33%) (Table 4 and Figure 4). The ARR difference in skin necrotic area between animal ADSCs and hADSCs was not statistically significant (difference in risk: 0.0433 [4.33%]; 95% CI –0.3447-0.4313; $P>0.05$).

4. Discussion

Preconditioning aims to increase a flap's surviving length [40]. The first preconditioning method proposed for flap surgeries was surgical delay, consisting in the partial interruption of a flap's blood flow before transfer. However, the need for an additional intervention, increased patient risk, and increased health-care costs made this approach unsuitable for clinical practice [40,41]. Ischemic preconditioning, which followed surgical delay, consists of applying a brief period of ischemia and reperfusion to the flap, increasing its resistance to reperfusion injury [40]. However, this approach was never fully adopted for the same reasons as surgical delay [40]. A different approach, remote ischemic preconditioning (rIPC), showed positive results in preclinical models and prevented endothelial dysfunction in humans [42]. However, a recent randomized clinical trial failed to show improved free flap outcomes [43].

More recently, preconditioning has also been achieved in preclinical models by inducing hyperthermia or hypothermia in the region of interest or using pharmacological agents, growth factors, and mechanical stress. Many studies evaluating these preconditioning approaches have been performed in preclinical animal models, with few published clinical trials. These interventions have decreased necrosis in preclinical models compared to control groups [8,40,44,45]. A recent systematic review on thermal preconditioning by Kankam *et al.* only found three clinical trials, randomized and non-randomized, showing a lower incidence of flap necrosis and surgical reintervention in those patients using hyperthermic preconditioning compared to sham controls [46-49].

The pharmacological agents used for flap preconditioning are therapies with known dose-dependent side effects. For example, although nitric oxide donors have proven effective for flap preconditioning in preclinical models, they can lead to a dose-dependent drop in blood pressure [50]. Furthermore, growth factors are not exempt from obstacles to their use. These molecules are limited by a short half-life, rapid diffusion from the delivery site, and low cost-effectiveness [51]. On the contrary, ADSCs' effects after administration are long sustained [52], providing a substantial advantage over other therapeutics.

Although initially, this review aimed to analyze the efficacy of both hBMSCs and hADSCs on skin flap necrosis prevention, there were no data on the former's use, and thus, the focus turned to hADSCs solely. This is most likely because hADSCs are easier to extract, have shorter replication times, secrete a higher number of cytokines, and yield a more consistent number when harvested from patients of different ages compared to hBMSCs [53]. An increased percentage of healthy skin was noted in eight out of 10 studies, confirming our working hypothesis. However, the fact that only five of those studies described a statistical analysis highlights the need for further studies with more rigorous methods.

The cumulative evidence in previous reviews shows that ADSCs increase skin flap survival through increased growth factor secretion, with a certain degree of ADSC endothelial differentiation [26]. However, these findings are based mostly on the use of animal ADSCs. Formal analyses of the differences between hADSCs and animal ADSCs are scarce in the literature. Nahar *et al.* recently found that 92% of the proteins expressed by hADSCs and mouse ADSCs were similar [21]. The clinical repercussion of this finding is still unknown. Understanding the clinical effect of hADSCs in animal models is crucial for translational medicine, since the cell products that will ultimately be used in patients will be of human origin.

Although animal ADSCs were associated with a higher reduction in flap skin necrosis than hADSCs, these values were not significantly different ($P>0.05$). Therefore, hAdMSC treatment is associated with a similar reduction in skin flap necrotic area compared to autologous or allogeneic animal ADSCs in preclinical animal models. Out of the six studies using hADSCs that were used to calculate the ARR in flap skin necrotic area, only two used immunosuppressed animals. Although hADSCs do not generate a substantial immunogenic reaction *in vitro* [28,54], it is unclear if the heterogeneity in the state of the immunologic systems of animal models influenced the results. Only three of

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Table 3. Summary of outcomes of studies using animal adipose tissue-derived mesenchymal stem cells in flap survival improvement.

