

Role of Leech Application in the Synthesis of Healthy Collagen in Non-Healing Ulcers (*Jalauka Awacharan in Dushta Vrana*)

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Abstract

Wounds form a very extensive and important class of the disease. Although a very extensive research work has been done on the nature of healing processes, yet the fundamental understanding of the mechanism of wound healing is not fully explored. However, the work done on the general pattern of the process of healing and on some of the factors which aid in wound healing are utilized in present study. Healthy collagen formation is one of the important factors for the process of wound healing. Therefore, keeping these points in mind a sincere review of Ayurvedic and Modern literature was done.

Leech application was mentioned by Sushruta and Charaka in the management of non-healing Ulcers (*Dushta Vrana*). Therefore a clinical study was done with patients divided in Leech applied group and control group, histobiochemical analysis was assessed on 'modified numerical scale of "Ehrlach and Hunt"'. Thorough observations and results were evaluated on chi-square statistical calculations it was found that Leech application is statistically significant in the synthesis of healthy collagen.

Keywords

Dushta Vrana, Leech application, Collagen



Greentree Group

Received 26/5/15 Accepted 30/6/15 Published 10/7/15

INTRODUCTION

Wounds are defined as breach in the continuity of epithelium due to tissue breakdown. Healing of a clean wound, unless influenced by adverse factors, starts a few hours after the wounding. Normally it does not require any specific treatment for the healing to proceed in its sequential pattern, but this natural process of healing is not very often allowed to happen, on account of various retarding agents. When these factors supervene, it becomes necessary to adopt certain measures of wound cleansing for the initiation of healing in contaminated wounds. For a thorough understanding of wound cleansing process, the mechanism of wound healing, which is related with this problem requires a detailed study.

1.1 Physiology of wound healing

There are essentially three phases of repair in all soft tissue wounds. In the first phase, sometimes referred to as the lag phase, there is inflammation of the wound and mobilization of the cells which will synthesize granulation tissue. In the second phase, sometimes referred to as the proliferative phase or phase of fibroplasia,

granulation tissue is formed in the wound; collagen and mucopolysaccharides are synthesized by the granulation tissue, and there is an increase in the mechanical strength of the wound. The third phase is the phase of maturation: the cells in the wound diminish in number, but there is extensive remodeling of wound collagen and a further increase in the mechanical strength of the wound. During second phase synthesis of extracellular collagen and mucopolysaccharides by fibroblasts is detectable both by histological and biochemical methods by the fourth or fifth day¹.

A major problem in clinical studies of wound healing is that the factors which may affect wound healing tend to co-exist in patients, and it may be difficult or impossible to determine the significance of any one factor.

1.2 Factors affecting wound healing:

Skin integrity and wound healing physiology are disrupted by the underlying pathology (intrinsic factors), by environmental influences (extrinsic factors), and by inappropriate management (iatrogenic factors), that influence whether

the wound will heal or will become chronic or refractory. Following is the list of factors-

Table 1: Factors affecting wound healing

Intrinsic	Extrinsic	Iatrogenic
Related to Medical Status	Related to Environment	Related to Wound Management
Age	Medication	local ischemia
Chronic disease	Nutrition	Inappropriate wound
Perfusion and Oxygenation	Irradiation and chemotherapy	Trauma
Immunosuppressant	Psycho physiologic stress	Wound extent and duration
Neurologically impaired skin	Wound burden and infection	

1.3 Perfusion, oxygenation and collagen formation

All phases of wound healing require adequate oxygen. Oxygen is carried in the blood and dissolved in the plasma by the red blood cells bound to the hemoglobin. In anemia, there is reduced hemoglobin and reduced oxygen-carrying capacity of the blood. However, research data suggest that anemia does not impair wound healing when there is adequate perfusion and blood volume². Hypovolemia, the lack of adequate

intravascular volume, has been shown to impair healing because of insufficient volume to transport the oxygen and nutrients to the tissues and remove waste products.

Prolonged hypovolemia impairs collagen production and diminishes leukocyte activities³. Hartmann and colleagues⁴ reported that fluid replacement, according to measurements of subcutaneous oxygen tension, improved accumulation of collagen in healing wounds by day seven in 29 patients after major abdominal surgery ($p < .05$).

Theories and research abound in looking at reasons why there is a failure to respond to the signals of injury. One theory of the etiology of venous ulcer chronicity attributes the problem to a dysfunctional fibrinolytic system. According to this theory, lipodermatosclerosis is a part of the pathogenesis of venous ulcers that impairs the progression of the inflammatory phase, progressing to the proliferative phase⁵.

An oxygen tension gradient develops across the wound that is used for regulatory purposes. Oxygen is essential to prevent infection and to meet the metabolic demands of the tissues, as well as the hydroxylation of proline necessary for useful collagen production in the remodeled wound⁶.

