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...
studies have shown protein expression differences α compared to those groups 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 capillary density in the groups treated with hADSCs either in vitro compared to control culture media or ex vivo 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 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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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
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 | 1×10^3 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 | hADSCs 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 15×10⁶, 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) | 1×10⁶ 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 cm²) | 5×10⁶ 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-Foxl⁻⁻/⁻ Cr/Narl 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 1×10⁶, medium-dose group received 1×10⁷, and high-dose group received 1×10⁸ (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 | 5×10³ 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...
Table 2. Percentage of surviving and necrotic areas of hADSC-treated flaps reported in the included studies.

| Author           | Necrotic/surviving flap area¹ | Perfusion¹ | 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 control media-treated (47 ± 4.5%) and control groups (43.7 ± 4.5%) | 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) | 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) | 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) |
| 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%) | 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 | 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 group² |
| Gong et al., 2014, China | The surviving area was significantly higher in hADSC-treated group (59.7 ± 0.03%) compared to control group (46.4 ± 0.038%) | N/A | VEGF levels were significantly higher in hADSC media supernatant (928.56 ± 105.24 pg/10⁶ cells) compared to those of DMEM without cells (21.05 ± 1.21 pg/10⁶ cells) | The number of capillaries was significantly higher in the hADSC-treated group (9 ± 1.5) compared to the control group (5 ± 1)² |
| Park et al., 2015, Korea | The necrotic area was lower in the hADSC-treated group compared to the control group³ | Perfusion units were higher in the hADSC-treated group compared to the control group³ | Protein levels and expression were higher in the hADSC-treated group compared to the control group (no specific values were provided) | The number of arterioles per mm² was significantly higher in the hADSC alone group compared to the control group (no specific value was provided) |
| Park et al., 2016, Korea | The necrotic area was lower in the hADSC-treated group compared to the control group³ | Perfusion units were higher in the hADSC-treated group compared to the control group³ | Protein levels and expression were lower in the hADSC spheroid-treated group (no specific values were provided) | The number of CD31+ vessel-like structures per mm² 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 group³ | Perfusion units were higher in the hADSC-treated group compared to the control group³ | Protein levels were lower in the hADSC monolayer-treated group compared to the hADSC spheroid-treated group (no specific values were provided) | The number of CD31+ vessel-like structures per mm² 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 treatment⁴ | 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)⁴ |
| 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)⁴ |
| 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%) | 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) | 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)⁴ |
| 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)⁴ | The number of CD31+ vessels was significantly higher in the hADSC-treated group compared to the control group (no specific values were provided) |

