Review Paper

Comparison and Evaluation of Seven Animal Ischemic Skin Wound Models

Running Head:- Comparison of Seven Animal Wound Models

ABSTRACT

Studies focusing on pathophysiology, prevention, and treatment of ischemic wound remain a priority for medical and basic science investigators in order to develop new clinical approaches and reduce number of problems. However it is not always easy for researcher to choose the optimal animal model for their particular assessment. This review provides concise information on all currently available ischemic animal models, including rabbits' ear ischemic model, axial skin flap (axial pattern flaps), burn, ischemic limb, localized ischemic wounds, pressure ulcer, and skin flap, along with their citations as a measure of their acceptance among other researchers.

Method: We searched the PubMed database. Key words used included ischemic wound, skin, and animals in combination in the advanced search option of the website.

Results: A detailed and concise description of the seven types of ischemia as well as their results are presented in Tables 1 -7. Table eight, presents the seven groups

of animal ischemic models, the number of studies, number of wounds, and total and average Google Scholar citations, and web of sciences citations. We found that rabbits' ear ischemic model, localized ischemic wound, and pressure ulcer have the highest total and average citations amongst the studied groups.

Conclusion: The authors believe that the rabbits' ear ischemic model and rat pressure ulcer models, and localized ischemic wound, have made the greatest contribution to our enhanced understanding of the pathophysiology of the ischemic wounds and increased production of new therapeutic protocols.

Key words

Chronic wounds, ischemic wounds, rabbits' ear ischemic model, localized ischemic wounds, pressure ulcer.

INTRODUCTION

Why tissue ischemia and skin repairs are important?

When the normal repair is disrupted, chronic wounds develop. Ischemia is one of the most common causes of chronic wounds (1) which fail to heal in an ''normal'' period of time. Clinical observations suggest that persistent tissue ischemia in the vicinity of the wound is an important underlying feature of chronic wounds and

severely impairs the healing process causing wound repair dysregulation ultimately threatening limb and life (1). Long term ischemia leaves wounds vulnerable to infection, inflammation, and necrosis and is an important factor in repair hindrance in many diseases (3). Chronic wounds are heterogeneous, and are clinically challenging because they strictly damage tissue repair (4-7). In the USA, 6.5 million people suffer from chronic wounds including ischemic wounds costing in excess of $25 billion in the management of chronic wounds (8).

Normal skin repair (wound healing process)

Understanding normal skin repair is necessary for effective prevention and treatment. Skin repair happens on a time continuum with steps including hemostasis, inflammation, proliferation, and remodeling (9). Each step is vital to achieve complete wound healing, and any alteration from the normal state can be associated with postponed or abnormal skin repair (9).

Ischemic skin repair

At first we should describe some important terms. **Hypoxia** refers to low organ oxygen tension, **ischemia** refers to when blood flow to a tissue or organ is limited, leading to low oxygen and nutrition levels (10), and an **ischemic ulcer (wound)** is an ulcer caused by diminished blood flow through an artery (11).

Low oxygen levels reduce neutrophils and fibroblasts functions, decrease collagen synthesis, and increase wound infection (12-14).

The need for animal models

Animal models are crucial to increase our knowledge (15), and serve as surrogates of the human condition in order to translate experimental findings into clinical use. The most critical factor is the requirement to mimic the clinical environment of the ischemic condition (16). Previous studies have shown that although more than 100 factors could be involved in non-healing wounds, one critical pathophysiology is associated with a deficient blood supply. Ischemia may not be the initiating factor for many chronic wounds, as most ulcers start from a combination of neuropathy, pressure loading, infection and/or trauma, but tissue ischemia is the main cause that hinders healing—wounds do not heal in tissue that does not bleed, whereas they always heal in tissue that bleeds extensively. Currently, the most common animal models of ischemia include: Rabbit ear ischemic model (REIM), axial skin flap (or axial pattern flaps) (ASF), burn, ischemic limb (IL), localized ischemic dermal repair (LIDR), pressure ulcer (PU), and different models of random patterns of blood vessels in skin flaps (SF).

Available animal models of ischemic wounds

Rabbits' ear ischemic model (REIM)

The REIM model was initially created using a microsurgical technique (17). Recently an improved version of this ischemic wound model that does not require microsurgery instruments has been reported (18).

Technique:

The technique creates incisions at the ear base, and the central and cranial arteries along with their accompanying nerves are severed and ligated, leaving the central vein and the caudal bundle intact. The subcutaneous tissues and muscles are also cut to reduce collateral formation. For wound study, two to four circular full-thickness wounds are created on the ventral side of each ear (18).

Axial skin flap (axial pattern flaps) (ASF)

This model is based on a direct cutaneous artery and vein providing a piece of skin. They provide a versatile option for big injury closure (19, 20). This model requires good surgical technique and careful attention to detail when inducing the flap (19, 20).

Technique:

The technique creates anterior abdominal skin flaps, based solely on the epigastric artery and vein, in the rat model. A unilateral axial pattern skin flap is elevated under direct microscopic vision. The flap is resutured into place and observed for a period of 3 to 4 days (20).

Burn

Cutaneous burns are dynamic injuries with a central zone of necrosis surrounded by a zone of ischemia (21). Acute tissue destruction occurs at the site of burn injury by direct thermal energy. In addition, a delayed loss of tissue occurs in the surrounding, uninjured skin as a consequence of progressive ischemia (22).

Technique

One common technique is the induction of a full-thickness burn by hot metal. Two burns are created on each animal's dorsum using a brass comb with four bars preheated in boiling water and used for 30 seconds, resulting in 4 full-thickness burns separated by 3 unburned interspaces (zone of ischemia) (21).

Ischemic Limb (IL)

Critical IL refers to the clinical state of advanced arterial occlusive disease, placing an extremity at risk of gangrene and limb loss (23). This is associated with significant morbidity including chronic wounds, infections, mortality, and health care resource utilization (24, 25, and 1).

Technique:

The technique involves a transient ligation of the femoral arteries, veins, and collateral vessels in rabbits using a microvascular clip. After a 2-hour period of ischemia, the clips are removed to allow reperfusion for 4 hours (26).

Localized ischemic dermal repair (LIDR)

Localized tissue ischemia is a key factor in the development and poor prognosis of chronic wounds (27). This ischemic wound model is reliable, relatively inexpensive, easy to perform, and reproducible (27).

Technique:

A dorsal, bipedicle skin flap was raised in the craniocaudal direction deep into the skin muscle (panniculus carnosus). Two adjacent excisional ischemic wounds were created in the center of the flap. Precut and sterilized nonreinforced medical grade sheeting is then placed underneath the flap. The skin flaps and silicone sheet are sutured to the adjacent skin edges. The silicone sheet inhibits wound contraction and internally controlled, non ischemic full-thickness wounds are created (Figure 1) (27). The excisional wounds provide sufficient tissue for laboratory tests, and are amenable to the evaluation of topical and systemic therapies that may induce angiogenesis or improve ischemic wound healing (27).

