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REVIEW ARTICLE

Aging increases the risk of flap necrosis in murine models: A systematic review

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Abstract

Background and Aim: Although a natural phenomenon, aging is a degenerative condition that promotes cellular malfunction and subsequent organ and body dysfunction. According to the World Health Organization, the elderly are the fastest growing age group worldwide. A 2012 population report stated that 43.1 million adults of 65 years or older lived in the United States, which is expected to jump to 83.7 million in 2050, placing an additional burden on an already stretched health-care network. Elderly patients broadly impact our health-care system, as reported in a 2014 wound report. 8.2 million patients were diagnosed with at least one type of wound, with patients 75 years or older making up most of the diagnoses. Aging affects all stages of the wound healing cascade. Although wound healing is downregulated in the elderly, scarce information exists regarding the effects of aging and flap survival in this group. Therefore, this study aims to report the impact of age on the survival of flaps in murine models. We hypothesize that increased aged animals will have decreased flap survival.
Methods: A systematic review was performed on February 1, 2022, following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis. We searched for full-text articles written in English, consisting of experimental murine models that compared flap survival between aged and young animals, in the following databases: PubMed, Scopus, CINAHL, and Web of Science. The terms "mice" OR "rats" AND "surgical flaps" AND "aging" guided our search. Models affected by chronic diseases were excluded from the study.
Results: Out of the 208 articles found by our search, seven were included according to our inclusion and exclusion criteria. Five studies used rats as experimental models, while the remaining two used mice. Local flaps were done in five studies, and two performed free flaps, transferring them from young and aged animals to young controls. Five articles reported lower flap survival in elder groups when exposed to ischemic insults. Three papers reported a deficiency in angiogenesis, vasculogenesis, and vascular reactivity as plausible causes for lack of survival, with one author correlating and verifying their results in human subjects. Although one article reported a lack of statistical power, they perceived a trend similar to the previous studies. Finally, one article reported inconclusive and variable results.
Conclusion: Evidence suggests that a lack of angiogenic and vasculogenic response in conjunction with decreased vascular reactivity are responsible for the diminished survival of flaps in the elder. Therapeutic means to boost the angiogenic, vasculogenic, and vascular reactivity response to improve patient outcomes require further research to understand the time course and mechanisms of flap survival in the elderly.
Relevance for Patients: All humans will feel the effects of aging one way or another. However, we can all agree that aging affects our basic biological processes, which negatively affects macroscopic appearance. One of the essential aspects downregulated in the elderly is their ability to respond to tissue injury and hypoxia, creating non-favorable circumstances for wound healing. Furthermore, to manage these non-healing wounds, flaps are raised to create a covering for these defects. However, age also impacts the ability of these flaps to survive, augmenting the problem and entering a vicious circle. To improve outcomes, we must focus our future research on understanding the basic principles of how aging affects the survival of flaps in elderly population.

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1. Introduction

Although a natural phenomenon, aging is a degenerative condition that promotes cellular malfunction and subsequent organ and body dysfunction [1].

According to the World Health Organization, elderly population is the world's fastest growing age group [2]. The number of people within this group is expected to increase to an estimated 2 billion and account for 22% of the world population [2]. Moreover, the United States (U.S.) report stated that 43.1 million adults aged 65 years or older lived in the U.S. as of 2012 and that number is expected to grow to 83.7 million by 2050 [3]. In addition, a 2014 study estimated that 8.2 million patients were diagnosed with at least one type of wound or wound-related infection, of which, 3% of the patients corresponded to non-healing surgical wounds [4]. The same report concluded that the costs to the U.S. health-care system to care for all wounds ranged from $28.1 to $69.8 billion [4]. Interestingly, Nussbaum *et al*. [4] reported that patients 75 years or older had the highest prevalence of nearly all types of wounds and, thus, accounted for the majority of the expenses.

As the world population ages, age-related diseases and impairments, including wound healing delays, exist at a higher incidence [4,5]. Although non-healing wounds are generally regarded as a consequence of chronic conditions, we have failed to consider age as a single and independent risk factor for wound healing impairment (Table 1) [6]. Aging affects all stages of the wound healing cascade, leading to impaired keratinocyte proliferation, dermal atrophy, including molecular and cellular mechanisms [7]. However, the wound healing process is complex and dynamic. Detrimental changes can additionally be seen in the hemostatic and inflammatory responses [6,7]. Most noticeably, angiogenesis, considered vital, seems to be downregulated in the elderly [6,8-10].