Author	Animal model	Flap characteristics	Cell number administration method	Necrotic or surviving flap measures	Flap perfusion	VEGF levels and HIF-1α expression	Capillary density
Lu *et al.*, 2008, Japan/China	Eight to 10-week-old ICR mice	Cranially based 1×3 cm flap. A 0.13 mm thick silicone sheet was inserted to separate the flap from the wound bed	1×10^6 cells in 0.1 mL of DMEM (level of injection is not specified)	Surviving flap length in AdMSC-treated groups significantly higher (1.45 cm±0.29 when injected on the pedicle's base; 1.87 cm±0.36 when injected 1.5 cm distal to the pedicle's base) compared to controls (0.93±0.11 cm) [a]	N/A	N/A	Number of capillaries significantly higher in AdMSC-treated groups (18±2.1 capillaries when injected on the pedicle's base; 33.5±1.7 capillaries when injected 1.5 cm distal to the pedicle's based) compared to controls (7.5±0.9 capillaries) [a]
Uysal *et al.*, 2009, Japan	10-week-old, albino, ICR mice	Cranially based random pattern skin flaps measuring 1×5 cm pedicle clamping to induce ischemia for 6 h	1×10^7 cells in 1 ml of PBS injected subdermally, distributed throughout the flap	Surviving flap length and area in AdMSC-treated group were significantly higher (24.4±2.9 mm and area of 21.8±3.7 mm^2) compared to controls (15.2±3.4 mm and area of 12.9±4.1 mm^2) [a]	N/A	Mean intensity of VEGF fluorescent antibody in AdMSC-treated group was significantly higher (27.53±3.57 pixels) compared to controls (13.87±1.12 pixels) [c]	Number of capillaries was significantly higher in AdMSC-treated group (7.5±0.68 capillaries per HPF) compared to controls (4.5±0.80 capillaries per HPF) [a]
Yang *et al.*, 2010, China	Six-week-old Wistar rats	Cranially based random pattern skin flap measuring 9×3 cm	4×10^6 cells in 0.5 ml of DMEM were injected subcutaneously, distributed in 10 points along the long axis of the flap	Surviving flap area in AdMSC-treated group was significantly higher (46.33±13.46%) compared to controls (26.33±13.46%) [a]	N/A	VEGF levels by *ex vivo* ELISA in AdMSC-treated group were not significantly higher (198.05±46 pg/ml) compared to controls (192.29±47.86)	Number of capillaries was significantly higher in AdMSC-treated group (27.56±5.80 capillaries) compared to controls (18.52±3.14) [a]
Li *et al.*, 2010, China	Adult male Wistar rats weighing 280-300 g	Caudally based abdominal rectangle peninsular flap based on the right femoral vessel pedicle	2×10^6 cells in five points around the vessel pedicle	Surviving flap area in AdMSC-treated group significantly higher (30.71±6.99%) compared to controls (17.53±4.38%) [b]	N/A	VEGF-A levels in hypoxic AdMSC media were significantly higher compared to normoxic AdMSC and chondrocyte media (no specific values provided)[a]; *ex vivo* ELISA of VEGF-A flap levels showed significantly higher levels in the AdMSC-treated group (1665.77±323.49 and 2821.82±654.88 pg/mL) compared to controls (923.20±115.54 and 1190±400.33 pg/mL) [a] at every time point	Number of capillaries significantly higher in AdMSC-treated group (27.85±13.64 capillaries per mm^2) compared to controls (7.63±4.24 capillaries per mm^2)[b]