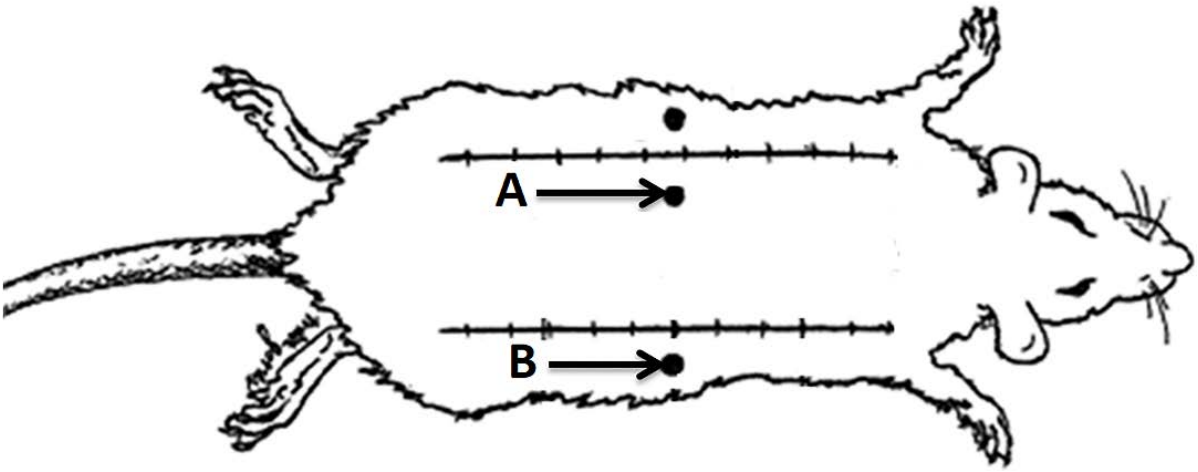

Figure 1.Localized ischemic wound model, A: ischemic wound; B:non ischemic control wound.

Pressure ulcer (PU)

Pressure ulcers develop as a result of a localized injury caused by to the skin and/or underlying tissue, or both, resulting from prolonged pressure on the skin. The

ulcers usually arise over a bony prominence, and are recognized as a common medical problem affecting people confined to a bed or wheelchair for long periods of time (28).

Technique

One approach is to gently pull up the dorsal skin of mice and trap it between two round ferrite magnetic plates for 12 hours. Once the plates are removed the mice develop two round ulcers separated by a bridge of normal skin (29).

Skin Flaps (SFs)

This technique has been considered an important procedure in plastic and reconstructive surgery in order to cover defects. Flap necrosis due to failure of blood circulation results in severe complications (30). SFs provide cutaneous coverage, and may be local, pedicled, or free (31). Nakajima and colleagues classified SFs into cutaneous, fasciocutaneous, and adipofascial, septocutaneous, and musculocutaneous (32). Random blood vessels pattern skin flaps (RSF) provide the greatest adaptability in reconstructive surgery (32).

Technique

In this technique, a random skin flap, including the entire thickness of the skin and panniculus carnosus is made. The base of the random skin flap is located on a horizontal line between the crest of the iliac bones. The dimensions of the flaps are

20×70 mm. After elevation, the flaps are immediately replaced. The surface area of the flap is measured immediately and seven days after surgery (33).

Necessity for doing current review study

A total of 6.5 million American patients suffer from chronic and ischemic wounds and would benefit from improvements in wound treatment. To achieve this goal, research scientists and physicians would benefit from appropriate and accurate animal model to research ischemic wounds (1). There are currently a limited number of review articles about animal models of chronic and ischemic wounds. Schäffer et al presented a limited review on SF, PU, and LIDR ischemic models in 2002, and concluded that animal model of ischemia are useful in developing information, although extending the application of these models into the human condition is an excessively lengthy and complex process (1). Salcido et al provided an outline of techniques used to induce PU in animal models in 2007 (15). They concluded that the mechanism of healthy tissue or organs progressing to PU remains unknown (15). Nunan et al (2014) classified all chronic wounds into one of three major categories: leg ulcers, diabetic foot ulcers,and PU. Nunan et al concluded that it should be possible to optimize animal models so that they better recapitulate the medical hallmarks of this situation and permit researchers to better understand its pathological mechanisms (10). McCafferty et al. described the development of ischemic conditioning strategies from lab to patient, and

highlighted where transition into patient investigations has been less successful compared to animal models (16). The present systemic review article provides concise information about all available studies on ischemic animal models using REIM, ASF, burn, IL, LIDR, PU, and SF, along with presenting their citations in order to determine their acceptance among other researchers. In this article, we aim to systematically review the scientific literature published in www.pubmed.gov using the keywords ischemic wounds, skin, and animals in combination in the advanced search option of the website. Besides presenting technical notes of the studies, our results also indicate the reliability of these techniques among peer review panels, and editors of journals based on the number of published papers in each item, and their citations in Google scholar and web of sciences.

METHODS

Search strategy

We first searched PubMed database using ischemic wound, skin and animals key words in the mesh term, and title and abstract icons of advanced search option of the website. Then, the titles and abstracts of all the selected studies published in English were evaluated. After that we categorized entire animal models of ischemic wounds into REIM, ASF, burn, IL, LIDR, PU, and SF categories. Finally, the full papers were found for full evaluation.

Study selection

All the papers using the key words ischemic wound and skin and animals in their titles and abstracts were incorporated. We found 440 articles in www.pubmed.gov between 1977 and 2017. Next we considered some criteria for inclusion of the selected papers in the review.

Inclusion criteria

1. The full text of paper should be available.

2. The language of the paper should be English.

3 Ischemia should be noted in the abstract.

4. Ischemia should be evaluated in skin.

5. The research should be performed in an in vivo model.

We confirmed the number of citations for each paper by reviewing the selected papers in the Google Scholar and Web of Sciences.

RESULTS

A detailed and concise description of the seven types of animal ischemic models(REIM , ASF, Burns, ischemic limb, localized ischemic wound healing, pressure ulcer, skin flaps as well as their results is presented in Tables 1 -7. In table

eight for each of seven groups of animal ischemic models, the number of studies, number of wounds, and total and average Google Scholar citations, and Web of Science citations are included. It is noted that all skin wounds in this review article were full thickness.

DISCUSSION

Finding an appropriate animal model for ischemic wound study has been a major challenge to scientists as well as clinicians (15,147). The choice of animal models to mimic the human condition is based on a compromise of cost, ease of use, reproducibility, and reliability of the data (25).

The ischemic wound in the rabbits' ear ischemic model has many characteristics of ideal ulcer model: ischemic enough to affect wound healing significantly, reproducible, quantifiable both in term of epithelialization and granulation tissue formation, associated with minimal contraction, viable without necrosis, comparable to reliable control, and analogous to clinical situation (147). This model is potentially useful to evaluate new therapeutic agents to promote healing such as growth factors (37, 39, 40, 41, 42, 44), and stem cell therapy (31,32,34).

A McFarlane - or bipedicled - skin flap on the dorsum of mouse or rats is frequently used as an ischemic cutaneous wound model (126,131). However, the amount of ischemia to each model differs with the extent and length of the flap,

new blood vessel progress rapidly within a short time, and blood perfusion proceeds to normal within near 14 days (3,148). The ischemic rabbit ear wound model is a better but not a perfect model because in three weeks even the healthy control wounds are healed (147). However dermal repair times in old and diabetic animals were extended, particularly when diabetic time was more than one year (147).

The modified minimally invasive procedure of rabbits' ear ischemic model (17,33,36,147) which was recently reported by Chien et al has several added advantages, such as less skin damage, simpler procedure, a higher success rate, and more flexible. Salcido et al at 2007 found that murine models were relevant models for understanding the causal factors as well as the wound healing elements of pressure ulcer. However Salcido et al concluded that no single method of induction and exploring pressure ulcer in animals can address all the aspects of the pathology of pressure ulcer. Each model has its particular strengths and weaknesses (15). In the current review, animal models of pressure ulcer have gained a second score in Google Scholar and Web of Science citations among seven animal models of ischemic wound tissue. It shows the importance of pressure ulcer morbidity and mortality among basic science researchers. Animal models that allow wounded tissue to be reperfused with blood following hypoxia might better recapitulate

human pressure ulcer in which perfusion has been restored (15). The reperfusion of ischemic tissue is crucial for survival, but is known to cause secondary tissue damage through inflammatory mediators and the release of free oxygen radicals (15). Hypoxic-ischemic injury with I/R is an important mechanism in PU development and that epidermal, dermal, and muscle damage occurs within several hours. However, the mechanisms of I/R injury are probably multifactorial and the actions of free radicals may be more complicated in the early stages of PU development in humans as compared to the rat model (15,113).