Although wound healing has been established as downregulated in the elderly, scarce information is found regarding the effects of aging and flap survival in this population, even more so regarding the molecular and cellular mechanisms involved in the impairment of flap survival in the elderly. Therefore, this study aims to report the consequences of old age on the survival of flaps in murine models as an independent risk factor.

Table 1. Factors with a negative effect on wound healing [11]

Local	Systemic
Infection and contamination	Age
Tissue hypoxia	Smoking
Trauma	Diabetes
Radiation	Obesity
Edema	Immune system compromise
	Stress
	Medication

2. Methods

2.1. Sources and search strategy

Following the PRISMA guidelines, a digital search of PubMed, Scopus, CINAHL, and Web of Science was performed on February 1, 2021, with no date limit. In addition, the following MeSH terms guided our search: "Mice" OR "Rats" AND "Surgical Faps" AND "Aging."

2.2. Eligibility criteria

A search was conducted in the existing literature with the following inclusion criteria; full-text availability, experimental murine models which compared flap (any type) survival between aged and young mice. Our exclusion criteria were the following: Non-English articles were excluded due to the risk of error in the translation; articles that utilized murine models affected by comorbidities or chronic conditions were also excluded from the study.

2.3. Study selection and data collection process

The first author performed the search and filtered duplicate articles using the computer program EndNote. Next, the remaining articles were selected based on their titles and abstracts compared to our established inclusion and exclusion criteria. Finally, the remaining studies were filtered by reading their full text (Figure 1). Finally, discordant papers were discussed between the authors for their inclusion or exclusion.

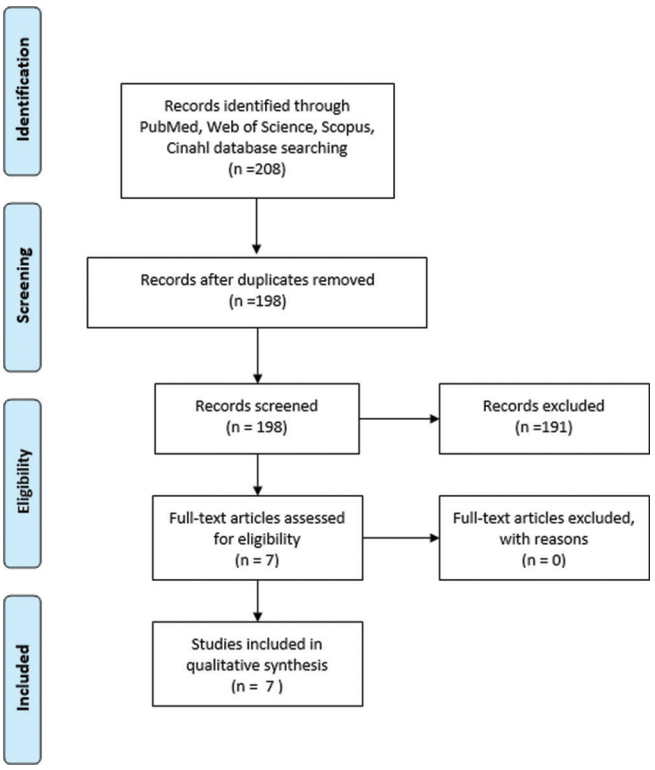

Figure 1. PRISMA flowchart.

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2.4. Risk of bias assessment

The risk of bias was assessed with the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) from the Cochrane Library, as shown in Figures 2 and 3.

3. Results

From the 208 articles yielded by our search, a total of seven studies were included in this review according to our inclusion and exclusion criteria. The papers reported their results on mice (2) [12,13] and rats (5) [14-18]. In addition, five studies utilized local flaps for their experimental models [12-16], while the remaining studies performed free flaps [17,18]. A summary of the included studies and their outcomes is found in Table 2.