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Table 3. *(Continued)*

Author	Animal model	Flap characteristics	Cell number administration method	Necrotic or surviving flap measures	Flap perfusion	VEGF levels and HIF-1α expression	Capillary density
Karathanasis *et al.*, 2013, Greece	Adult Wistar rats weighing 300-450 g	Cranially based random pattern skin flaps measuring 8×2 cm	1×10^6 cells in 1 ml of PBS were administered intradermally in the upper and lower halves of the flap	Surviving flap area in AdMSC-treated groups were significantly higher (81% in unlabeled AdMSCs and 85% in GFP-labeled AdMSCs) compared to controls (51-56%) [a]	N/A	N/A	N/A
Reichenberger *et al.*, 2012, Germany (a)	Adult male Lewis rats weighing 290-350 g	Extended epigastric adipocutaneous flap based on the left superficial epigastric vessel	1×10^6 cells by topical administration in 100 µl of the thrombin component of fibrin glue, between subcutaneous layer of skin flap and wound bed	Surviving flap area in AdMSC-treated group was significantly higher (93.6±6.7%) compared to controls (84.7±5.4% in group without fibrin glue and 81.9±4.2% in group with fibrin glue) [a]	Perfusion index in AdMSC-treated group was significantly higher (93.5±6.9%) compared to controls (82.3±3.9% in group without fibrin glue and 84.5±5.2% in group with fibrin glue) [c, d]	Significantly higher expression of VEGF-A and HiF1a genes in AdMSC-treated group compared to controls by *ex vivo* semi-quantitative RT-PCR [a]	Number of capillaries was not significantly higher in AdMSC-treated group (32.5±7.4 capillaries per HPF) compared to controls (31.1±8.1 in group without fibrin glue and 25.1±6.2 in group with fibrin glue)
Reichenberger *et al.*, 2012, Germany (b)	Adult male Lewis rats weighing 290-350 g	Modified extended epigastric island flap of 6×10 cm of the left superficial epigastric vessels subject to 3 h of ischemia	5×10^6 cells in 300 µL of culture media were injected intravenously into the right superficial epigastric vein	Surviving flap area in AdMSC-treated group was significantly higher (73.9±8.9%) compared to untreated controls (33.3±8.9%) [c]	Perfusion index in AdMSC-treated group was significantly higher (78.4±6.8%) compared to untreated controls (34.2±7.7%) [c,d]	Significantly higher expression of VEGF-A and HiF1a genes in AdMSC-treated group compared to controls by *ex vivo* semi-quantitative RT-PCR [a]	Number of capillaries was not significantly higher in AdMSC-treated group (27±7.8 capillaries per HPF) compared to untreated controls (19±6%)
Hollenbeck *et al.*, 2012, United States of America	Adult male Lewis rats	Cranially based random pattern skin flap measuring 1×3 cm	1×10^6 cells in 300 µL of DMEM were injected in the distal 1 cm of the skin flap	Surviving flap area in AdMSC-treated group significantly higher (37.4±18%) compared to controls (11.2±7%) [a]	N/A	ELISA on conditioned media from hypoxic cells had increased levels of VEGF (3215±173.1 pg/mL) compared to cells in normoxia (2476±108 pg/mL) [a]	N/A
Yue *et al.*, 2013, China	Adult male Lewis rats weighing 400-450 g	Caudally based pedicled flaps based on the circumflex iliac artery subject to ischemia by ligation of the perforators of the lateral thoracic and posterior intercostal arteries	2×10^6 cells were injected subcutaneously, divided in eight points of the distal third of the flap	The effective survival percentage* was significantly higher in the group receiving both hAdMSCs and artery ligation when performed 3 and 7 days before flap elevation compared to those receiving either treatment alone [a] (no comparisons were made with untreated groups)	N/A	Protein levels of VEGF and HIF-1α were significantly higher in the group receiving both hAdMSCs and artery ligation when performed 3 and 7 days before flap elevation compared to those receiving either treatment alone [a]	The number of capillaries was significantly higher in the group receiving both hAdMSCs and artery ligation when performed 3 and 7 days before flap elevation compared to those receiving either treatment alone [a] (no comparisons were made with untreated groups)

