

*Original Research***Preparation of Recipient Wound Bed by Ultrasound Therapy for Skin Flaps in Dogs****M. Gokulakrishnan^{1*}, C. Ramani², T. A. Kannan³ and Mohamed Shafiuzamma⁴**^{1,2&4}Department of Clinics, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, INDIA³Centre for Stem Cell Research and Regenerative Medicine, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, INDIA***Corresponding author:** drcool_vet@yahoo.co.in

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Abstract

Open wound management, until the wound is considered suitable for reconstruction or until it has healed by second intention, has been the treatment of choice for centuries. The aim of wound healing is to promote rapid wound closure and prevent excess scar formation. Wound healing stimulated an optimum microenvironment for successful reconstruction which can be created by employing modern method such as ultrasound therapy. In the present study, the wounds were treated with Low Intensity Pulsed Ultrasound (LIPUS) using water soluble ultrasound gel for 15 minutes on 0, 3rd, 7th and 14th day, until a proper and healthy granulation tissue was formed. The flaps performed were single pedicle and bipedicle advancement flaps, flank and elbow rotational flap, transposition flap and caudal superficial epigastric flap. The flaps were selected based on the location and site of the wound. Subjective evaluation of wound healing based on the physical observations such as colour, odour and presence of exudates for recipient wound bed and skin flap respectively were performed. Clinical photography, wound planimetry studies were evaluated. Additionally, hematological, bacteriological, biochemical, and histopathological evaluation were done for the recipient wound bed and skin flap. A subjective analysis of vascularity of the donor site was performed through Colour flow Doppler ultrasonography. Ultrasound therapy increased the vascularity and uptake of skin flap through enhanced capillary growth, increased wound strength and hastened wound closure. It also provided better protection of granulated tissue and wound against rupture and infection.

Key words: Dogs, Recipient Wound Bed, Skin Flap, Uptake, Ultrasound Therapy**How to cite:** Marudhamuthu, G., Kannan, T., & Shafiuzama, M. (2018). Preparation of Recipient Wound Bed by Ultrasound Therapy for Skin Flaps in Dogs. International Journal of Livestock Research, 8(6), 123-132. doi: 10.5455/ijlr.20180226105043

Introduction

Full-thickness skin loss injuries often results in granulation tissue formation, contraction leading to restriction of function and adverse aesthetics. The aim of wound healing is to promote rapid wound closure and prevent excess scar formation (Al – Bagdadi, 1993). Open wound management, until the wound is considered suitable for reconstruction or until it has sealed by second intention has been the treatment of choice for centuries. In open wound management, it is customary to prevent infection using antibacterial, antiseptic agents, and sometimes hygroscopic powders (Schultz *et al.*, 2005). Wound healing stimulated an optimum microenvironment for successful reconstruction which can be created by employing modern methods such as electrical stimulation and ultrasound (Shakespeare, 2001).

Ultrasound therapy has been noted to effect fibroblasts and stimulate them to secrete collagen, in addition continuous ultrasound at higher therapeutic intensities provided an effective means of heating deep tissues prior to stretching. (Robertson and Baker, 2001) A pedicle graft or skin flap is a partially detached segment of skin and subcutaneous tissue where the base or pedicle of the flap maintains circulation to the skin during its elevation and transfer to a recipient location (Hunt *et al.*, 2001). They represent one of the most practical methods of closing defects that cannot be approximated by simple undermining and suturing. The wound assessment was a complex activity which aimed to collect a large quantity of information to make appropriate decisions for treatment which was the first step in identifying the suitable treatment objectives for the management of wound (Plassmann, 2005). Tracking wound size was an essential part of treatment. The wound's surface area (S) and surface area-to-perimeter (S/P) ratio were useful to document healing (Mayrovitz and Soontupe, 2009). Though, lot of work on conservative management for wound healing using routine, standard protocols viz., cleansing, lavaging and debridement were carried out in dogs. Whereas, the present study aimed in preparation of recipient wound bed using elctro-physio modalities like ultrasound therapy.