In the current review, localized ischemic wound healing has gained a third score in Google Scholar and Web of Science citations among seven animal models of ischemic wound tissue. However there are few differences with pressure ulcer ischemic wound model, and both the pressure ulcer ischemic wound and localized ischemic wound model achieved equal scoring in Web of Science citations.

The localized ischemic wound model is easy to perform, reliable to reproduce tissue ischemia, and is amenable to studying therapeutic modalities. The ischemic rabbit ear dermal ulcer model, while elegant in design, requires use of an operating microscope in some models (32, 43, and 44). This model depends on the large rabbit ear and has not been successfully adapted to either rats or mice (43, 44). Furthermore, rabbits impose more housing and handling difficulties than small animals such as rats and mice, and are consequently more expensive. The

localized ischemic wound model is a longitudinally oriented, dorsal, bipedicle flap model that addresses these criteria and will prove to be a valuable model for studying tissue ischemia (35,149). The rat model has the advantages of ease of use, low cost, small, and easily attainable (149). However, wound healing in rats has been subject to scrutiny because of their ability to heal infected wounds and the high rate of inter animal variability (25). This rat skin wound model has a molecular profile similar to that of chronic human wounds (109). It has been reported that the 2.5 cm flap without silicone is not ischemic compared with controls, but does have a slower rate of healing. The addition of an intervening silicone sheet decreases tissue oxygen slightly, but does not impact upon other parameters of wound healing. By further narrowing the flap to 2.0 cm, Gould et al have provided some biochemical and mechanical evidence that correlates with tissue ischemia (25). However recently Gould et al have made some changes in their procedure to make 10.5×3-3.5 cm ischemic wounds in F344 rats (97, 96).

The laboratory ASF model was reported in 1965 by McFarlane et al (150), but the most popular is a H-shaped cutaneous flap model developed by Quirinia et al (151). The technique has been modified numerous times since then and is still commonly used for ischemic wound studies not only in rats but also in other animals (152,153, 154). Several problems have been reported for this model. McFarlane et al pointed out in their original study that the occurrence of skin-flap

necrosis was unpredictable and might occur in more than 90% of rats (155). Schaffer et al (1) and Martson et al (156) pointed out the existence of natural cranio-caudal differences in granulation tissue formation in small animals like mice or rats, which added to the complexity in making comparisons. Dunn and Mancoll pointed out that there are major differences in skin blood flow patterns between "loose-skin" and "tight-skin" species such as the rat and human, respectively (155), and this difference also contributes to higher skin contractions in small animals. Gould et al also pointed out that rats have a higher ability to heal infected wounds and a higher rate of inter animal variability (27). The major problem is the short period that the flap can maintain ischemia. Studies by Nakajima indicated that although perfusion to the flap was immediately reduced, new vascular channels were present around the entire wound margin and also developed from the recipient bed within 2-3 days (157). Blood perfusion increases in a linear fashion to normal at postoperative days 14-16(2). The rapidity of perfusion recovery precludes extended testing of potential vulnerary agents (158).

Finally we should note that pressure sore models as well as burn models are not quite the same thing as excisional wounds in ischemic tissues as the former are surrounded by well visualized healthy tissue.

It was concluded that the ischemic wound in the rabbits' ear ischemic, pressure ulcer, and localized ischemic wound models have obtained the highest Google

Scholar and Web of Science citations among the seven animal models of ischemia. The information presented here would help researchers to select the right animal model in order to study ischemic wound healing. The authors of this review paper believes that the rabbits' ear ischemic model and the rat models of pressure ulcer, and localized ischemic wound, have been proven beneficial to our increased understanding of the pathophysiologies of the ischemic wounds and designing new therapeutic protocols.

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Tables

Ref. no& 1st Author's	Target organ or	Incision /Excision (wound)	Intervention	No of evaluating methods	Main results/ Conclusions	Number of Google

Name & Published year, Animal	Tissue, Technique	/Nothing				scholar& Web of sciences citations
34. Reyes-ortega, 2015, Rabbit	Ear, One artery and vein were ligated	1circular excisional wound	A 2 layer dressing for repair of non healing wounds	Macroscopic &Microscopic tests	The dressing enhanced wound repair in both ischemic and non-ischemic injuries	20,12
35. García-Honduvilla, 2013, Rabbit	Ear, one artery and vein were ligated	A circular wound, 2 cm in diameter	Topical treatment with proadrenomedullin N-terminal 20 peptide (PAMP), Alone, or with stem cells	Macroscopic & Microscopic tests	The treatments improve healing both in normoxic and ischemic conditions.	8,5
36. Said, 2009, Rabbit	Ear, Division of the different arteries	7 wounds In each ear	They postulated that ischemic situation could activate hypoxic signalling paths	Luciferase assay	The biologic systems for hypoxic signalling could be applied to show local ischemia	2,0
37. Wang, 2009, Rabbit	Ear, The two arteries were ligated	Four round full-thickness wounds	ATP-vesicles was used	Histologic studies, Wound Tissue Angiogenesis	The treated-wounds exhibited extremely fast granular tissue growth.	16,9
38.Volk, 2007, Rabbit	Ear, One or more of the arteries or veins were ligated & circumferential	Four 6mm diameter wounds	Stromal progenitor cell (SPC) therapy	4	Treated -wounds showed significantly accelerated wound	15,13

	incisions made.				healing	
39. Kloeters 2007, Rabbit	Ear, Two arteries were dissected	Four 7 mm full-thickness punch wounds	Ad-Smad3 or Ad-LacZ was administrated.	-Histological analysis	Reepithelialisation Was enhanced in an ischemic wound mode	14,7
40. Chien, 2007, Aged Rabbit	Ear, Two arteries were ligated, & a circumferential tunnel was made.	2 to 4 circular 6-mm -full-thickness wounds	An occlusive Dressing with ATP	-Measurement of ATP by high-performance liquid chromatography (HPLC)	ATPs were higher in the normal ear than in the ischemic ear.	18,12
41.Sun, 2007, Rabbit	Ear, One or more of vessels were ligated& circumferential incisions	Two 8-mm excisional dermal ulcers	Collagen-based Platelet-Derived Growth Factor (PDGF) targeting delivery system	-Histological test for new collagen deposition,& capillary lumens	PDGF-BB could effectively promote ulcer healing	47,29
42. Mogford, 2006, elderly Rabbit	Ear, Division of 2 arteries, with preservation of the 3 veins	Three to five 6-mm full-thickness dermal punches	Treating wounds by gene delivery of human telomerase reverse transcriptase (hTERT)	4	hTERT significantly improved ischemic wound healing in old rabbits	23,18
43. Breitbart, 2001, Rabbit	Ear, Division of the two of 3arteries	3 Eight-millimeter-diameter excisional wounds	Treating by cultured fibroblasts enriched with growth factors	-Immuno histochemistry	Treatment modulates ischemic wound healing	46,27
44. Xia, 1999, Rabbit	Ear, Division of two arteries & circumferential incisions	Three 6-mm full thickness dermal ulcers	Topically applied Keratinocyte growth factor-2 (KGF-	-Histological analysis	KGF-2 is effective.	133,63