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Table 3. *(Continued)*

Author	Animal model	Flap characteristics	Cell number administration method	Necrotic or surviving flap measures	Flap perfusion	VEGF levels and HIF-1α expression	Capillary density
Suartz *et al.*, 2014, Brazil	Adult male Wistar rats weighing 250-300 g	Cranially based random pattern skin flap measuring 10×4 cm including deep and superficial fascia, panniculus carnosus, subcutaneous tissue, and skin. A plastic barrier was inserted to separate the flap from the wound bed	5×10^6 in 0.5 mL of PBS were injected intravenously in the caudal vein	Surviving flap area in AdMSC-treated group was significantly higher (58.14±4.46%) compared to controls (38.86±5.021%) [a]	N/A	N/A	N/A
Xu *et al.*, 2015, China	New Zealand long-eared white rabbits weighing approximately 3 kg	Axial skin flap measuring 3×6 cm based on the central auricular artery, vein and nerve pedicle	2×10^6 cells divided in 5 cutaneous injections (unspecified level)	Surviving flap area was higher in the AdMSC-treated group compared to controls (no exact value was provided) [a]	N/A	N/A	Capillary numbers were higher in the AdMSC-treated group compared to controls (no exact value was provided) [a]
Han *et al.*, 2015, Korea	Eight-week-old adult male Sprague Dawley rats	Epigastric flap of 6×4 cm of the right superficial epigastric vessel subject to 4 h of vein clamping	5×10^5 cells/mL in 0.1 mL of DMEM were injected subcutaneously and 5×10^5 cells/mL in 0.5 mL of DMEM were injected intraperitoneally	Surviving flap area was significantly higher in AdMSC-treated group (51.6±13.6%) compared to untreated controls (31.2±11.9%) [a]	N/A	N/A	N/A
Izmirli *et al.*, 2016, Turkey	Adult male albino Wistar rats weighing 200-350 g	Cranially based 6×5 cm flap with a central vascular pedicle, interpolated to a nearby site of 3×5 cm after five, eight, 11, or 14 days	3×10^6 cells in 1 mL of PBS were injected under the skin in two sites of the distal flap and four on the wound edges before transferring the flap to the defect area	Survival flap area was significantly increased in AdMSC-treated group (55.6±19.87%; 714.93±220.00 mm^2) compared to controls (39.7±12.37%; 459.59±175.28 mm^2) [a]	N/A	N/A	Number of capillaries was significantly higher in AdMSC-treated group (102.81±13.09 capillaries per HPF) compared to controls (80.5±13.27 capillaries per HPF) [b]
Ballestín *et al.*, 2018, Spain	Adult male Wistar rats weighing 290-350 g	Superficial caudal epigastric skin free flap measuring 3×6 cm subject to 8 h of ischemia by cutting the artery and vein prior to revascularization	5.5×10^6 cells were seeded on a collagen scaffold, inserted between the flap and the wound bed	Surviving flap area was significantly increased in AdMSC-treated group (73.09±16.32%) compared to controls (41.82±15.99%) [a]	N/A	N/A	N/A
Foroglou *et al.*, 2019, Greece	Wistar rats 30-50 weeks old weighing 200-250 g	Cranially based 8×2 cm flap. A 0.13 mm thick silicone sheet was inserted to separate the flap from the wound bed	1×10^6 cells in 1 mL of PBS were injected intradermally	Necrotic flap area was significantly lower in the AdMSC-treated group (3.1±2.8 cm^2; 19±18%) compared to controls (6.9±4.2 cm^2; 43±26%) [b]	N/A	N/A	N/A

*The effective survival percentage is defined as the survival rate of the experimental flap minus that of the control flap and indicates the effect of the exogenous intervention on flap survival. [a]$P<0.05$. [b]$P<0.01$. [c]$P<0.001$. [d]Perfusion is provided as mean perfusion index of the whole flap area in percentage±standard deviation in relation to normal surrounding skin. VEGF: Vascular endothelial growth factor; HPF: High-power field; GFP: Green fluorescent protein; DMEM: Dulbecco's Modified Eagle Medium

the studies analyzed in this systematic review present information regarding the immunological reaction after xenogeneic stem cell transplantation in immunocompetent hosts. Gong *et al.* stated that there was no evident macroscopic reaction (e.g., erythema and fever) in the animals treated with hADSCs [32]. In addition, the number of CD3+ cells and the CD4/CD8 ratio in pathology slides of treated and control groups were not statistically different ($P>0.05$) [32]. Furthermore, IFN-γ, IL-2, IL-4, and IL-10 levels were also not statistically different between groups ($P>0.05$) [32]. In Toyserkani *et al.* study, CD68+ cells were observed in all treated

Table 4. Percentage of necrosis with respect to the total flap area in the experimental and control groups observed in studies using ADSCs of animal and human origin.