Materials and Methods

The study was carried out on six dogs that were brought to Madras Veterinary College Teaching Hospital, Chennai with large wound requiring skin flaps. Low Intensity Pulsed Ultrasound (LIPUS) probe was directly applied against the dogs skin and wound bed using water soluble ultrasound gel for 15 minutes on 0, 3rd, 7th and 14th day, until a proper and healthy granulation tissue was formed. The Ultrasound machine (Fig. 1) was set to 20 % duty cycle at a frequency of 1-3 MHz. The intensity was set to less than 0.5 watts per square centimetre (0.3 watts/cm^2) if the animal experienced pain or heat. Periwound tissues were treated with 1 MHz, continuous ultrasound (Fig. 2). An ultrasound applicator 1.5 to 2 times the size of the treatment area was used. With an aqueous coupling medium in place, the probe was placed lightly against the skin surface and moved in a slow and deliberate manner. The intensity was typically set

between 1 and 1.5 watts per square centimetre. This parameter was extremely variable and depended on the animal's circulatory, sensory and mental status.



Fig. 1: Ultrasound therapy instrument



Fig.2: Ultrasound application in periwound area

After proper granulation, appropriate skin flap technique for the wound was decided based on the wound healing parameters (Ojingwa and Isseroff, 2003). The following skin flaps were performed viz., single pedicle advancement flap, flank and elbow rotational flap, transposition flap and caudal superficial epigastric flap. Subjective evaluation of wound healing based on the physical observations such as colour, odour and presence of exudates for recipient wound bed and skin flap respectively were performed (Table1) (Fig. 3).

Table 1: Clinical observation of the recipient wound bed and skin flap

Parameters	Days	1	2	3	4	5	6
Colour of open wound	Day 0	Y	Y	Y	B	B	Y
Y-Yellow, SR-Slight Red, R-Red, B-Black	Day 3	Y	Y	Y	Y	Y	R
	Day 7	SR	SR	SR	R	R	R
	Day 14	R	R	R	R	R	R
Colour of skin flap	Day 3	B	P	P	B	B	B
	Day 7	P	P	P	P	P	P
	Day 14	P	P	P	P	P	P
Odour of open wound	Day 0	O	P	O	O	O	P
P-Putrid, O-Offensive	Day 3	M	M	M	O	M	M
M-Mal, N-No odour	Day 7	M	N	N	M	M	M
	Day 14	N	N	N	N	N	N
Odour of flap	Day 3	M	M	M	N	M	M
	Day 7	M	N	N	N	N	N
	Day 14	N	N	N	N	N	N
	Day 0	E	E	E	E	E	E
Exudate of open wound	Day 0	E	E	E	E	E	E
E-Exudate, N-No Exudate	Day 3	E	E	E	E	ME	E
ME-Moderate Exudate	Day 7	ME	ME	ME	Me	ME	ME
	Day 14	N	N	N	N	N	N
Exudate of the flap	Day 3	E	E	Me	N	E	Me
	Day 7	Me	Me	N	N	Me	N
	Day 14	N	N	N	N	N	N

Clinical photography, wound planimetry studies were evaluated as per Bohling *et al.* (2006) during the period of ultrasound therapy. Additionally biochemical and histopathological evaluation were done for the recipient wound bed and skin flap. The total protein content from wet granulated tissue samples were performed as per Porat *et al.* (1980). A subjective analysis of vascularity of the donor site was performed through Colour Flow Doppler ultrasonography. After reconstructive surgery, skin flap vascularity and uptake were analysed by the same procedure on 3rd, 7th and 14th day respectively. The procedure was repeated post operatively to assess the cutaneous arteries on the skin flap on 3rd, 7th, 14th day.



Fig.3: Subjective evaluation of wound healing based on the physical observations

Results and Discussion

The wound planimetry studies of the recipient wound bed are presented in Table 2.

Table 2: Wound planimetry values of recipient wound bed

Case No.	Characteristics	Wound Healing %			
		Day 0	Day 3	Day 7	Day 14
1	Epithelisation	19.32	20.36	30.66	49.90
	Contraction	20.99	21.32	31.99	48.56
	Wound Healing	22.54	23.65	35.68	49.65
2	Epithelisation	20.09	21.36	31.25	50.23
	Contraction	19.54	20.26	30.99	48.56
	Wound Healing	23.65	24.36	36.99	50.23
3	Epithelisation	22.58	23.99	32.00	50.99
	Contraction	21.59	22.36	32.98	51.99
	Wound Healing	24.48	25.68	37.89	52.14
4	Epithelisation	21.82	22.92	34.56	52.00
	Contraction	22.05	23.03	33.58	53.00
	Wound Healing	25.09	26.35	39.05	53.65
5	Epithelisation	22.47	23.65	35.62	52.31
	Contraction	23.48	24.65	35.98	56.84
	Wound Healing	26.07	27.84	38.54	54.26
6	Epithelisation	22.14	23.65	36.65	52.96
	Contraction	20.14	21.02	31.69	54.25
	Wound Healing	20.13	21.03	33.26	48.29

The percentage of epithelisation, contraction and wound healing of recipient wound bed treated by ultrasound therapy are presented in Table 3.