¹Every 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. ²Perfusion units are arbitrary units and are therefore not comparable among studies. ³Ratio of post-operative PU to pre-operative PU: *P < 0.01; †P < 0.05; ‡P < 0.001. *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 [29,30,32]. 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 skin flap 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...
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 and HIF-1α expression | Capillary density                                                                 |
|-------------------|-----------------------------|-----------------------------------------------------------|-----------------------------------|------------------------------------------------------------------------------------------------------|--------------------------------------|----------------------------------------------------------------------------------|
| Lu et al., 2008,  | Eight to 10-week-old ICR   | Cranially based 1×3 cm flap. A 0.13 mm thick silicone sheet was inserted to separate the flap from the wound bed | 1×10⁶ 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)⁹ | 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 base) compared to controls (7.5±0.9 capillaries)⁹ |
| China             | cranially based 1×3 cm flap. | A 0.13 mm thick silicone sheet was inserted to separate the flap from the wound bed | 1×10⁶ 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)⁹ | 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 base) compared to controls (7.5±0.9 capillaries)⁹ |
| Uysal et al., 2009 | 10-week-old, albino, ICR   | Cranially based random pattern skin flaps measuring 1×5 cm pedicle clamping to induce ischemia for 6 h | 1×10⁶ 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 mm²) compared to controls (15.2±3.4 mm and area of 12.9±4.1 mm²)⁴ | 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)⁹ |
| Japan             | cranially based random pattern skin flaps measuring 1×5 cm pedicle clamping to induce ischemia for 6 h | 1×10⁶ 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 mm²) compared to controls (15.2±3.4 mm and area of 12.9±4.1 mm²)⁴ | Surviving flap length and area in AdMSC-treated group were significantly higher (24.4±2.9 mm and area of 21.8±3.7 mm²) compared to controls (15.2±3.4 mm and area of 12.9±4.1 mm²)⁴ | 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)⁹ |
| Yang et al., 2010 | Six-week-old Wistar rats    | Cranially based random pattern skin flap measuring 9×3 cm | 4×10⁶ 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%)⁴ | N/A                                  | VEGF levels by ex vivo ELISA in AdMSC-treated group was not significantly higher (198.05±46 pg/ml) compared to controls (192.29±47.86) | |
| China             | cranially based random pattern skin flap measuring 9×3 cm | 4×10⁶ 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%)⁴ | Surviving flap area in AdMSC-treated group was significantly higher (46.33±13.46%) compared to controls (26.33±13.46%)⁴ | N/A                                  | VEGF levels by ex vivo ELISA in AdMSC-treated group was not significantly higher (198.05±46 pg/ml) compared to controls (192.29±47.86) | |
| Li et al., 2010,  | Adult male Wistar rats      | Caudally based abdominal rectangle peninsular flap based on the right femoral vessel pedicle | 2×10⁶ 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%)⁹ | N/A                                  | VEGF-A levels in hypoxic AdMSC media were significantly higher compared to normoxic AdMSC and chondrocyte media (no specific values provided); 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)⁹ at every time point | |
| China             | caudally based abdominal rectangle peninsular flap based on the right femoral vessel pedicle | 2×10⁶ 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%)⁹ | Surviving flap area in AdMSC-treated group significantly higher (30.71±6.99%) compared to controls (17.53±4.38%)⁹ | N/A                                  | VEGF-A levels in hypoxic AdMSC media were significantly higher compared to normoxic AdMSC and chondrocyte media (no specific values provided); 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)⁹ at every time point | |

(Contd...)
| 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 | 1×10⁶ 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 was significantly higher (81% in unlabeled AdMSCs and 85% in GFP-labeled AdMSCs) compared to controls (51-56) | 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 | 1×10⁶ 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) | 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) | Significantly higher expression of VEGF-A and HIF1α genes in AdMSC-treated group compared to controls by ex vivo semi-quantitative RT-PCR | 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 | 5×10⁶ 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%) | Perfusion index in AdMSC-treated group was significantly higher (78.4±6.8%) compared to untreated controls (34.2±7.7%) | Significantly higher expression of VEGF-A and HIF1α genes in AdMSC-treated group compared to controls by ex vivo semi-quantitative RT-PCR | 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 | 1×10⁶ 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%) | 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) | 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 | 2×10⁶ 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 (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 (no comparisons were made with untreated groups) | 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 (no comparisons were made with untreated groups) |

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the studies analyzed in this systematic review present information regarding the immunological reaction after xenogenic 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 3. (Continued)

| Author            | Animal model                      | Flap characteristics                                                                 | Cell number administration method | Necrotic or surviving flap measures                                                                 | Flap perfusion | VEGF levels and HIF-1γ 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%) | 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) | N/A            | N/A                                | N/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 | 5×10^6 cells/mL in 0.1 mL of DMEM were injected subcutaneously and 5×10^6 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%) | 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 mm²) compared to controls (39.7±12.37%; 459.59±175.28 mm²) | N/A            | N/A                                | N/A              |
| Ballestin 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%) | 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 cm²; 19±18%) compared to controls (6.9±4.2 cm²; 43±26%) | 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.

*P<0.05. **P<0.01. ***P<0.001. *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

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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.

### 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 |
|                           | Saartz 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 |
|                           | Ballestin 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.
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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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/cm², for a total fluence of 30 J/cm². (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.
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 tourniquet. (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.