Studies using animal ADSCs			
Author	**Necrosis % in experimental group (%)**	**Necrosis % in control group (%)**	**Absolute risk reduction (%)**
Uysal *et al.*, 2009	56.40%	74.20	17.80
Yang *et al.*, 2010	53.67	73.67	20
Li *et al.*, 2010	69.29	82.47	13.18
Karathanasis *et al.*, 2013	17	46.50	29.50
Reichenberg *et al.*, 2012 (a)	6.40	16.70	10.30
Reichenberg *et al.*, 2012(b)	26.10	66.70	40.60
Hollenbeck *et al.*, 2012	62.60	88.80	26.20
Suartz *et al.*, 2014	41.86	61.14	19.28
Han *et al.*, 2015	48.40	68.80	20.40
Izmirli *et al.*, 2016	44.40	60.30	15.90
Ballestín *et al.*, 2018	26.91	58.18	31.27
Foroglou P *et al.*, 2019	19	43	24
Mean	-	-	22.37
Studies using hADSCs			
Author	**Necrosis % in experimental group (%)**	**Necrosis % in control group (%)**	**Absolute risk reduction (%)**
Gao *et al.*, 2011	16.80	56.30	39.50
Lee *et al.*, 2014	45.95	60.80	14.85
Gong *et al.*, 2014	40.30	53.60	13.30
Toyserkani *et al.*, 2018	49.60	54.30	4.70
Feng *et al.*, 2020	20.71	52.62	31.91
Pak *et al.*, 2020	36.64	40.60	3.96
Mean	-	-	18.04

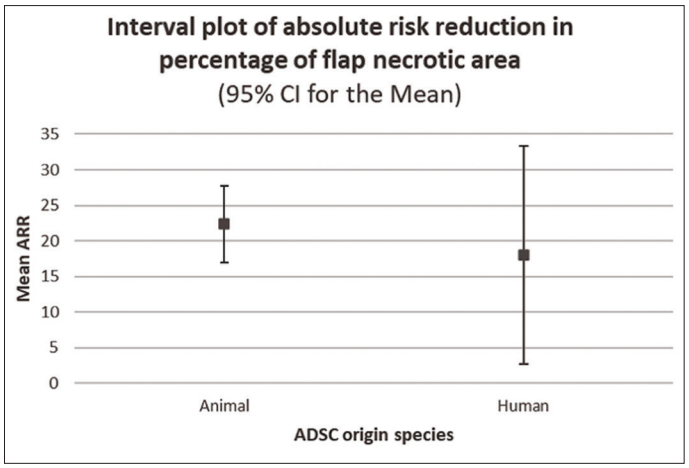

Figure 4. Percentage of skin flap necrotic area reduction attributable to cell therapy. The ARR in the percentage of skin flap necrosis was obtained by subtracting the mean percentage of skin flap necrosis of the experimental group from that of the control group for each study. The studies were grouped in those using ADSCs of animal or human origin, and the mean ARR was calculated for each group. The mean ARRs in flap necrosis were 22.37% (95% CI 16.98-27.76%) and 18.04% (95% CI 2.74-33.33%) for studies using animal ADSCs or hADSCs, respectively. The means are not significantly different (difference in risk: 0.0433 [4.33%]; 95% CI – 0.3447-0.4313; $P>0.05$). ADSC: Adipose-derived mesenchymal stem cell; ARR: Absolute risk reduction.