3.1. Studies performed in rats

In 1992, Li *et al*. [17] raised 95 cutaneous maximus muscle free flaps, which were transferred to young recipients from young, middle-aged, and aged donors after being stored at room temperature for 1, 6, or 10 h. After 10 h of ischemia, four flaps failed in the young group. In addition, three flaps in the middle-aged group and five in the aged group were reported to fail after the 6 and 10 h ischemia intervals. Furthermore, tissue viability was assessed with nitroblue tetrazolium assay, which demonstrated that aged flaps had slower staining times after 1, 6, and 10 h of ischemia ($P < 0.005$, $P < 0.01$, and $P < 0.05$, respectively), indicating a higher degree of necrosis.

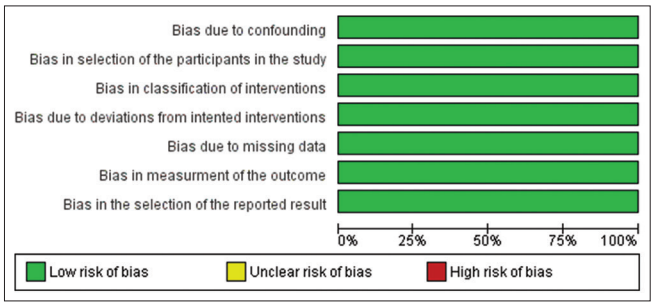

Figure 2. Risk of bias graph.

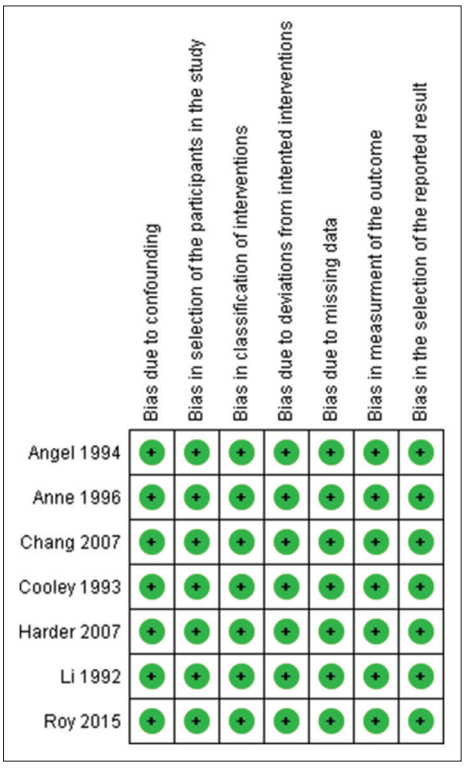

Figure 3. Risk of bias summary.

Following these results, Cooley and Gould [17] raised unilateral groin free flaps in similarly aged groups as Li *et al*. [18]. The team stored them in hypothermia (4°C) for 72 h immediately post-surgery. After transplantation to young recipients, 58% of the young flaps survived compared to 42% and 26% of the middle-aged and old-aged flaps, respectively. However, after analyzing the data, only the survival difference between old versus young flaps was significant ($P < 0.03$).

Moreover, Angel *et al*. [15] designed a study in 1994 to determine the effect of age on the survival of skin flaps subjected to ischemia by either primary or secondary venous obstruction. Unfortunately, even though a difference in survival rates was obtained, a lack of statistical power caused the study to report no statistical significance in survival among groups.

After the unsuccessful experiment by Angel *et al*. [15], Quirinia *et al*. [14] raised an H-shaped double flap on the rat's dorsum consisting of a caudally and a cranially based flap. Contradictory results were obtained. Cranially based flaps had a significantly shorter necrosis length in older rats ($P < 0.001$). However, the caudal flaps exhibited more considerable necrosis in the aged group compared to the young ($P < 0.01$).

Most recently, in 2015, Roy *et al*. [16] explored the effects of aging on fasciocutaneous flaps. They raised an axial flap that utilized the superficial inferior epigastric bundle to perform their experiment. On gross examination, older rats had impaired wound healing and flap integration with apparent necrosis of the distal and middle segments of the flap compared to the younger animals. After planimetric analysis, young flaps had, on average, 4.2% necrotic tissue compared to 49.17% necrosis in older rats ($P < 0.046$). In addition, the distal third of the older rats displayed full-thickness necrosis, involving the adnexal structures of the dermis and hypodermis, which were not observed in the younger distal segment.

