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Research Article

PREPARATION OF RECIPIENT WOUND BED BY MANUKA HONEY FOR SKIN FLAPS IN DOGS

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ABSTRACT

The aim of wound healing is to promote rapid wound closure and prevent excess scar formation. Honey has been used for cleansing and accelerating the healing of wounds for centuries. The use of honey to treat wounds on animals has been slow to come into acceptance. Application of manuka honey remain a viable option for enhancing the granulation tissue formation and early wound bed preparation before large wounds could be reconstructed with skin flaps.

The flaps performed in the present study were flank and elbow rotational flap, transposition flap and caudal superficial epigastric flap, single pedicle and bipedicle advancement flaps. 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. Manuka honey decreased inflammatory edema, attraction of macrophages to further cleansed the wound, accelerated sloughing of devitalized tissue, provision of a local cellular energy source, and formation of a protective layer of protein over the wound and a healthy granulation bed that led to fibroblast recruitment, proliferation and matrix remodelling and ultimately good granulation and wound healing in the cases

INTRODUCTION

The aim of wound healing is to promote rapid wound closure and prevent excess scar formation. To stimulate skin healing, a variety of methods have been used, such as the topical application of herbal remedies like Aloe vera extract, the use of soft laser, natural honey and electromagnetic pulses (Houghton and Campbell, 1999). Even though good results have been achieved by these methods, the customary approach remains the prevention of infection using antibacterial and antiseptic agents, and sometimes hygroscopic powders (Schultz *et al.,* 2005). Manuka Honey is an excellent cellular energy source, provides a viscous barrier to wound invasion, and has a hygroscopic effect, which reduces edema and enhances wound healing.

Skin flaps or free grafts should be considered for larger defects, for which healing by second intention might be prolonged or expensive. Those reconstructive techniques also were advisable when wound contracture or a fragile epithelialised scar might occur (Hunt, 1995).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). Routine histopathological evaluation of collagen measurement and staining pattern of wound healing processes were performed by using Hematoxylin and Eosin and Masson's trichrome stains.

Ultrasonography and colour-flow Doppler Ultrasonography were relatively easy, noninvasive and inexpensive methods to assess the integrity of the direct cutaneous arteries and could

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potentially be helpful when planning an axial pattern flap for flap survival in clinical cases as performed in the present study. The aim of the present study was to determine the uptake of skin flap in chronic wounds treated with Manuka honey in dogs.

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. Manuka honey was applied to the wound on 0, 3rd, 7th and 14thday respectively. 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 (Table 1). Clinical Photography, Wound Planimetry studies were evaluated (Fig 1). 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 method of Porat *et al*. (1980).

Table 1 Clinical wound cases treated by Manuka Honey

Parameters	Days	Group III					
		1	2	3	4	5	6
Colour of open wound	Day 0	Y	B	Y	B	Y	Y
	Day 3	SR	Y	SR	Y	SR	R
	Day 7	R	SR	R	SR	R	R
	Day 14	R	R	R	R	R	R
Colour of skin flap	Day 3	P	P	P	B	P	P
	Day 7	P	P	P	P	P	P
	Day 14	P	P	P	P	P	P
Odour of open wound	Day 0	P	O	P	O	O	P
	Day 3	M	M	M	M	M	M
	Day 7	N	M	N	M	N	N
	Day 14	N	N	N	N	N	N
Odour of flap	Day 3	M	N	M	M	M	M
	Day 7	M	N	M	M	N	N
Exudate of open wound	Day 14	N	N	N	N	N	N
	Day 0	E	E	E	E	E	E
	Day 3	E	E	E	E	E	E
Exudate of the flap	Day 7	ME	ME	ME	E	ME	ME
	Day 14	N	N	N	ME	N	N
	Day 3	N	Me	N	N	N	N

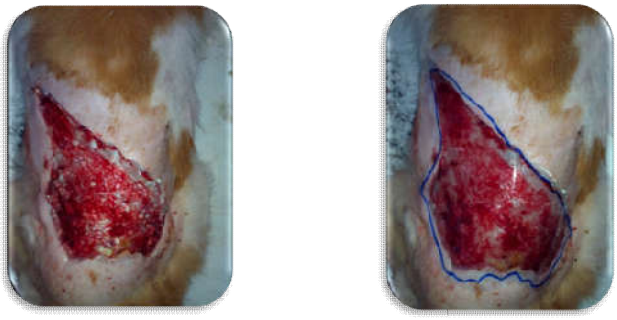

Fig 1 Wound Planimetry drawing of open wound margin on a transparent sheet

A subjective analysis of vascularity of the donor site was performed through Colour flow Doppler ultrasonography (Fig 16). 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.

RESULTS

The wound planimetry studies of the recipient wound bed are presented in Table 2. The percentage of epithelisation, contraction and wound healing of recipient wound bed treated by Manuka honey are presented in Table 3.