Table 3: Percentage of wound epithelisation, contraction and wound healing of recipient wound bed (Mean $\pm$ S.E.)

Group/Days	Recipient Wound Bed			
	0 Day	3 rd Day	7 th Day	14 th Day
Epithelisation	21.40** $\pm$ 0.54 ^{ay}	22.66** $\pm$ 0.60 ^{by}	33.50** $\pm$ 0.98 ^{cy}	51.40** $\pm$ 0.40 ^{dy}
Contraction	21.17** $\pm$ 0.60 ^{ay}	22.11** $\pm$ 0.65 ^{by}	32.87** $\pm$ 0.73 ^{cy}	52.49** $\pm$ 0.20 ^{dy}
Healing	23.63** $\pm$ 0.86 ^{ay}	24.75** $\pm$ 0.93 ^{by}	36.90 $\pm$ 0.87 ^{cy}	51.44 $\pm$ 0.92 ^{dy}

Means bearing different superscripts in rows and columns vary significantly (* P <0.05) or (** P <0.01)

The percentage of epithelisation, contraction and wound healing on 0, 3rd, 7th and 14th day prior to skin flap, revealed a statistically significant increase. There was significant increase in epithelisation, contraction and wound healing on 3rd, 7th and 14th day respectively. Single Pedicle advancement flap was performed in one case with chronic wound on the dorsolumbar, region. The flap acceptance was moderately good, advancement was accompanied taking advantage of the elasticity of the skin however the single pedicle advancement flap did not exhibit additional “loose” skin to the wound site, successful closure required the flap to be stretched over the defect. Bipedicule advancement flap was performed in a case with chronic wound on the frontal region. Bilateral application of this flap was preferred in the wound which were too wide for unilateral application of the technique as observed in the above cases. Although bipedicule advancement flaps had two sources of circulation, the “Central Body” of the flap usually undergo mild ischemic necrosis when flap were excessively long. This was avoided by creating

flaps that were short as possible, minimizing surgical trauma during flap elevation and transfer, and incorporating a direct cutaneous artery and vein into one pedicle when feasible (Hedlund, 2006)

Transposition flap was performed in a dog that had a wound on the right medial and lateral aspect of the Hindlimb. (Fig. 4) Transposition flap was performed as it relied upon the stretching of elastic skin as it brought additional skin to the defect unlike the single pedicle advancement flap. However mild flap necrosis was observed at the extreme end of the flap. A pretension method of suturing was performed as flap contraction occurred at the distal extremity on 12th day postoperative. Proper anchorage with slings and padded bandages were applied to prevent wound dehiscence.

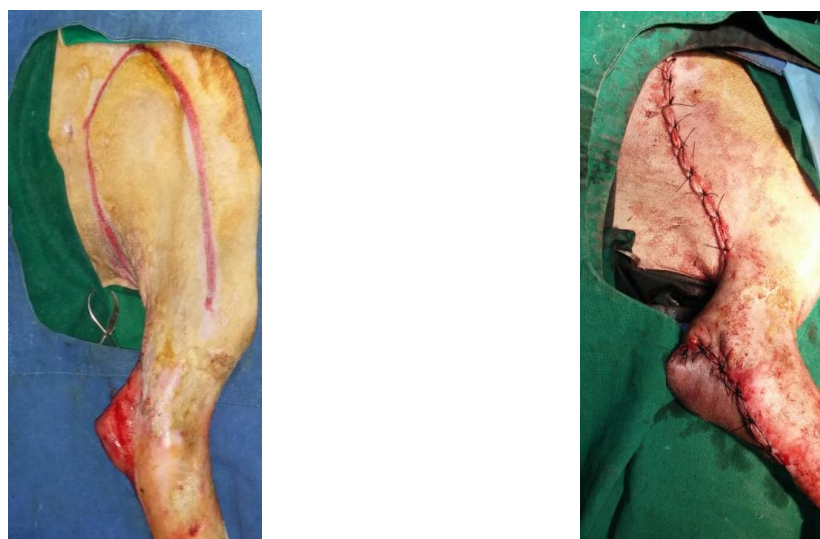


Fig. 4: Transposition flap (flap planning and final outlook of skin flap)

Caudal superficial epigastric flap was performed in a case that had a large lacerated wound on the left medial thigh region. (Fig. 5) Wider flaps were possible due to adequate skin that remained close to the donor bed. Adequate planning was undertaken prior to dissection of axial pattern flaps. Preoperatively, measurements were taken to ensure the length and size of flap reached and covered the wound in the above cases. The mean $\pm$ S.E. values of total protein of granulation tissue are presented in Table 4.