Furthermore, the flap segments from the older group exhibited drastically reduced VEGF 164/165, Akt/protein kinase B, and Thr308, indicating lower proliferation secondary to a diminished angiogenic capability. To further understand these results, Roy *et al*. [16] measured VEGF levels on days 2–9. VEGF expression on all flap segments decreased 46% in the older flaps ($P < 0.01$), confirming a decreased angiogenic response.

3.2. Studies performed in mice

In 2007, Chang *et al*. [12] reported an experiment designed to investigate the function of young and old endothelial progenitor cells (EPC) and their response to an ischemic injury. For this

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study, a peninsular skin flap was elevated in the dorsum and sutured back with a silicone sheet between the wound bed and the skin flap. The group observed impairment of flap survival and tissue oxygenation after 7 days in aged mice compared to the young mice ($P < 0.005$). In addition, older animals showed a diminished mobilization of systemic Flk-1+/CD11b- (EPC) progenitor cells compared to younger animals ($P < 0.005$), indicating a vasculogenesis impairment. Similarly, aged animals displayed a decreased vascular density compared to younger animals ($P < 0.005$).

Bone marrow EPC neovascularization was also assessed. Older animals demonstrated a 5-fold decrease of EPC in vascular structures versus younger mice ($P < 0.05$). To confirm that bone marrow EPCs were not different due to age exhaustion or malfunction, an examination for a more primitive form of EPCs was conducted. These results showed no difference between aged and young animals ($P > 0.05$). In addition, a known EPC stimulant (stromal-derived factor-1a [SDF-1a]) was injected to understand the migration potential of aged EPCs. Surprisingly, both young and aged EPCs mobilized in similar quantities ($P > 0.05$), suggesting an impairment of EPC signaling and not numbers or function in the elderly.

Human EPC from young and aged adults was examined to confirm the previous findings. No difference was reported regarding baseline quantities of EPCs in circulation ($P > 0.05$). Similarly, no distinctions were detected in colony formation, migration, or proliferation by hypoxic insult ($P > 0.05$).

Furthermore, Chang *et al*. [12] performed age and gender mismatched reciprocal bone marrow transplants. Bone marrow transplants from aged to young recipients demonstrated a similar EPC response as the young control under ischemic insult ($P > 0.05$). However, when the opposite was performed (young donor/aged recipient), the bone marrow exhibited impairments in accordance with aged controls ($P > 0.05$). Moreover, elder animals displayed decreased SDF-1a and hypoxia-inducible factor-1a (HIF-1a) ($P < 0.005$).

Table 2. Summary of included studies

Author	Type of flap	Type of animal	Conclusion
Angel *et al.,* Australia, 1994	A 3 cm×6 cm abdominal skin flap from the vessels of the epigastric region	50 Sprague-Dawley rats Young: 2–3 months old Aged: 18–22 months old	Although variance in survival rates was perceived, due to insufficient statistical power, no significance was reported
Anne *et al.,* Denmark, 1996	H-shaped double flap, with a cranially and a caudally based flap 2 cm wide×4 cm long	Male Wistar rats Old: 102–104 weeks old Young: 10 weeks old	Older rats had significantly shorter length of necrosis in cranially based flaps compared to younger rats Caudally based flaps had a longer area of necrosis in the aged group
Chang *et al.,* USA, 2007	A dorsal peninsular skin flap (2.5 cm×1.25 cm). A silicone sheet was adhered in between the flap and the wound bed to produce an ischemic insult	C57/BL6 mice Young mice were 4–6 months old. Old mice were 18–24 months old.	Aged mice displayed a significant impairment in tissue survival and tissue oxygenation Aged mice have similar number and function of primitive EPCs compared to young mice Failure in EPC recruitment signaling occurs in old age mice Reduced HIF-1a may be the cause of decreased SDF-1 in aged tissue
Harder *et al.,* Great Britain, 2007	Proximally based axial pattern skin flap within the ear The base neurovascular bundle of the ear was transected to create an ischemic insult	30 immunocompetent homozygous hairless mice Adolescent age: 2±1 months old. Adult age: 10±2 months old Senescent age: 19±3 months old Additional mice were used to investigate secondary aims	Aging increases tissue susceptibility to ischemic necrosis through reduction of vascular reactivity and not by a decrease in ischemic tolerance
Roy *et al.,* USA, 2015	A fasciocutaneous pedicled axial flap utilizing the superficial inferior epigastric vessels was elevated The flap had a W-L ratio of 3:8	18 male Sprague Dawley rats Young: 2 months old Old: 6 months old	Tissue necrosis was increased in aged rats due to decreased levels of VEGF and decreased signaling of PI3K/Akt and STAT3
Cooley *et al.,* USA, 1993	Unilateral free flaps from the groin were raised, wrapped in saline soaked gauze, inside a plastic bag, and stored in hypothermic storage at 4°C	48 male Lewis rats Young: 2–3 months old Middle aged: 10–12 months old Old: 20–22 months old	Flaps from younger rats had a significant higher survival rate compared to aged rats
Li *et al.,* USA, 1992	Cutaneous maximus muscle free flap	84 male Lewis rats Young: 2–3 months old Middle aged: 10–12 months old Old: 20–22 months old	Muscle flaps from older rats had increased necrosis compared to flaps from younger rats