Table 2 Wound Planimetry Studies of the Recipient Wound Bed

Case No.	Characteristics	Wound Healing %			
		Day 0	Day 3	Day 7	Day 14
1.	Epithelisation	22.87	22.54	36.00	51.26
	Contraction	25.21	23.56	36.50	51.69
	Wound Healing	23.54	27.45	37.02	57.96
2	Epithelisation	21.36	23.89	35.68	49.56
	Contraction	22.99	24.98	36.99	52.31
	Wound Healing	24.12	27.89	38.54	55.05
3	Epithelisation	20.31	24.99	35.89	52.69
	Contraction	24.25	25.98	37.78	54.36
	Wound Healing	24.36	25.44	34.65	51.26
4	Epithelisation	23.24	26.00	36.99	52.69
	Contraction	25.48	26.54	39.23	56.98
	Wound Healing	25.36	27.99	38.99	55.99
5	Epithelisation	25.14	27.56	36.15	54.68
	Contraction	23.47	28.00	39.99	57.00
	Wound Healing	26.54	26.54	36.54	55.02
6	Epithelisation	24.54	24.36	36.58	49.65
	Contraction	25.34	25.69	36.23	50.26
	Wound Healing	24.12	26.59	36.58	55.36

Table 3 Percentage of wound epithelisation, Contraction and Wound healing of recipient wound bed of different Groups (Mean ± S.E.)

Cases / day	Recipient Wound Bed		
	3	7	14
1 to 6	4.59±0.24	5.00±0.10	5.37±0.15

Cases/Days	Wound epithelisation			
	0 Day	3rd Day	7th Day	14th Day
1 to 6	23.91±0.75	24.89±0.71	36.22±0.20	51.76±0.81
Wound contraction				
1 to 6	24.47±0.44	25.76±0.61	37.79±0.20	53.77±0.15
Wound healing				
1 to 6	24.67±0.45	26.98±0.40	37.06±0.64	55.11±0.89

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 (Fig 3 and 4).

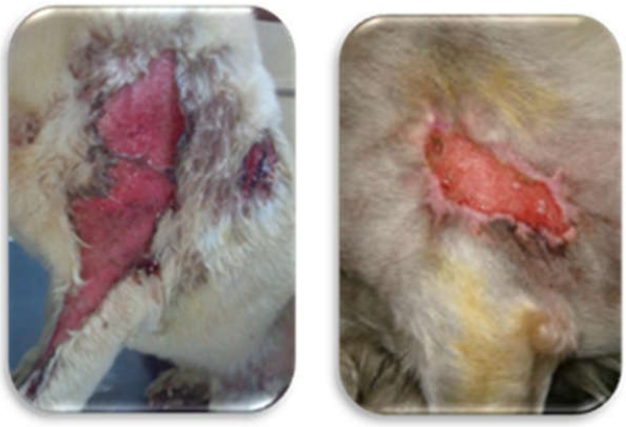

Fig 3 and 4 Clinical observation of Recipient Wound Bed of animals treated by Manuka Honey

Transposition flap were performed in one case which had chronic wounds in the left lateral thorax (Fig 5 and 6). Bi Pedicle advancement flap were performed in one case with wound on the upper eyelid. The Elbow rotational and flank fold flap were performed in one case each respectively. Caudal epigastric flap was performed in one case.

Fig 5 and 6 Clinical observation of skin flap of animal (Transposition Flap)

The mean ± S.E. values of total protein of granulation tissue are presented in Table 4. There was a significant increase ($P < 0.05$) in total protein content in granulation tissue on 3rd, 7th and 14thday. The mean ± S.E. values for collagen proliferation, epithelisation and angiogenesis was 2.28 ± 0.83, 2.40 ± 0.72, 2.49 ± 0.62 and 1.80 ± 0.78, 2.27 ± 0.62, 2.90 ± 0.00 and 2.43 ± 0.91, 2.56 ± 0.63, 2.69±0.12 on 3rd, 7th and 14th day respectively.

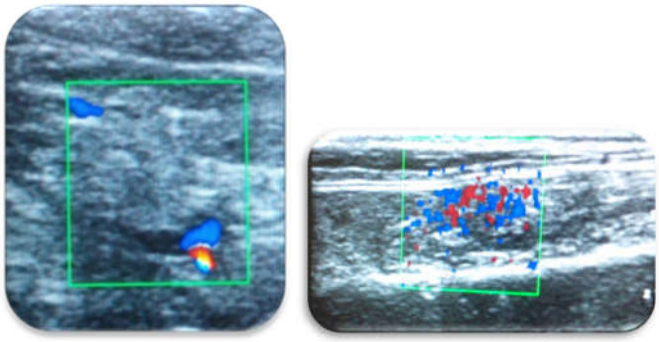

Fig 7 and 8 Color flow Doppler images of skin flap at 3rd, 7th and 14th day

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., scapula, paralumbar, sacral, thorax and flank. A moderate level of confidence was observed for vessel that was located within 3 to 5 minutes on donor site viz., parascrotal, axilla. A low level of confidence (> 5 minutes) was not encountered in the study on the donor sites.