Table 4: Mean $\pm$ S.E. of total protein (mg/50mg) value of granulation tissue

Cases 1 to 6/ day	Recipient Wound Bed		
	3	7	14
1-6	3.91 $\pm$ 0.21 ^a	4.75 $\pm$ 0.17 ^b	4.96 $\pm$ 0.078 ^c

Means bearing different superscripts in rows and columns vary significantly ($P < 0.05$) or ($P < 0.01$)

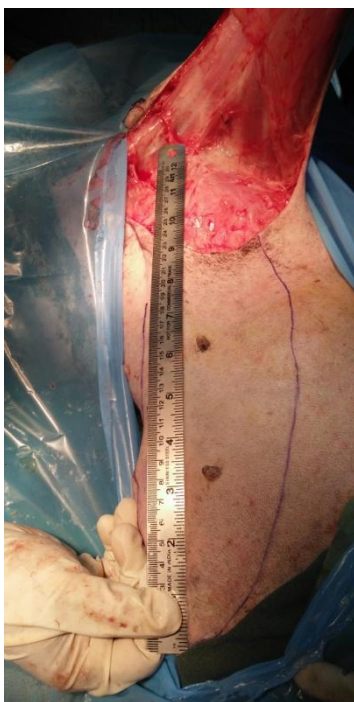


Fig.5: Caudal epigastric flap (flap planning and final outlook of skin flap)

There was a significant increase ($P < 0.05$) in total protein content in granulation tissue on 3rd, 7th and 14th day. The mean $\pm$ S.E. values for collagen proliferation, epithelisation and angiogenesis was 2.00 ± 0.64 , 2.18 ± 0.61 , 2.21 ± 0.53 and 1.65 ± 0.52 , 1.73 ± 0.52 , 2.81 ± 0.62 and 2.03 ± 0.72 , 1.63 ± 0.73 and 1.34 ± 0.22 on 3rd, 7th and 14th day respectively (Fig. 6).

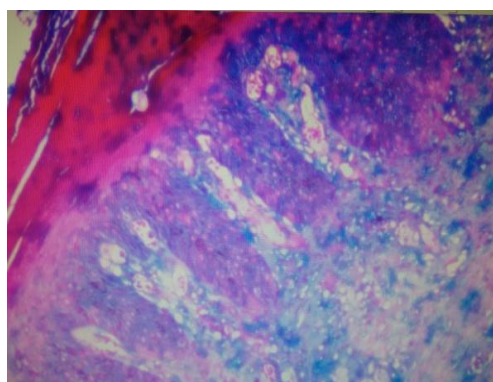
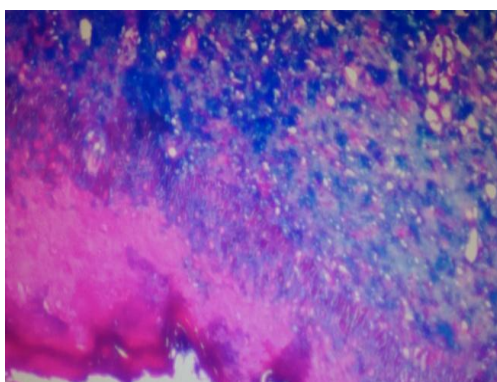


Fig. 6: Wound Bed- Moderate epithelisation with fibrin and immature capillaries on 7th and 14th day
Masson's Trichrome Stain x 50

The slight red colour of wound bed on 3rd day indicates the early neovascularisation and granulation as observed by Pavletic (2003). Ultrasound could modify plasma membrane permeability to ions such as calcium, and that this might act as a stimulus to cell activity. Thus, repeated stimulation of these cells

accounts for the observed acceleration of the resolution of inflammation and progress through the subsequent phases of repair. Ultrasound therapy stimulated the synthesis and maturation of collagen, thus resulting in an increased tensile strength of the healed skin. Tensile strength has commonly been associated with the organization, content, and physical properties of the collagen fibril network and was one of the necessary parameters for determining the pharmacological effects of potential wound healing agents as opined by Pol *et al.* (2004).