EPCs: Endothelial progenitor cells, HIF: Hypoxia-inducible factor, SDF: Stromal-derived factor, VEGF: Vascular endothelial growth factor

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Harder *et al*. [13] created a proximal-based axial pattern skin flap in the ear by transecting the distal and central neurovascular bundle in a separate mice study. At 5 days post-flap elevation, the adolescent group of mice presented 31% necrosis compared to 42% necrosis in the adult mice ($P < 0.05$) and 49% in the aged mice ($P < 0.001$). In addition, marked hyperemia in the proximal pedicle at day 5 was significantly decreased in adult and aged mice ($P < 0.05$). On elevation of the flaps, all groups had an immediate remaining blood flow to the central pedicle of 20–35%. However, adolescent mice recovered 5 days postoperatively, whereas the adult and aged mice experienced an almost complete blood flow cessation ($P < 0.05$).

Similarly, the adolescent mice maintained a 5% residual perfusion to the distal flap, whereas the remaining groups ceased flow altogether. Moreover, aged and adult animals had a significant decrease in functional capillary density in both the central ($P < 0.05$) and distal flap ($P < 0.001$) segments after 5 days. Finally, HO-I protein was expressed similarly among all three groups at 5 days post-flap elevation.

4. Discussion

The wound healing process is a complex and dynamic process that contains four interconnected and overlapping phases (Figure 4) [7]. Hemostasis, inflammation, proliferation, and tissue remodeling must all function appropriately to provide an optimal wound healing response [19]. Immediately following a break in the skin, vessels at the site of injury undergo contraction to aid hemostasis and clot formation [20]. In addition, platelets are activated as they meet the extracellular matrix proteins such as collagen and Von Willebrand factor [20]. As thrombin is released, it elicits a conformational change in platelets, releasing granules with pro-coagulation molecules. Platelets also contribute to the other phases of wound healing, as their granules contain chemokines and growth factors stimulating immune cells, fibroblasts, and keratinocytes [20]. As the immune phase begins, the innate response oversees the initial defense against pathogen invasion of the wound [20]. Resident immune cells are then activated by damage-associated molecular patterns and pathogen-associated molecular patterns [20]. Further down the road, pro-inflammatory molecules stimulate neutrophil