groups; however, no statistical analysis was done [38]. Finally, Feng *et al.* found that TNF-α, IFN-γ, and IL-6 levels were lower in groups treated with a low cell dose compared to controls [30]. In this same study, groups receiving a high dose of stem cells showed levels similar to those in control groups [30]. Although these studies point to an absence of a substantial inflammatory reaction and in some cases, a decrease in pro-inflammatory cytokines, a conclusion cannot be drawn at this time due to lack of information. Further studies using hADSCs to prevent skin flap necrosis should measure the immunologic reaction, both *in vivo* and *ex vivo*, after cell transplantation.

Although some studies did not find differences between the hADSC-treated groups and the controls [38,39], the absence of a statistically significant difference between the hADSC-treated groups and the best performing groups of each study imply that improvements in the methodologies (e.g., increased number of animal models or transplanted cell number adjustments) could yield conclusive results. Some studies' primary outcome was to study the addition of hypoxia preconditioning methods (e.g., low-level light therapy [LLLT] or remote ischemic preconditioning), either on the cells or the skin, on flap survival [35-37,39]. The fact that these studies found that using these methods increased flap survival, proangiogenic cytokine secretion, and capillary density to a higher degree than hADSCs alone ($P<0.05$) is an important finding that should be further studied.

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The results of the included studies suggest improved small vessel vascularity using hADSCs. The results of Park *et al.* [35,37] imply that LLLT, applied to flaps transplanted with either monolayer hADSCs or hADSC spheroids, could enhance these cells' secretory capacity and survival, thereby increasing the capillary number and flap perfusion. However, it should be noted that LLLT-treated hADSC spheroids had increased levels of endothelial markers [37]. The excessive use of hypoxia preconditioning methods might compromise hADSCs to the vascular endothelial lineage before transplantation.

In vitro comparisons of hADSCs and rat ADSCs have shown a better endothelial differentiation potential of the latter when cultured in commercial endothelial differentiation methods [55]. When evaluated *ex vivo*, most of the included studies found that hADSCs differentiated to endothelial cells to a low degree [29,30,32-37]. One study calculated that hADSC differentiation to endothelial cells contributed to 15.4% of the flaps' neovascularization [32]. However, one study did not find human cells in the flap even though it found an increased number of vessels in the hADSC-treated group, concluding that this increase was due to paracrine effect [38]. The contribution of hADSCs to flap neovascularization should be quantified in further studies.

Although the studies had favorable results, the lack of protocol standardization might pose a substantial bias. In 2014, Lee *et al.* [34] compared the effectiveness of different hADSC delivery routes to improve the viability of ischemic flaps and found that their application with a collagen sponge provided the best results. In 2020, Feng *et al.* [30] proved that intra-arterial delivery of hADSC through the femoral artery is also an efficient method to prevent flap ischemia and necrosis. Studies comparing these two methods are required to elucidate the delivery route that shows the best therapeutic efficacy.

Studying the best route of administration and the influence of the immunologic response should be further analyzed since only two studies evaluated these factors [34,38]. However, the study of hADSCs to prevent flap necrosis should first focus on elucidating the general contribution of the direct cell differentiation to endothelium and the paracrine effect on neovascularization. This might further derive in comparative studies between hADSCs and their cell products.

Finally, most studies evaluated random pattern skin flap necrosis, with few focusing on ischemia/reperfusion injuries. Ischemia/reperfusion injury is an acute process characterized by mitochondrial damage and cell death due to an abrupt increase in reactive oxygen species and other inflammatory mediators after an ischemic tissue regains perfusion [56,57]. This being an acute pathologic process, stem cells of any type might not be particularly suitable for treating it since their effects are more gradual and sustained. Therefore, pathologies requiring increased perfusion and gradual evolution are more suitable for stem cell treatment. Soft-tissue defect reconstruction and wound healing fit these requirements, and thus, stem cell research in plastic surgery has mainly focused on those processes [58,59].