			2)on wound			
45. Liechty, 1999, Rabbit	Ear, One or more of three arteries or veins were divided and circumferential incisions.	6 mm wounds	Topical treatment by an adenovirus containing the PDGF-b	4	Platelet-derived growth factor-B overcame the ischemic defect in wound healing .	143,98
46. Wu, 1997, Young rabbits	1,2, or 3 arteries or veins were divided and circumferential incisions.	Four 6 mm diameter full-thickness circular wounds	Treating by recombinant human Macrophage colony-stimulating factor (rh-M-CSF)	1.Histology, 2.Reverse transcription - polymerase chain reaction (RT-PCR)	M-CSF increases dermal ulcer ischemic wound healing	51,42
47.Uhl, 1993, Mice	Two of the three principal neurovascular bundles were ligated	A (6.6 mm2) full-thickness dermal layer was excised	Treatment with hyperbaric oxygen	1.Measurement of wound surface area, 2.Laser Doppler imaging	Hyperbaric oxygen therapy improves reepithelialization in normal and ischemic skin tissue	114, 76
48.Uhl, 1993, Mice	two of the three principal neurovascular bundles were ligated	a (5 mm2) full-thickness dermal layer was excised	Injection of basic fibroblast growth factor (bFGF)	1.Measurement of wound surface area, 2.Morphological studies	bFGF decreas wound surface area of ischaemic tissue.	58,44
16. Ahn, 1990, Rabbit	1,2, or 3 arteries or veins were divided and circumferential incisions.	Four 6-mm Surgical punch biopsies	To test effects of blood flow changes on dermal repair	4	This ischemic ulcer model is reliable & quantifiable.	140, 102

Table one. Specifications of rabbits' ear ischemic model in the reviewed papers; abbreviations: proadrenomedullin N-terminal 20 peptide (PAMP), stromal progenitor cell (SPC) , Platelet-derived growth factor(PDGF), high-performance liquid chromatography (HPLC), human telomerase reverse transcriptase (hTERT), Keratinocyte growth factor-2

(KGF-2), recombinant human Macrophage colony-stimulating factor (rh M-CSF), Reverse transcription - polymerase chain reaction (RT-PCR), rh basic fibroblast growth factor (bFGF).

Ref. no& 1st Author's Name & Published year, Animal	**Target organ or Tissue, Technique**	**Incision /Excision (wound) /Nothing**	**Intervention**	**No of evaluating methods**	**Main results/ Conclusions**	**Number of Google scholar & Web of sciences citations**
49. Leng, 2017, Rat	Skin , Abdominal perforator skin flaps	No wound	Treating by a new method of the "stem cells-gene" combination therapy.	1.Evaluation of flap surface, 2. Evaluation by HE staining 3. Evaluation of platelet endothelial cell adhesion molecule	This stem cells therapy can effectively improve the repair of ischemia-reperfusion(I/R) injury	0,0
50. Sönmez, 2013, Mice	Skin, A lateral thoracic artery pedicled island skin flap was made & arteries were occluded .	No wound	Treating by platelet rich plasma (PRP)	-In vivo bioluminescence imaging, - Histology and immunohistochemistry	This study shows the angiogenic effects of PRP	18,10
51.Leng, 2012, Mice	Skin, Axial skin flap(ASF), using clamp for epigastric artery for inducing ischemia	No wound	Treating by human umbilical cord matrix stem (HUCMS) cells	4	HUCMS cells could progress the viability of ASF by promoting vascularization	12,4
52. Mirabella, 2012,	Skin, A ASF elevated in the Abdominal	No wound	Amniotic fluid stem cells (AFSC) derived conditioned	-Histological analysis, - Recruitment studies and	ACM is good for patients	31,19

Rat	region & inferior epigastric vessels was ligated.		media (ACM) delivered topically into a ASF	progenitors isolation		
53. Plock, 2008, Mice	Skin, Two flaps were made on both sides of mices, then the related arteries were ligated	Incision	To target healing and survival of flap by application of liposomal hemoglobin vesicles (HbVs).	-Histological examination, - Laboratory analysis	HbVs may improve the viability and wound healing in ASF	24,16
54. Schlaudraff, 2008, Rat	Skin, Random dorsal skin flaps were made, and related arteries were divided	No wound	To test the effects of leeches in mixed arterio-venous insufficiency	-Macroscopic and Planimetric Analysis, - Laser-Doppler Flowmetry	Application of leeches can be hazardous to flap viability	15,8
55. Fujihara, 2008, Rat	Skin, Dorsal island skin flap based on the related artery were made	No wound	Delivereing basic fibroblast growth factor (bFGF) to flap	4	Delivery of bFGF to the flap area enhances the viability of an ASF.	33,16
56. Michlits, 2007, Rat	Skin, A flap was made, and the related vessels were ligated.	No wound	To evaluate the effect of topical administration of a vascular endothelial growth factor (VEGF)-A plasmid to the flap bed	4	This protocol may also enhance wound healing in post trauma skin lacerations or in skin grafts	49,34
57. Giunta, 2005, Rat	Skin, A flap was made and inferior and superior epigastric arteries were dissected	No wound	To test the effect of preoperative injection of adenoviral vectors encoding (Ad)VEGF(1	4	Results confirm the important role of VEGF(165) on angiogenesis in ASF	54,28

			65).			
58. Huemer, 2005, Rat	Skin, An epigastric skin flap model were made, next the related vessels were ligated	No wound	To compare the effect of gene therapy with transforming growth factor-beta (TGF-beta) and extracorporal shock waves (ESW) to treat ASF	- Evaluation of flap survival, - Microscopic flap analysis	Treatment with ESW enhances ASF viability significantly more than TGF-beta	60,27
59. Harder, 2004, Pig	Skin, skin flap was made on each side of the gluteals, next the related vessels were ligated	No wound	to test, if ASF survival may be improved by local heat preconditioni ng	- Histological examination, - Apoptosis	Necrosis and apoptosis rate of ASF could be reduced significantly in treatment group	44,27
60. Furuta, 2004, Mouse	Skin, The related artery was ligated, later the zoned skin was incised, and elevated	No wound	to test ASF viability, &angiogenes is whilst under pharmacologi cal or genetic inhibition of nitric oxide synthase (NOS)	- Flap survival, -Histology, - immunoreactivity	NOS has a significant role in promoting wound healing/angiogenesis in its early stages	17,0
61. Mitterm ayr, 2003, Rat	Skin, Denervated epigastric island skin flaps were elevated, tolerated ischemic for 8 hours, then reperfused	No wound	to test whether S-nitroso human serum albumin(oxide-donor) improves ASF survival	4	Nitric oxide has as an key mediator in the defence against ASF I/R injury	31,17
62. Cottler,	Skin, An ASF were made & the related	No wound	Two 18-gauge needle-puncture	-Assessment of flap perfusion and viability	Two spatially separated outlets are as effective as one leech in improving	25,14

1999, **Rat**	artery was ligated temporarily		outlets , or two sessions of leech therapy		flap viability	
22. Taub, **1998,** **Rat**	Skin,Unilateral axial pattern skin flaps(6×3cm) was made, based on the epigastric artery, & temporary occlusion	No wound	To test the effect treating with the gene for VEGF	1. Dye fluorescence, & 2. planimetry	Treatment can improve the survival of ASF	42,33
63. Taub, **1998,** **Rat**	Skin,Unilateral ASF based on the related artery, & temporary occlusion	No wound	Treatment with the gene encoding of VEGF	1. Dye fluorescence, & 2. immunohistochemical analysis	The treatment improved flap viability	135,85
64. Ueda, **1998,** **Rat**	Skin, the ASF were made, next the related vessels were ligated	No wound	To test the effect of sulfatide on I/R injury	4	The treatment has a significant defensive effect against I/R	17,12
65. Lees, **1991,** **Horse**	Skin, An ASFflap in the horse were made. End-to-end anastomoses were used in some flaps	No wound	To review authors experimental work with the ASF flap in the horse	Follow-up	Horse must be highly susceptible to I/R injury	3,1

Table two. Specifications of axial skin flaps in the reviewed papers; abbreviations, ischemia-reperfusion (I/R), platelet rich plasma (PRP), Axial skin flap (ASF), human umbilical cord matrix stem (HUCMS) cells, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-beta) and extracorporeal shock waves (ESW).