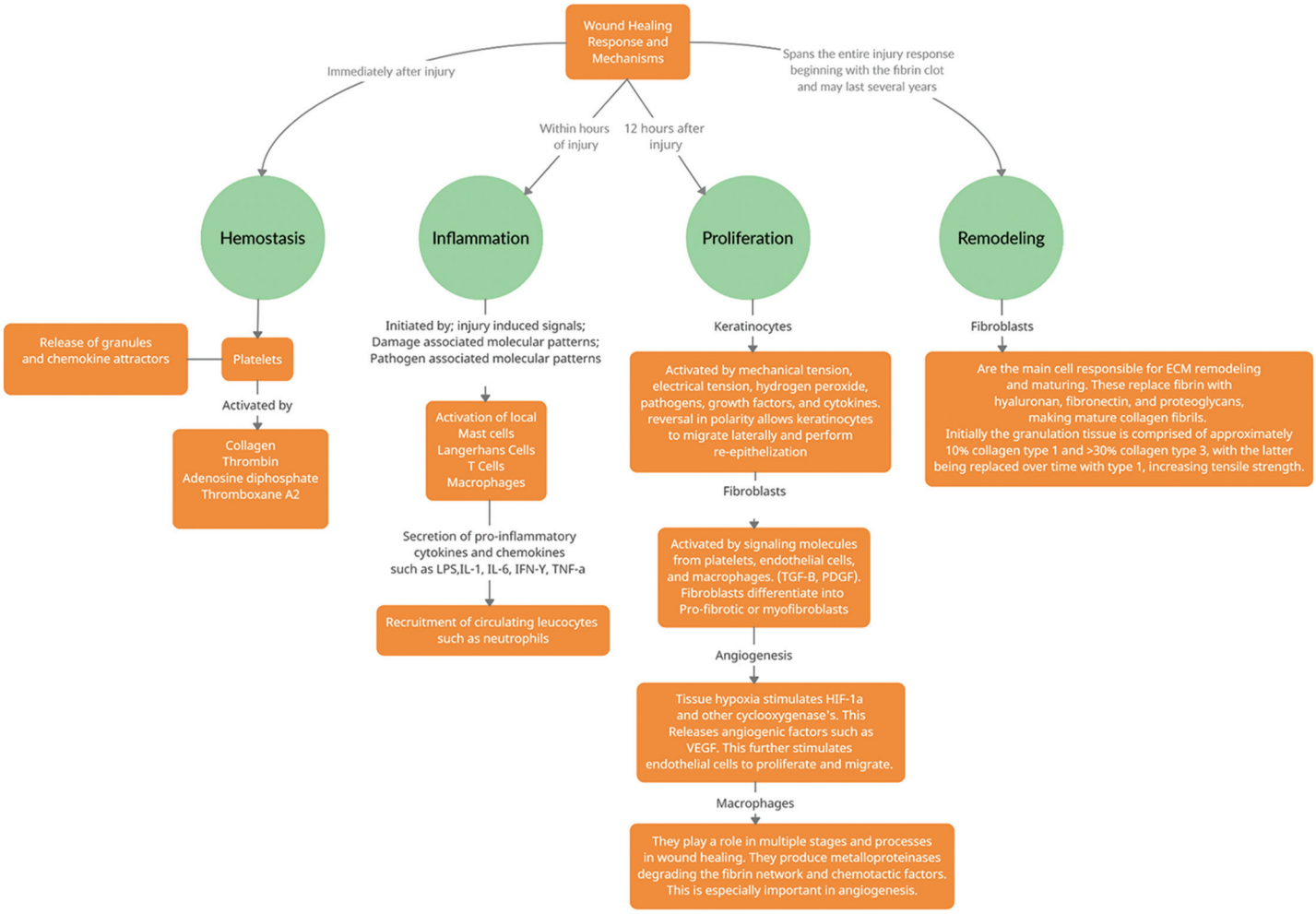

Figure 4. Depiction of the wound healing cascade and its mechanisms.