Hunt⁷ and his colleagues suggested tissue PO₂ levels which are significantly lower than 15 mmHg may be a rate limiting factor in the synthesis of collagen during the normal process of wound repair.

1.4 Recent advances of the use of collagen in wound healing

- Application of bioengineered skin substitutes⁸. Apligraf (Organogenesis; Novartis) is a bilayered skin substitute produced by combining bovine collagen and living cells derived from tissue-cultured human infant foreskins. One study of diabetic foot ulcers demonstrated 12-week healing rates of 39% for patients who received only standard wound care versus 56% for those who were treated by application of an Apligraf after a period of standard wound care.
- Oasis (Healthpoint, Ltd), a relatively new product, is a xenogeneic acellular collagen matrix derived from porcine small intestinal submucosa in such a way that an extracellular matrix and natural growth factors remain intact. This provides a scaffold for inducing

wound healing. We should not use this in patients with allergies to porcine materials.

2. MATERIALS AND METHODS

Total 20 patients, belonging to department of *Shalya Tantra*, were selected randomly from outpatient department and indoor ward and divided into two groups. Group I was treated with Leeches and Group II with conventional therapy (control). The criteria for selection of cases were based in the symptomatology presented by the patients in accordance with the criteria described by the Sushruta. Local and general examination of the wound was then done in detail. Wound's anatomical site, size, colour, margins, presence of discharge, associated pain, tenderness, depth and edema etc., were noted down. Routine laboratory investigations were done as below;

- Hb%
- TLC, DLC, ESR
- Blood urea
- Blood sugar
- Blood albumin, globulin
- Urine
 - Routine
 - Microscopic
 - Culture

- Pus  Sensitivity

2.1 Leech Application

2.1.1 Material

- Two small glass jar/plastic jar, kidney tray, one big glass bowl and turmeric powder.
- Sterilized gauze, swab and gloves.
- Sterile needle, sterile dispovan (10 ml).
- Normal saline and dressing material.

2.1.2 Applying Leeches

- First the Leeches were purified by putting them in turmeric mixed water for 15 minutes;
- After that Leeches were kept in plain water for 5 minutes.
- Then patient's wound was cleaned thoroughly with plain water. (If sterile water available it is best suited for this purpose).
- Then adequate numbers of leeches were applied to the general area of maximal congestion.
- Then a wet gauze or thin cotton pad was placed covering the leech's body (head and mouth remained

uncovered) and continuously pouring of fresh water was done. Once the leech attached, it will remain safely in place until fully distended and then detaches itself. (30-45 mm.)

- After that Leeches were purified in the same manner described as above, the wound was cleaned with normal saline and dressing was done.
- Same leeches were again applied to the same patient on 4th day.
- So two times leeches were applied to a single patient with dressing with normal saline.

2.2 Histobiochemical Analysis

Ten patients of Group I (Leech applied) and 10 patients of Group II (control) were evaluated on histobiochemical basis. Wound biopsy was taken on the 6th day and histobiochemical markers were graded according to modified numerical scale of Ehlach and Hunt.

- 0- No evidence
- 1- Occasional evidence
- 2- Light scattering
- 3- Abundant
- 4- Confluent or fibers

2.3 Parameter of assessment

Observations were evaluated by using statistical calculations. Chi-square test has been applied to test the significance of difference between proportions of two groups. Wherever the expected frequency came less than 5, chi-square has been computed after suitably pooling the rows or columns.

3. OBSERVATION AND RESULTS

Observations were done based on chi-square test in both the groups and graphical representation is given in Table 2.

4. DISCUSSION:

For treating chronic, non healing ulcers leech application was prescribed in Sushruta and Charaka Samhita^{9,10}.

- In modern medicine, leech application is commonly use in procedures such as skin graft, reattachment of body parts and reconstructive surgery.

Table 2: Collagen deposition

Collagen deposition Grade (0-4)	No. of Patients		Chi-square test Intergroup
	Group I	Group II	
0	0 (0%)	0(0%)	$\chi^2 = 8.67$ P<0.02
1	1(10%)	2(20%)	
2	1(10%)	4(40%)	
3	6(60%)	0(0%)	
4	2(20%)	4(40%)	

Comment

Leech application patients had abundant deposition of collagen (80%) in comparison to control group and this was statistically significant as seen in figure 1.