Ref. no& 1st Author's Name & Published year, Animal	Target organ or Tissue, Technique	Incision /Excision (wound) /Nothing	Intervention	No of evaluating methods	Main results/ Conclusions	Number of Google scholar & Web of sciences citations
66. Fourman, 2014, swine	Skin, 4 burns were made	Wound	To test the capability of two methods of angiography in the estimation of burn progression	-Perfusion analysis, - wound assessment	Indocyanine green dye angiography has markedly beneficial potential in the estimation of burn development	12,8
67. Soto-Pantoja, 2014, mice	Skin, Burns were induced by using a 95 °C heated brass rod	Wound	To test absence of CD47 on the rate of wound closure	4	Affecting CD47 may accelerate burn healing process	18,9
68. Tobalem, 2014, Rat	Skin,The burns were made using comb burn model	Wound	To evaluate the effect of local warming on burn progression	4	Treatment improved the microcirculatory perfusion	11,9
69. Hanjaya-Putra, 2013, mouse	Skin, Third degree burn was generated.	Wound	To test engineered human vasculatures when embedded in a burn model	Histology and Immunohistochemistry	Vasculature was improved in ischemic conditions	22,16
70. Bader, 2012,	Skin, A zone on the back of the mice was	Wound	To know the effect of nanosized recombinant human erythropoietin (rhEPO) in burn	1.Histological analysis, 2.Gene expression Analysis,	rhEPO improved burns	21,10

Mice	burned by hot water		healing	3.Western blot analysis		
71. Lanier, 2011, domestic pig	Skin, 4 full thickness burns were made using a comb model	Wound	To understand the rates of cellular death and apoptosis in the area of ischemia close to burn	5	It was demonstrated pathological signs of cell death	32,23
72. Singer, 2009, Rat	Skin, A brass comb burn makes3 burns	Wound	To test A brass comb burn model	- Photography -Histopathologic studies	the progression of most unburned ischemic zones to necrosis were happened	26,18
73. Singer, 2008, Rat	Skin, 4 burns were made by a brass com b model	Wound	To test the involvement of necrosis and apoptosis to cell death in the ischemic part.	Immunohistochemistry	Both apoptosis and necrosis are present in the ischemic part	50,29
74.Singer, 2007, Rat	Skin,4 burns were made with a brass comb	Wound	To test the effect of curcumin on the conversion of the ischemic zone to necrosis.	-Photography, -Histopathologic studies	curcumin reduced the percentage of unburned skin that progressed to necrosis.	44,25
75. Penington, 2006, Rat	Skin, Two burns was made with a brass block, them a axial skin flap(ASF) was made	Wound	To define whether the area of stasis displays a clear phase of sensitivity to a sublethal exposure to ischemia	Histological examination	Area of stasis shows amplified sensitivity to ischemia one to two days after burning	3,2
76. Cassuto, 2005, Rat	Skin, Partial and full thickness burns were induced in the abdominal skin	Wound	To test the effect of alpha- and beta-adrenoceptors in blood circulation of normal and burned skins	-Blood pressure and heart rate, - Blood flow	Activation of alpha(2)-receptors meaningfully impair skin circulation	10,7

77. Arslan, 2005, Rat	Skin, At first a dorsal skin flap was made, next 9cm2 of the flap was burned	Wound	To test the effects of mixture of L-carnitine and vitamin C on partially burned skin flap	- Macroscopic examination	The treatment decreased risk of ischemia-induced necrosis in flap.	15,6
78. Tan, 2002, Guinea pig	Skin, The skin of the guinea pigs were burned by hot water	Wound	To test the effect of Ibuprofen on inhibiting post burn ischemia	- Determination of depth of capillary stasis - Assessment of 6-keto-PGF1α and TxB2 in skin tissue	Ibuprofen did not inhibit progression of ischemia after burning	13,8
79. Lindblom, 2000, rat	Skin,A full-thickness burn was made in the abdominal skin	Wound	To test the effects of Vasoactive intestinal polypeptide (VIP)on post burn skin perfusion and progressive ischemia	- Laser Doppler measurements of skin blood flow	VIP application significantly impaired skin perfusion	7,3
80. Jönsson, 1999, Rat	Skin,A full-thickness burn was made in the abdominal skin	Wound	To test the effects of lidocaine on eicosanoid formation by normal and burned skins	-Eicosanoid analysis	The absence of effect of vascular route of lidocaine could relate to the severe burn trauma	19,12
81. Cetinkale, 1997, Rat	Skin, 4 burns were induced.	Wound	To examine the responses in the adjacent zone of burn damage that might cause to more necrosis	- Assessment of muscle perfusion, - Assessments of tissue oedema	Neutrophils might be contributed in the pathogenesis of local reaction to burn injury	38,23
83. Tarnow, 1996, Rat	Skin,A burn injury was induced in the abdominal	Wound	To test the effects of D-myo-inositol-1,2,6-trisphosphate (IP3), on the development of ischemia	- Skin blood flow	IP3 improved local dermal perfusion in burned skin	16,12

	skin					
84. Battal, 1996, Rat	Skin,A combo brass burn model was made	Wound	To test the effects of a prostaglandin I2 analogue, on burn injury	4	Prostaglandin I2 plays an important role in burn injury	21,13

777 Table three. Specifications of burn models in the reviewed papers; abbreviations:
778 recombinant human erythropoietin (rhEPO), topical lidocaine/prilocaine cream
779 (EMLA), D-myo-inositol-1,2,6-trisphosphate (IP3).

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Ref no& 1st Author's Name & Published year, Animal	**Target organ or Tissue, Technique**	**Incision /Excision (wound) /Nothing**	**Intervention**	**No of evaluating methods**	**Main results/ Conclusions**	**Number of Google scholar& Web of sciences citations**
89.Cremers, 2017, Mice	Skin, 3 cycles hind limb ischemia was induced, next 2 full-thickness excisional wounds were made on the dorsal side of mice	Excision	To improve skin wound repair by remote ischemic preconditioning (RIPC) treatment via induction of the heme oxygenase-1 (HO-1).	- Immunohistochemical staining & test, - Real Time-Quantitative-PCR	RIPC did not accelerate wound closure.	0,0
26.Park, 2016, Rat	Skin, Hindlimb ischemia was induced by femoral artery ligation, then an excisional wound were made	Excision	To study the role of E-twenty six (ETS) factor Ets variant 2 (ETV2)on vascular regeneration	5	A novel obligatory role for the ETV2 was reported	15,12
90. Spallotta,	Skin, Femoral artery was excised.	No wound	To test the effect of Histone deacetylase inhibitors (DIs)	7	Class-selective DIs interfere with	18,13

2013, **Mice**			during tissue regeneration following acute peripheral ischemia		normal mouse ischemic hindlimb regeneration	
91. Nishimoto, **2013,** **Rabbit**	Skin, All related arteries were ligated.3 weeks later, a 2×2 skin defect created on both legs	Excision	To test effect of Platelet rich plasma (PRP) derived from bone marrow aspirate (bm-PRP) and from peripheral blood (pb-PRP) on wound healing of persistent ischaemic rabbits' limbs	-DiI staining, -wound observation	Injection of bm-PRP is good for treating wounds on ischaemic limbs.	4,2
92. Porporato, **2012,** **Mice**	Skin, After resection of femoral vessels, a circular wound a 5- or an 8-mm diameter were made.	Excision	Whether the pro-angiogenic potential of lactate may be exploited therapeutically to accelerate wound healing	4	Poly-D,L-lactide-co-glycolide (PLGA) promoted angiogenesis and accelerated wound closure	52,40
93. MacLauchlan, **2011,** **Mice**	Skin, Ischemia was induced in one leg of mice by ligation of both the femoral and saphenous arteries, next 2 full-thickness excisional wounds were made	Excision	Providing evidence that nitric oxide (NO) induces angiogenesis	6	Modulation of thrombospondin2 (TSP2) expression is a major function of NO	44,18
94. Alizadeh, **2007,** **Rat**	Skin, After femoral arterial resection, a full-thickness skin area of 1.2×0.8 cm was removed from rats' foot	Wound	A new animal model designed to assess the impact of ischemia on wound healing	4	A significant delay in wound closure was observed	30,20
95. Bauer, **2006,** **Mice**	Skin, The related vessels were ligated, next the mice received bilateral excisional wounds with a 3-mm punch biopsy on the hindlimb.	Excision	To test the relationship between bone marrow-derived endothelial progenitor cells (BMD EPCs) and wound healing.	5	BMD EPCs were incorporated into the neovessels in the granulation tissue	74,50
96.	Skin, In a model of hindlimb ischemia on	Wound	To test, whether new blood vessel	-Histology, Immunohist	Arteriogenesis is enhanced	46,40