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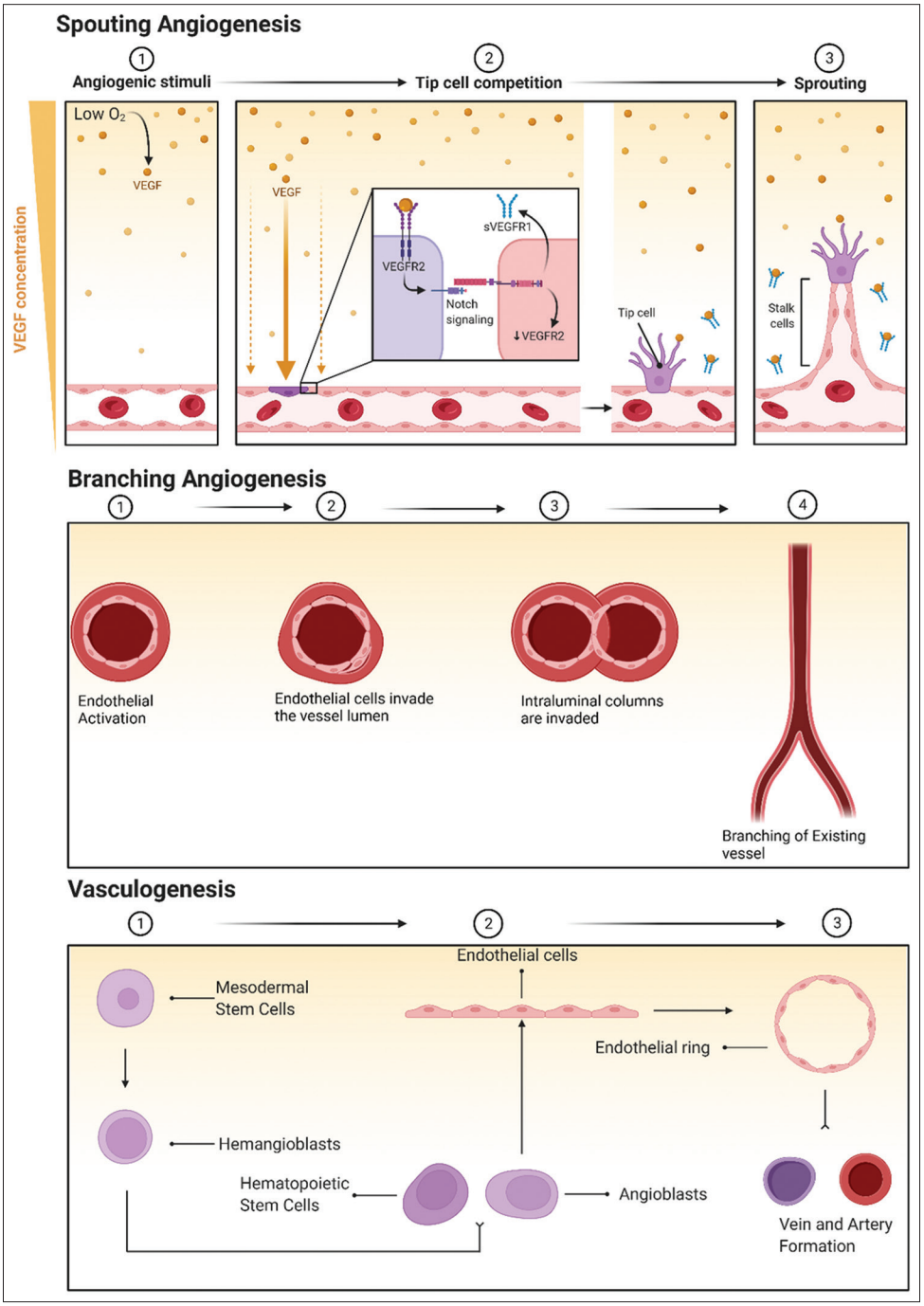

Figure 5. Types of angiogenesis.

and monocyte migration, which will remove necrotic tissue and pathogens (neutrophils and macrophages), along with aiding in tissue repair (macrophages) [20]. Following the immune phase, tissue repair mechanisms are activated. Keratinocytes, fibroblasts, macrophages, and endothelial cells work together to promote angiogenesis, re-epithelization, and scar formation [20]. Finally, during the remodeling phase, fibroblasts begin to replace the type 3 collagen deposited earlier for a much stronger and durable type 1 collagen, ensuring the recovery of around 80% of the pre-injury tensile strength over time (Figure 4) [20]. When any step of the cascade is flawed, wounds experience characteristic changes resulting in delayed acute wound healing or chronic non-healing wounds [21].

Although non-healing wounds are generally a consequence of chronic diseases, age has failed to be considered a single and independent risk factor for wound healing delay [6]. Age has negatively impacted all phases in the wound healing cascade [7]. During the initial hemostatic response, age seems to increase the adhesion of platelets to the endothelium and increase the release of growth factors from degranulation [22]. In addition, the response

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rate of the inflammatory cells seems to be altered, with an earlier neutrophil migration and a delayed monocyte response [22]. Macrophages are crucial elements in multiple phases of the wound healing response; therefore, a delayed reaction has been shown to hinder wound closure, granulation tissue formation, angiogenesis, collagen and growth factor production, and the number of myofibroblasts. During proliferation, keratinocytes, fibroblasts, and endothelial cells have displayed age-related changes, including reduced proliferation rate and migration, decreased response to growth factors, and diminished cytokine secretion, resulting in delayed wound closure and decreased angiogenesis [22]. The current literature suggests that these alterations may be attributed to a reduced response to hypoxia at the time of injury [22]. Evidence indicates that keratinocyte migration from older donors is significantly decreased in hypoxic conditions compared to younger donors.