5. Conclusion

The effect of hADSCs in flap viability improvement is being increasingly studied. The results provided in this review show that hADSCs prevent flap necrosis to the same extent as animal ADSCs in rodent and rabbit models of random pattern skin flaps and pedicled flaps.

6. Limitations

This study has several limitations. Since only studies published in English were included in this review, some studies may have been missed. Other limitations include the scarcity of studies reporting on this topic, the potential bias of misinterpreting data and results, and the study selection process, the latter being a potential source of bias common to systematic reviews. Specific for this systematic review, the absence of high-quality data, and the relative absence of information regarding the animal's immunologic response to human cells preclude us from analyzing if the immunologic status of the animal model should be considered for these studies. The major use of one flap technique (i.e., McFarlane flap) poses a risk of bias when extrapolating these findings to other types of flaps (e.g., pedicle flaps). Finally, the lack of a reason for using cells of human origin in the studies also poses a substantial risk of bias.

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Conflict of Interest

The authors report no conflicts of interest.

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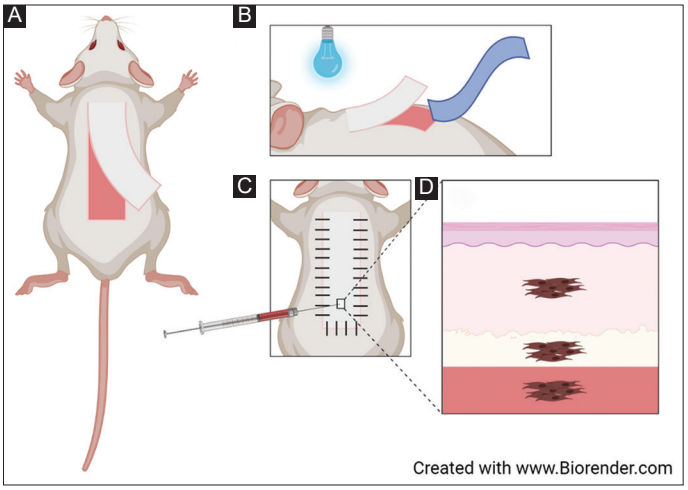
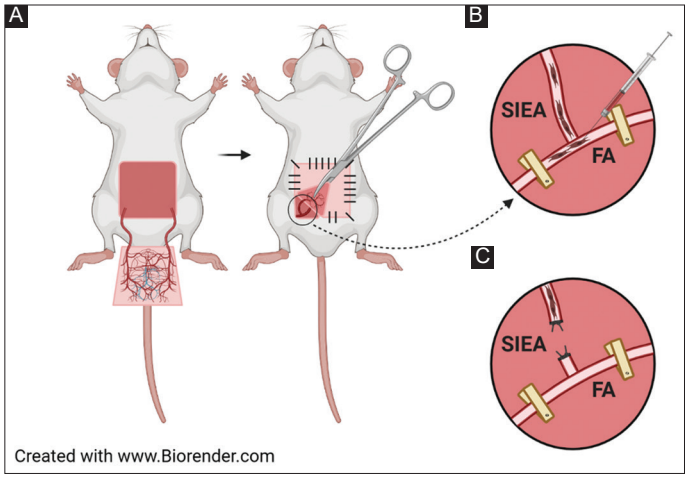
REVIEW ARTICLE

Human stem cells prevent flap necrosis in preclinical animal models: A systematic review

Supplementary Figures



Supplementary Figure 1. Modified McFarlane flap. (A) Elevation of a cranially, or caudally (not shown in the figure), based random pattern flap. The included studies used flaps of different sizes. (B) After flap elevation, some authors positioned a silicone sheet between the flap and wound bed to avoid neovascularization emanating from the latter. In addition, Park *et al.* [35,36] treated the mice's flaps with LED light from day 0 to day 14 at a distance of 8 cm. The light had a wavelength of 660 nm and a power density of 50 mW/cm^2, for a toral fluence of 30 J/cm^2. (C) After elevation and additional therapy, the flap is sutured back in place and hADSCs are injected. (D) Cells are injected either in the dermis, subcutaneous tissue, or in the muscle below. The figure was created using www.Biorender.com. LED: Light-emitting diode; hADSC: Human adipose-derived mesenchymal stem cell.