Straino, 2004	femoral artery ligation, a full-thickness wound of 3.5 mm diameter was created		development was altered in mdx mice. mdx mouse is a model for studying Duchenne muscular dystrophy (DMD)	ochemistry, - Angiogenes is Assays	in mdx mice both after ischemi a and skin wounding and in response to growth factors	

Table four. Specifications of ischemic limb models in the reviewed papers; Abbreviations: remote ischemic preconditioning (RIPC) , the heme oxygenase-1 (HO-1), histone deacetylase inhibitors (DIs) , bone marrow platelet rich plasma (bm-PRP), peripheral blood platelet rich plasma (pb-PRP), E-twenty six (ETS) factor Ets variant 2 (ETV2), poly-D,L-lactide-co-glycolide (PLGA), nitric oxide (NO), thrombospondin 2 (TSP2), bone marrow-derived endothelial progenitor cells (BMD EPCs), Duchenne muscular dystrophy (DMD).

Ref. no& 1st Author's Name & Published year, Animal	**Target organ or Tissue, Technique**	**Incision /Excision (wound) /Nothing**	**Intervention**	**No of evaluating methods**	**Main results/ Conclusions**	**Number of Google scholar& Web of sciences citations**
97.Wang , 2017, Mice	Skin,2 parallel incisions were made, next 2 full-thickness, 6 diameter wounds were made on the midline	Wound	To test the effect of mu opioid receptor (MOPr)-on healing of full thickness ischemic wounds using MOPr or kappa opioid receptor knockout (KO) mice	4	MOPr plays an important role in the proliferation phase with the formation of granulation tissue	0,0
98. Trujillo, 2015,	Skin , A bipeicled flap were made, next two 6-mm-	Wound	To show an ischemic flap model that permits a prolonged reduction of blood flow resulting	4	This model presents a valuable alternative	9,1

Rat	circular "ischemic" wounds were made, A silicone sheet were placed under flap.		in wounds that resemble a ischemic&chronic wound model		to previously developed ischemic skin flap models.	
99. Moor, 2014, Rat	Skin, 2 full-thickness 6-mm excisional wounds were created in the center of a 10.5× 3.5-cm flap	Wound	To test the hypothesis that age and tissue ischemia alter the balance of endogenous antioxidant enzymes	6	Deficiencies in two antioxidant pathways in aged rats observed that become exaggerated in ischemic tissue	12,8
100. Zhang, 2014, Rat	Skin, A dorsal, bipedicle skin flap was raised , then two 6 mm full-thickness excision wounds were created in the center of the flap	Excisio n	To test whether hyperbaric oxygen treatment (HBOT) modulates reactive oxygen species (ROS) and matrix metalloproteinase (MMP) regulation in ischemic wound tissue	-Western blot, - Histology and immunohistoch emistry	HBOT acts via the ROS / mitogen-activated protein kinases (MAPKs)/ MMP signaling axis to improve ischemic wound repair	21,12
101. Ruedrich , 2013, Rat	Skin, At first a 3 × 11.5-cm dorsal pedicle flap were made, Next four, 6-mm wounds were created symmetrically	Wound	To determine the most stable reference gene for studying gene expression in a rat ischemic wound-healing model using reverse transcription-quantitative polymerase chain reaction 9RT-qPCR)	- Real-Time PCR	Results provide insight on dependence of reference-gene stability on experimenta l parameters	3,0
102. Howe, 2011, Rat	Skin, An ischemic tissue flap was created . Two ischemic wounds were created	Wound	A electrical stimulation bandage has been developed for use with an established rat ischemic wound model	Clinical observation	The device has been successfully demonstrate d using the rat ischemic wound model for a period of	1,0

					seven days	
103. Weinreich, 2010, Rat	Skin, A pedunculated ischemic skin flap (3 × 7 cm) was lifted, One 8-mm wound was made in the flap	Wound	To test the hypothesis that systemic administration of isoniazid or niacin can enhance ischemic wound healing in	- PCNA immunohistochemistry, - Angiogenic assays,	Isoniazid stimulates wound-healing in ischemic tissue to the level of nonischemic wounds	2,1
104. Roy, 2009, Pig	Skin , 4 full-thickness bipedicle skin flaps (15 × 5 cm) were made, one 8-mm excisional wounds were made in the each flap	Excision	To develop and characterize the first porcine model of ischemic wound utilizing pig	8	This study serve as a base to develop hypotheses aiming to elucidate the biology of ischemic chronic wounds	82,45
105. Xue, 2009, Pig	Skin, An ischemic tissue flap is created. One ischemic wound was made	Wound	To develop a mathematical model of ischemic dermal wounds.	4	Ischemic conditions limit macrophage recruitment to the wound-site and impair wound closure	90,52
106. Zhang, 2008, Rat	Skin, A dorsal, bipedicle skin flap was raised , then two 6 mm full-thickness excisional wounds were created in the center of the flap	Wound	To test the effect of HBO on ischemic wound healing	6	HBO improves wound healing by downregulation of hypoxia-inducible factor-1alpha (HIF-1alpha)	115,71
107. Poonawala, 2005, Rat	Skin, 2 parallel incisions were made , then two 8-mm wounds were inflicted between	Wound	To test the effect of topically applied opioids on the healing of ischemic wounds in rats	1-Histological evaluation, 2-Immunofluorescent staining, 3-Western blot	Opioids accelerate wound healing	93,62

	incisions.			analysis		
27. Gould, **2005,** **Rat,**	Skin, Bipdicle skin flap were made, Two 6mm excisional wounds were created in the the flap, and a sheet was inserted into wound bed.	Excision	To develop a reproducible ischemic model for use in wound-healing studies	Wound-breaking strength, Lactate test, PDGF test	The excisional wounds provide sufficient tissue for biochemical and histologic analysis	40,28
108. Canapp, **2003,** **Rat**	Skin, 6-mm-diameter, wounds were created within an ischemic bipedicle skin flap	Wound	To test the effects of topical glycyl-L-histidyl-L-lysine tripeptide-copper complex (TCC)2% gel on tumor necrosis factor alpha (TNF-alpha) , &matrix metalloproteinases (MMP) in a ischemic open wound	TNF-β Concentrations, MMP-2 and MMP-9 Concentrations	Topical TCC resulted in accelerated wound healing in ischemic open wounds.	59,29
109. Zhang, **2003,** **Rat**	Skin, Normal incisional wound and H-shaped double flaps were made, The ischaemic test wound was the horizontal bar in the H-shaped double flap	Incision	To test the effect of exogenous vascular endothelium growth factor (VEGF) on wound healing in an ischaemic skin flap model	VEGF level determination, Tensile strength test, CD31 immunohistochemical staining	Treatment can increase early angiogenesis and tensile strength	90,45
110.Lee, **2000,** **Sheep**	Skin, Bilateral 10 × 15 cm dermal flaps were created, flap was then divided into 3 fields and Staphylococcus aureus was injected to each field	No wound	To study the effect of a noncontact radiant heat bandage in controlling an ischemic soft tissue infection	Bacterial quantification	Treatment controls ischemic soft tissue infections	16,6
111. Chen, **1999,** **Rat**	Skin, Six wounds were made within a bipedicled dorsal flap	Wound	To provide molecular and mechanistic evaluation of an ischemic wound model	4	This model will likely prove to be useful in chronic wound research.	90,56

792 Table five. Specifications of localized ischemic wound healing in the reviewed
793 papers; abbreviations: mu opioid receptor (MOPr), kappa opioid receptor knockout
794 (KO), hyperbaric oxygen (HBO), hypoxia-inducible factor-1alpha (HIF-1alpha),
795 glycyl-L-histidyl-L-lysine tripeptide-copper complex (TCC), tumor necrosis factor
796 alpha (TNF-alpha), matrix metalloproteinases (MMP), vascular endothelium
797 growth factor (VEGF).