Figure 1

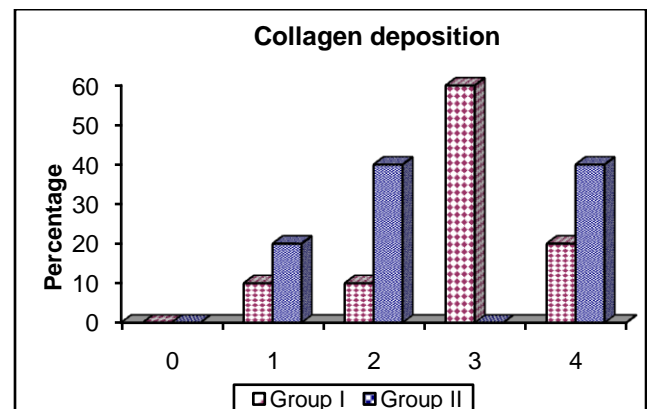
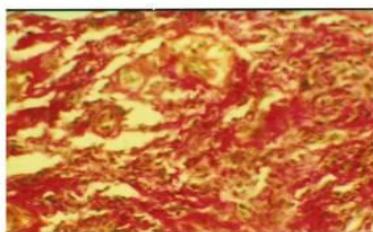
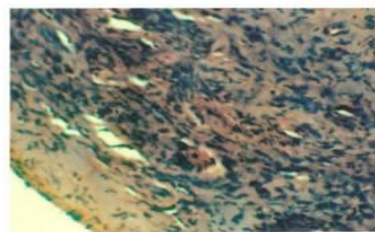


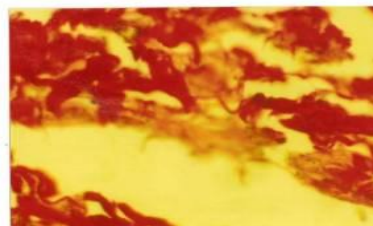
Figure 1: Histo-biochemical photograph showing evident collagen in leech applied group



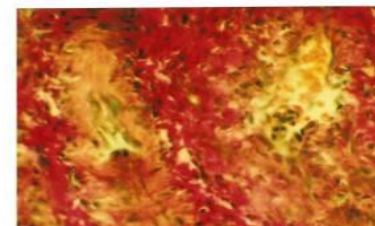
Diabetic wound, showing abundant collagen, blood vessels with thickened wall and scattered mononuclear cell infiltration. (Modified Van Gieson Stain $\times 500$)



Diabetic wound, showing abundant fibroblasts, spindle cell nuclei and occasional evidence of mononuclear cells. (H&E $\times 500$)



Infected wound showing confluent fibers of collagen without any inflammatory cell infiltration (Modified Van Gieson stain $\times 500$)



Diabetic wound, showing blood vessels with thickened wall surrounded by abundant collagen. (Modified Van Gieson stain $\times 500$).

Histobiochemical evaluation of leech applied group

- As a matter of fact FDA has formally classified medicinal leeches as medical device. It has more than 100 proteins in saliva¹¹.
- Leech has hirudin in saliva which is a potent thrombolytic as well as fibrinolytic agent; it has also an antibiotic like substance, equally potent to penicillin¹².
- Leech has potent anesthetic agent in saliva, which is equally potent to morphine¹³.
- Leech has a potent vasodilator agent and hyaluronic acid present in saliva¹⁴.
- Leech application is useful in methicillin resistant Staphylococcus bacterial infection in wounds¹⁵.

Collagen deposition, fibroblast proliferation and angiogenesis can be accelerated by improving oxygenation in the wound tissue. For that purpose many mechanical tools are evolved but they are very expensive and they need specialized persons for their handling. Furthermore their role is very limited in those wounds where micro vascular thrombi are present. Recent tissue O₂ studies indicate that most occurrences of non-healing in wounds are due to tissue hypoxia, tissue hypoxia causes increased edema, ischemia and infection. It affects all phases of normal wound healing including phagocytosis, angiogenesis, wound contraction epithelialization and collagen synthesis. On other hand, increase oxygen

tension accelerates the healing process, improves phagocytic capabilities of leucocytes to destroy bacteria and other foreign material. It enhances epithelialization and wound contraction and also enhances collagen synthesis as well as improves cross linking of collagen fibers, thus improving wound tensile strength¹⁶.

4.1 Objective Assessment:

Objective assessment was performed on 20 patients. Two groups were made as Group I and Group II. Biopsy sample was taken on 6th day as collagen proliferation is abundant between 3 to 8 days.

On inter-group comparison between group I and group II, the effect of therapy was statistically significant. In leech applied group maximum number of patients (80%) had abundant collagen deposition in comparison to control group. This is because leech improves the oxygenation of tissues by rectifying the micro vascular thrombi with the effect of Hirudin, thus it helps in procollagen hydroxylation and improves the quality of collagen (mature collagen). Procollagen easily degrades by enzymatic means, so it does not provide any mechanical strength to the wound tissue.

CONCLUSION

We conclude that the Leech application was statistically significant in the synthesis of healthy collagen fibers in non-healing ulcers (*Dushta Vrana*) by promoting increased oxygenation in the wound surroundings.

I hope this study will further enlighten the new areas of research for healing of non-healing ulcers.

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