Supplementary Figure 2. Mouse superficial inferior epigastric artery flap model. This model consisted in elevating a 3×3 cm left SIEA. (A) Flap elevation including both the right and left SIEAs. After elevation, the flap was sutured back in place, leaving the right FA and SIEA exposed. (B) hADSCs were injected into the FA, which was previously clamped proximal and distal to the origin of the SIEA to secure flowing of the cells into the flap. (C) Posteriorly, the right SIEA was ligated and cut. The figure was created using www.Biorender.com. SIEA: Superficial inferior epigastric artery; FA: Femoral artery; hADSC: Human adipose-derived mesenchymal stem cell.

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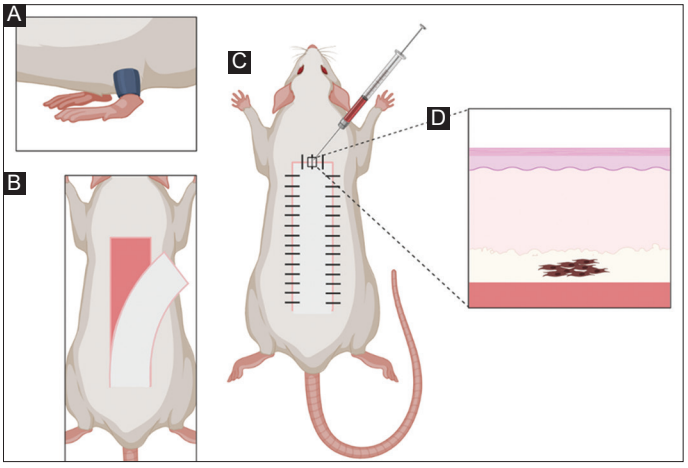
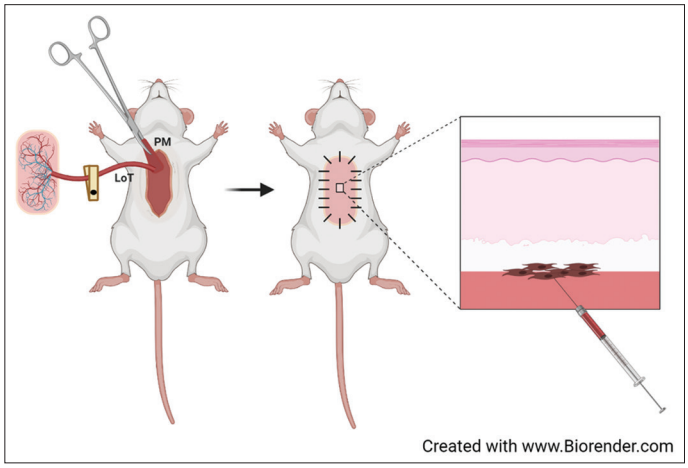


Supplementary Figure 3. Rat modified McFarlane flap. (A) rIPC was performed in the rats' hind limb. The model consisted of 3 cycles of 5 min of occlusion followed by 5 min of reperfusion using a torniquet. (B) A caudally based 3 × 9 cm dorsal flap was elevated. (C) After suturing the flap back in place, hADSCs were injected at the border of the flap. (D) A subcutaneous injection positioned the cells in the subcutaneous tissue. The figure was created using www.Biorender.com. hADSC: Human adipose-derived stem cell.



Supplementary Figure 4. Mouse long thoracic artery pedicled flap model. This model consisted in elevating a 4×1 cm right LoT pedicled flap. The flap's artery was then clamped for 3 h and then sutured back in place. Human adipose-derived stem cells were injected subcutaneously between the subcutaneous tissue and the underlying muscle in three sites (not shown in the figure). The figure was created using www.Biorender.com. LoT: Long thoracic artery.

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