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Ref. no & 1st Author's Name & Published year, Animal	**Target organ or Tissue, Technique**	**Incision /Excision (wound) /Nothing**	**Intervention**	**No of evaluating methods**	**Main results/ Conclusions**	**Number of Google scholar& Web of sciences citations**
112.Romana-Souza, 2016, Mice	Skin, Dorsal skin was pulled up and placed between a pair of magnet for 2 periods	Wound	To test the effect of the administration of celecoxib [a selective Cyclooxygenase-2 (COX-2) inhibitor] in wound healing of pressure ulcers.	5	Celecoxib administration improves the wound healing of pressure ulcers	4,0
29.Uchiyama, 2015, Mice	Skin,The skin was pulled up and trapped between two round ferrite magnetic for 12 hours	Wound	To assess the role of MFG-E8 in the formation of skin ulcers	Real time -PCR	Exogenous application of MFG-E8 is good for I/R injuries	14,9
113. Assis de Brito, 2014, Mice	Skin,16-hour period of magnet placement, followed by a release period of 8 h for 2 cycles.	Wound	To test the effect of β1-/β2-adrenoceptor blockade in wound healing of pressure	6	β1-/β2-Adrenoceptor blockade delays wound healing	12,10

			ulcers			
114. Jiang, 2011, Rat	Skin, One of four Ischemia-reperfusion (I/R) cycles [70 mm HG of pressure for 2 hours followed by 1, 2, 3, or 4 hours of reperfusion].	Wound	To explore the possible mechanism of I/R injury in begining of pressure ulcer(PU) development using clinically relevant amounts of pressure and pressure duration	- Biochemical test, - Histological test	a minimum of 4 hours pressure relief may be helpful for PU prevention	30,15
115. Nakagami, 2010, Rat	Skin, Ischemic wounds were created between the 2 incisions by applying an indenter for 3 hours	Wound	To test the usefulness of laser speckle flowgraphy (LSFG) for assessing skin blood flow in PU	Skin Blood Flow Measurements, Light Microscopic Assessment	LSFG measurements were useful for assessing tissue circulation	11,8
116. Erbayraktar, 2009, Rat	Skin, 5 recurring 2-h ischemic episodes, each separated by 30 min of reperfusion (12 h total), were performed, followed by a period of 12 h of ischemia	Wound	To test the effect of receptor-selective derivatives of Erythropoietin (EPO) in an PU	Wound size measurement	Wound healing is mediated by the tissue protective receptor isoform	44,28
117. Tsuji, 2005, Mice	Skin, 4 cycles of compression release. One cycle consisted of 2 hours of compression and 1 hour of release.	Wound	To establish a PU model that visualizes the microcirculation	Intravital microscopic images	Significant contribution of I/R injury to the pathophysiology of PU observed.	118,74
118. Stadler, 2004, Mice	The skin was gently pulled up and placed between 2 magnetic plates that had 12 mm diameter for 3 cycles	Wound	This paper reports the development of a reliable mouse model of I/R injury by the external application of magnets.	Macroscopic evaluation, Recording skin temperature	This method will facilitate the development of new prevention and management strategies.	75,48

119. Peirce, 2000, Rat	Skin, I/R injury was induced by applying and removing a permanent magnet to a rat skin under which a ferromagnetic steel plate was implanted	Wound	To develop and characterize a reproducible model of cyclic I/R injury in the skin of small un-anesthetized animals	4	Using this model, the biological markers of I/R-induced wound development can be studied	265,121
120. Houwing, 2000, Pig	Skin, Pressure application was achieved with a newly developed computer-controlled pressure device	Wound	To investigate the role of I/R in pressure-induced tissue necrosis in the trochanteric region	Pathological examination, Biochemical analysis	Administration of vitamin E may prevents PU in humans undergoing elective surgery	103,?
121. Lauritzen, 1981, Rabbit	Skin folds were located in chambers with temperature of (36°C&10°C) during cuff compression (200 mmHg) for 4 h	Wound	To quantitate the skin injury caused by the pressure ischemia	-The breaking load of the wounds	Cooling may preserve the reparative capacity in skin subjected to pressure ischemia	1,1

Table six. Specifications of pressure ulcers in the reviewed papers; abbreviations: Cyclooxygenase-2 (COX-2), Ischemia-reperfusion (I/R), pressure ulcer (PU), laser speckle flowgraphy (LSFG), Erythropoietin (EPO)

Ref. no& 1st Author's Name & Published year, Animal	**Target organ or Tissue, Technique**	**Incision /Excision (wound) /Nothing**	**Intervention**	**No of evaluating methods**	**Main results/ Conclusions**	**Number of Google scholar& Web of sciences citations**
122. Chenu,	Skin, A U-shaped peninsular skin	No woun	To study the role of steroid hormones in	4	Testosterone provides males with a	2,0

2017, **Mice**	incision was made	d	male mice		strong protection against cutaneous necrosis	
123. Seyed Jafari, **2017,** **Rat**	Skin, A random dorsal skin flaps (modified McFarlane) were made	No woun d	To test effect of electroporation-mediated hepatocyte growth factor (HGF) gene delivery to random dorsal skin flaps	4	Electroporation-mediated HGF gene delivery enhanced viability and vascularity of the ischemic skin flap	3,0
124. Zellner, **2015,** **Pig**	Skin, four random skin flaps(RSF) per animal were made	No woun d	To determine if skin flap failure rates could be improved with the use of a dissolved oxygen wound dressing	Histology	Treated-flaps had fewer clinical failures and improved histological profiles	5,2
125. Scioli, **2015,** **Rat**	Skin, A 10 × 3 cm l skin flap was elevated. An excisional wound in other rats were made.	Excisi on	To investigate the effects of Propionyl-L-carnitine (PLC) in rat skin flap and cutaneous wound healing	6	PLC treatment improved rat skin flap viability, accelerated wound healing and dermal angiogenesis	13,6
126. Cao, **2015,** **Rat**	Skin, A McFarlane flap model (9 × 3 cm) was designed	No woun d	To investigate the effects of lidocaine on RSF survival in rats.	4	Lidocaine increased flap viability	7,7
127. Silva, **2015,** **Rat**	Skin, A modified McFarlane flap model (2.5 ×8) cm was made	No woun d	To investigate the protective effects of L-Arginine (LA) and Kaurenoic acid (KA), against ischemia reperfusion	Biochemical Assays	KA may attenuate the oxidative stress and the inflammation	12,6