Furthermore, fibroblasts from aged donors were reported to have a diminished response to hypoxia and a loss of migration activity [22]. In addition, hypoxia is a very potent angiogenic stimulator. However, an inadequate response to hypoxia suggests a decrease in HIF1a, SDF-1, and vascular endothelial growth factor, decreasing the angiogenic capabilities displayed in the elderly, similarly to the articles mentioned in this review [22]. Finally, an imbalance of matrix metalloproteinases (MMP) and inhibitors of MMPs suggests that aged skin is prone to tissue breakdown [22].

Even though aging negatively impacts wound healing, scarce information is found regarding the effects of age on flap survival. Five studies (Table 2) reported lower flap survival in elderly rats and mice in this review [12,13,16-18]. Three papers reported a deficiency in angiogenesis, vasculogenesis, and vascular reactivity as plausible causes for lack of survival [12,13,16]. The process of angiogenesis starts as a response to tissue injury and acts as a transport system for necessary materials, including oxygen and other growth factors required to repair the injured tissue (Figure 5) [23]. Hence, relevant angiogenic stimulating factors, such as HIF-1a, vascular endothelial growth factor, and angiopoietin, are essential to the process [23]. Chang *et al*.'s [12] data indicate that aged mice have a similar number of primitive EPCs that retain similar function and migration as the younger mice EPCs, which was confirmed using human subjects. This indicates that signaling failure for EPC recruitment occurs in the aged mice. Future studies should focus on the EPC recruitment signaling cascade to understand decreased signaling mechanisms better.

Similarly, Harder *et al*. [13] added evidence that aging is associated with augmented necrosis. However, this may partly be due to a loss of microvascular reactivity to endogenous ischemia protective proteins, such as HO-1. As mentioned previously, angiogenesis plays a crucial role in wound healing; hence, we expect it to be critical in flap survival. Roy *et al*. [16] demonstrates this as they were able to measure direct angiogenic pathways, including endogenous VEGF, activating phosphorylation of Akt on Ser473 and Thr308, among others. Their findings stipulate that age may directly reduce the angiogenic response and thus decrease the transport of necessary material to sustain wound repair.

Interestingly, Cooley and Gould [17] and Li *et al*. [18] reported similar findings even though their approach involved free flap transfers. Although performed with simple designs, they observed that aging directly produces a greater extent of necrosis in flap survival. In addition, Angel *et al*. [15] lacked statistical power, yet the group could still detect a trend toward less flap survival in the elder rats. Outbred rats, insufficient venous obstruction time, and normothermic ischemic insults are possible reasons for the lack of power. Finally, the contradicting results gathered by Quirinia *et al*. [14] may be due to their H-shaped double flap, as this was the only study with this design.

This review aimed to report the effects of old age on the survival of flaps in murine models. The included studies confirm that age harms flap survival. Although the articles included in this review suggest a lack of angiogenesis and vasculogenesis as the leading cause of lower flap survival in aged animals, other mechanisms may also be affected and contribute to the increased necrosis rate. Therefore, research must continue to explore how age affects flap survival. As age is a very complex mix of multiple cells and molecular processes, this creates a unique challenge for researchers to understand every aspect affected by age and test which of these has the highest impact and potential for clinical and translational research. Given the importance of flap-based surgical reconstruction on elderly patients, we must continue to research flap survival therapies and techniques to enhance quality of life of our patients.

5. Conclusion

Age affects the wound healing capability of elderly population and has a detrimental effect on flap survival. The articles included in this review suggest that a lack of angiogenic and vasculogenic response in conjunction with decreased vascular reactivity are responsible for the diminished survival of flaps in the elderly. Large studies are needed in the field to advance our understanding of flap survival's molecular and cellular function in the elderly. Gaining this knowledge will allow us to guide future research focused on therapeutic means to boost molecular and cellular mechanisms diminished in the elderly.

6. Limitations

Related studies may have been excluded due to our English and full-text requirement. Another possible limitation includes misinterpreting data, results, and the study selection process common for systematic reviews.

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Conflicts of Interest

None.

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Further Disclosure

Figures 1 and 5 were created using our purchased BioRender.com license. Figures 2 and 3 were created with the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) from the Cochrane Library. Figure 4 was created under the free license with Creately.com.

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