Mayhew and Holt (2003) described the use of ultrasonography to evaluate the integrity of the caudal superficial epigastric arteries prior to bilateral transposition of caudal superficial epigastric flaps to close a traumatic wound in a dog. In some instances, the trauma that was caused on the initial wound, also damaged the area where the cutaneous vessels originated. Thus, it was important to assess the integrity of the cutaneous vessels before constructing an axial pattern and a subdermal plexus flap to reconstruct a traumatic wound. This technique was relatively easy, non-invasive, and inexpensive method of assessing the integrity of cutaneous arteries and was potentially helpful when planning a flap in the clinical cases. Because of relatively small diameter of these vessels and their superficial location, 10 to 12MHz linear transducer was best for obtaining diagnostic information. However, a 7.5 MHz linear transducer was used successfully in a previous report by Mayhew and Holt, (2003). With superficial vessels, such as caudal superficial epigastric artery, a stand-off pad was used, that minimized the effects of near-field artifacts on image quality. Although not necessary in every instance, color-flow Doppler ultrasonography greatly facilitated vessel identification. Unless an artery was pulsating, it was difficult to differentiate a small vessel from parallel hyperechoic connective septa in the subcutis as opined by Reetz *et al.* (2006). The degree of confidence in locating cutaneous artery was subjectively graded as high, moderate, or low. A high level of confidence was observed for vessel that was located within 1 to 3 minutes and that its identity was not questioned on donor site viz., thorax and flank. A moderate level of confidence was observed for vessel that was located within 3 to 5 minutes on donor site viz., frontal. A low level of confidence (> 5 minutes) was not encountered in the study on the donor sites. This may be due to the selection of established donor sites and better perfused areas adjacent to the flap designed by subdermal plexus and collateral blood vessels (Fig.7) Electro-magnetic modality like ultrasound therapy reduced the infection at the wound site periodically (Lorenzo *et al.*, 2013). Thus, the preparation of a healthy wound bed was one of the most important aspects of reconstructive surgery. Ultrasound caused degranulation of mast cells resulting in the release of histamine (Christopher *et al.*, 2009).

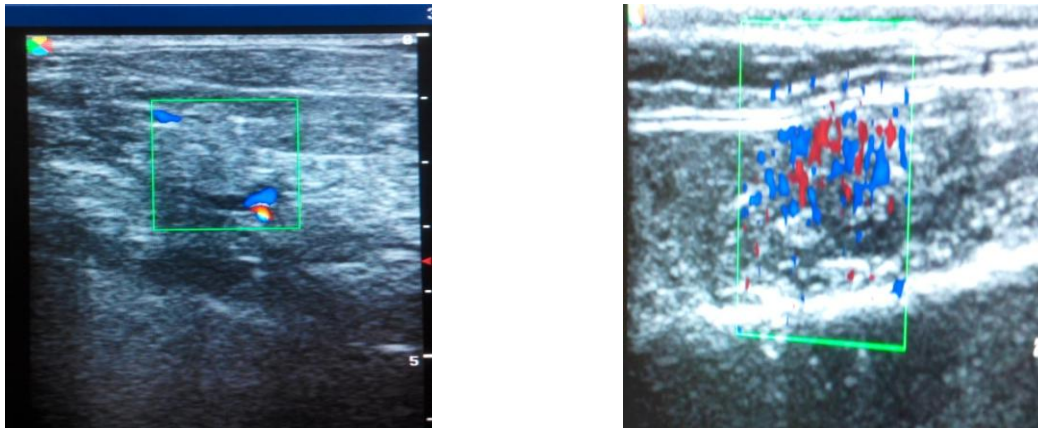


Fig.7: Colour flow doppler ultrasound images day 0 and day 14

Histamine and other chemical mediators released from the mast cell played a role in attracting neutrophils and monocytes to the injured site which was evident in histopathology in the present study (Selkowitz *et al.*, 2002). These and other events appeared to accelerate the acute inflammatory phase and promoted wound healing (Ennis *et al.*, 2006). Since both heat and stretch were combined in the present therapy, connective tissue elongated better. Continuous ultrasound at higher therapeutic intensities provided an effective means of heating deeper tissue prior to stretch (Busse *et al.*, 2002). As the frequency of ultrasound increased, the penetration of the signal decreased in all the cases. In addition to the above, Ultrasound has been noted to effect fibroblasts and stimulate them to secrete collagen as per Diana *et al.* (2004). This accelerated the process of wound contraction and increased tensile strength of the healing tissue.

Conclusion

The present study concluded that preparation of recipient wound bed by using ultrasound therapy augmented skin flap uptake through hastening granulation, neo-vascularisation and re-epithelisation in dogs.

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