			(I/R)injury			
128. Harder, 2014, Mouse	Skin, A randomly perfused flap(11×15mm) Were made	No wound	To present a well-established model to directly visualize microvascular architecture	Intravital Epifluorescence Microscopy	The model has proven reliability in several published experimental studies	9,4
129. Khan, 2013, Mouse	Skin, A peninsular flap (3×1.5cm) by making three soft tissue incisions were made.	No wound	To study the significance of monocyte heterogeneity in physiologic neovascularisation and flap	4	Loss of function of chemokine ligand and receptor genes influenced the transcription of local genes involved in monocyte chemotaxis and wound angiogenesis	7,4
130. Shafighi, 2012, Rat	Skin, A caudally pedicled flap measuring 3 ×9 cm was made	No wound	To investigate the influence of of topical E2 on the survival of skin flap	Flap survival assessment, measuring distribution of blood flow	Treatment significantly increase survival of ischemically challenged skin flaps	11,6
131. Fayazzadeh, 2012, Rat	Skin, Full-thickness rectangular skin incisions (2×8) were made	No wound	To investigate the effects of fibroblast growth factor-2 (FGF-2 or bFGF) and erythropoietin (EPO) in prevention of skin flap necrosis	-Measuring survival rate, -Histology	Treatment of skin flaps could remarkably increase tissue viability and accelerate the wound healing process	6,4
132. Polito, 2012, Rat	Skin, H-shaped flap(2×4cm) were used	No wound	To assess the ability of polydeoxyribonucleotide (PDRN) to restore blood flow and improve	4	PDRN restores blood flow and tissue architecture	19,14

			wound healing			
133. Milch, 2010, Rat	Skin, A modification of the single pedicle dorsal skin flap were made.	No wound	To determine if monocytes activated toward an angiogenic phenotype can be used to improve ischemic tissue healing	Macroscopic Evaluation, Histology	Delivery of activated pro-angiogenic monocytes enhance histologic evidence of vascularity	5,5
134. Ferraro, 2009, Rat	Skin, The RSF was elevated and sutured back to its bed	No wound	Plasmid DNA encoding VEGF(165) (pVEGF) was delivered to the ischemic skin	5	pVEGFE+ increase perfusion and healing of skin flaps and ischemic wounds	69,39
135. Kuo, 2009, Rat,	Skin, A random-pattern extended dorsal-skin-flap (10 × 3 cm)	No wound	To assess whether extracorporeal shock wave (ESW) treatment rescues the compromised flap tissue	5	ESW treatment exerts a positive effect of rescuing ischemic extended skin flaps	60,42
136. Uema, 2008, Rat,	Skin, A RSF (10×4)were made.	No wound	To evaluate the possible benefits of eletroacupuncture stimulation of the points over the skin flap	Macroscopic appearances	Treatment preserved vitality and decrease RSF necrosis	8,6
137. Liapakis, 2008, Rat	Skin, A full thickness dorsal flap (10 × 2 cm) was designed	No wound	To determine the effect of local application of exogenous leptin on the survival of full thickness skin flaps	Histological and immunohistochemical evaluations	Treatment increases early skin flap angiogenesis	13,0
138. Liebano, 2008,	Skin, The random skin Flap(10×4 cm) was raised and	No wound	To determine the effect of low-frequency (2 Hz)	Estimating the percentage of necrotic	Treatment was effective in improving the viability	39,22

Rat	a plastic barrier was placed between the flap and its bed		transcutaneous electrical nerve stimulation (TENS) on the viability of ischemic skin flaps	area	of skin flap.	
33. Bayat, 2006, Rat	Skin, A RSF(20×70)were made	Incision	To clarify the histological, & ultrastructural effects of pentoxifylline (PTX) on the survival of RSF	4	Thirty days of pretreatment of RSFs with PTX significantly increased the survival of RSF	19,8
139. Park 2004, Rat	Skin,A flap (1.25× 2.5-cm) was elevated in the athymic nude mice,then a silicone sheet was separated the flap from the bed	No wound	To determine whether circulating endothelial stem cells might selectively traffic to regions of tissue ischemia	- Assessment of the Flap, -Histologic Assessment,	Systemic delivery of endothelial progenitor cells increased neovascularization	94,58
140. Babuccu, 2004, Rat	Skin, a 3 × 3 cm skin flap was elevated	No wound	To find out the effect of cerebrospinal(CSF) fluid leakage on wound healing after flap surgery	- Radioactivity in the CSF collection - Macroscopy, -Histology	CSF leakage itself has effects on wound healing.	14,4
141. Buemi, 2002, Rat	Skin, The RSF (3×9) were cut on the skin of the rat.	No wound	To ascertain whether erythropoietin (EPO) plays a role in repair processes following ischaemic skin flap injury	5	Treatment improve the wound healing process	78,50

142. Quirinia, 1997, Rat	Skin, The horizontal wound joining the two single flaps of a(2×8 cm)H-shaped double flap were made,	Incision	The influence of diclofenac and indomethacin on the healing of normal and ischemic incisional wounds using a flap model were studied.	- Biomechanical test, - Measurement of surface necroses	The treatments may be used for reducing superficial necroses of skin flaps.	26,19
143. Quirinia, 1996, Rat	Skin, H-shaped double flaps (2×8 cm), as well as suture sites were made on the skin each animal	Incision	The effect of buflomedil and isoxsuprine on the healing of ischaemic wounds was investigated	- Measurement of surface necrosis, - biomechanical test	The treatments were not effective in the treatment of ischaemic wounds or flaps	13,10
144. Quirinia, 1995, Rat,	Skin, H-shaped double flaps (2×8cm) were created.	Incision	To test the effect of 100% oxygen (2.4 ATA) on different phases of healing ischaemic and normal incisional wounds	- Biomechanical testing, - Measurement of length of surface necrosis	When hyperbaric oxygen was given on days 4-9 there was a tendency towards a decrease in the biomechanical parameters	30,27
145. Cheung, 1994, Rat	Skin, A McFarlane skin flap (3 × 9 cm) was raised	No wound	A localized 31P nuclear magnetic resonance (NMR) spectroscopy were examined	-NMR Coil Design and Construction, -Phantom Studies, - Flap Spectroscopy	This technique may facilitate a better understanding of cutaneous metabolic derangements	5,3
146. Rees, 1994, Rat	Skin, A McFarlane flap model (10 ×4cm) was made	No wound	To test the hypothesis that xanthine oxidase (XO) activity was increased along an ischemic gradient of a	-Xanthine oxidase and dehydrogenase	XO activity as source of free radical injury in skin necrosis seen in RSF.	45,21

			skin flap	assay,- Myelopero xidase assay		

Table seven. Specifications of flap skins in the reviewed papers; abbreviations: hepatocyte growth factor (HGF), random skin flaps (RSF), Propionyl-L-carnitine (PLC) , L-arginine (LA), Kaurenoic acid (KA), 17β-estradiol (E2), nitric oxide (NO), fibroblast growth factor-2 (FGF-2 or bFGF) and erythropoietin (EPO), polydeoxyribonucleotide (PDRN), plasmid DNA encoding VEGF(165) (pVEGF), pentoxifylline (PTX), cerebrospinal (CSF), erythropoietin(EPO) nuclear magnetic resonance (NMR), xanthine oxidase (XO).

GROUPS	**NUMBER OF PAPERS**	**NUMBER OF SAMPLES WITH WOUNDS**	**GOOGLE SCHOLAR CITATIONS**		**WEB OF SCIENCES CITATIONS**	
			TOTAL	**AVERAGE**	**TOTAL**	**AVERAGE**
1. RABBITS' EAR ISCHEMIC MODEL	**16**	**16**	**848**	**53**	**557**	**34.8**
2. AXIAL SKIN FLAPS	**18**	**1**	**610**	**33.8**	**351**	**19.5**
3. BURN MODELS	**18**	**18**	**378**	**21**	**233**	**12.9**
4.ISCHEMIC LIMB MODELS	**9**	**8**	**282**	**31.3**	**195**	**21.7**
5. LOCALIZED ISCHEMIC WOUND HEALING	**16**	**16**	**633**	**39.6**	**371**	**23.2**
6. PRESSURE ULCERS	**11**	**11**	**677**	**61.5**	**371**	**28.5**
7.SKIN FLAPs	**29**	**5**	**612**	**23.5**	**367**	**14.1**

Table eight. Number of papers, number of wounds, and total and average Google scholar citations, and web of sciences citations of studied groups.

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