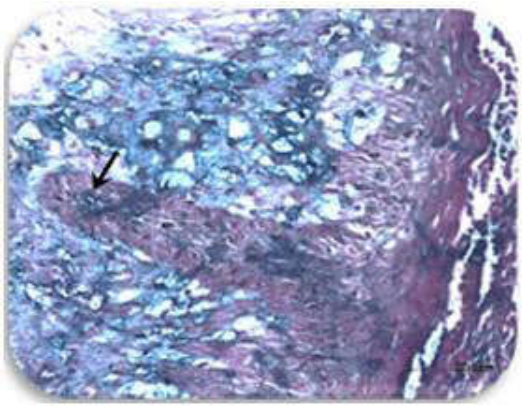

Fig 3rd Day Wound Bed-Granulating tissue with increased immature collagen fibres (arrows) Masson's Trichrome Stain

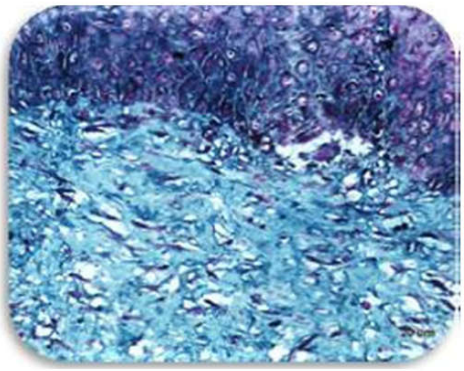

Fig 14 14th Day Wound Bed- Increase Neovascularisation with matured collagen fibres Masson's Trichrome Stain

DISCUSSION

Manuka Honey enhanced hemostasis, angiogenesis, growth, vascular fibroblast proliferation that was denoted by slight red color of the wound bed on 3rd day. Bright shiny red colour of the open wound was due to micro vascular network throughout the granulation tissue with neovascularisation the early neovascularisation and granulation as observed by Pavletic (2003).

In the present study, the colour of skin flap on 3rd day was predominantly pink, portions of the flap passed from red to pink overtime without necrosis. Colour changes in the early stages of circulatory obstruction could have been due to various reasons as opined by Slatter (2003).

Honey has antibacterial properties that have been attributed to its high osmolarity, acidity, and hydrogen peroxide H_2O_2 content. The effects of osmolarity in contaminated wounds is based on the low water content (or high osmolality) created in the wound. As the high osmolarity of honey draws lymph from a wound, dissolved nutrients within the lymph provide nutrition for regenerating tissue. Manuka honey is composed of approximately 40% glucose; 40% fructose; 20% water; and trace amounts of amino acids, vitamins (i.e., biotin, nicotinic acid, folic acid, pentotenic acid, pyridoxine, and thiamine), enzymes (i.e., diastase, invertase, glucose oxidase, and catalase), and minerals (i.e., potassium, iron, magnesium, phosphorus, copper, and calcium). The generation of low levels of hydrogen per oxide stimulates angiogenesis and the growth of fibroblasts. This increased angiogenesis increases oxygen delivery to tissues, which is a limiting factor for tissue generation. Topical acidification of wounds has been shown to promote healing therefore, manuka honey's low pH (3.6 to 3.7) will accelerate healing as well as increase antibacterial effects.

Although the skin was considered as a potential "donor" area for wound closure, the closed donor site was more prone to dehiscence in an active pet but proper immobilisation technique and rest prevented the above complications in the study. In view of the fact that 100% flap survival occurred in single pedicle and transposition flap but caudal epigastric flap showed survival of at least as good, if not better, than the other result. Vessel kinking and shortening through rotation and transposition become major obstacles when these types of flaps are rotated more than 90 degrees which was considered as precautionary step when transposition flap was done (Connery and Bellenger, 2002). Angiogenesis and Fibroplasia was

higher on the recipient wound bed and skin flap. The rate of granulation tissue formation and epithelialisation of wounds may be enhanced by the various constituents of honey. Manuka Honey is an excellent cellular energy source, provides a viscous barrier to wound invasion, and has a hygroscopic effect, which reduces edema. Honey also has high levels of antioxidants: which protect wound tissues from oxygen radicals that may be produced by the hydrogen per oxide.

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. Because of relatively small diameter of these vessels and their superficial location, 10 to 12MHz linear transducer was best for obtaining diagnostic information. 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 (Reetz *et al*., 2006)

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