Efficacy of Topical Hemocoagulase in improving the characteristics of Surgical Wounds: In Vitro Study

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Abstract

Wound Healing Is a Complex Process Involving A Series Of Overlapping Events. Hemocoagulase Is A hemostatic agent that is recently studied for its wound healing property. Though its role in secondary wound healing has been researched, its role in primary wound healing is not studied yet. This in-vitro study aims to evaluate the efficacy of topical hemocoagulase in improving the healing of surgical wounds. This study included 6 healthy male or female (total of 12 sites) rodents weighing at least 160 grams. Under sterile aseptic conditions, the rodents were anesthetized, and a 1 cm length vertical skin incision was made on the right and left side of the vertebral column, exposing the fascia and muscles underneath. Topical hemocoagulase (test) was used on the right side of the rodent while the left side served as control. The rodents were sacrificed on postoperative days 3 and 7, and the control and test sites were excised and sent for histological analysis. The outcomes assessed were histological wound healing parameters with the wound healing scale which includes the amount of granulation tissue, inflammatory infiltrate, collagen fiber orientation, the pattern of collagen, amount of early collagen, and amount of mature collagen. Our study results showed no difference in the wound healing parameters between the test and control groups. Topical hemocoagulase, though proven to play a role in improving secondary wound healing, has no significant role in primary wound healing among the rodent species.

Keywords: wound healing, topical hemocoagulase, botroclot

1. Introduction

By acting as a mechanical barrier to microorganisms and foreign bodies, the skin and mucosa contribute to innate immunity. 1 Insult to the skin or mucosa disrupts the barrier function, and its restoration is directly related to wound healing. 2 Wound healing is a complex process involving a series of overlapping events that begin at the time of injury or insult and proceed in the following order: hemostasis (0–several hours after injury), inflammation (1–3 days), proliferation (4–21 days), and remodeling (21 days–1 year). Hindrance to even one of these events will affect the function, aesthetics, and mental status

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of the patient if related to the skin. 3 In general, patient-related factors, intraoperative management, and postoperative wound care all have an impact on postoperative wound healing. Interventions are intended to alter the aforementioned factors in favor of improved wound quality. 4

Hemocoagulase is a hemostatic agent isolated from the venom of poisonous snakes like Bothrops jararaca or Bothrops atrox. It is a coagulative and antihemorrhagic enzyme complex that intervenes in the first stage of wound healing (hemostasis). 5 The mechanism of action is that it speeds up the transfer of fibrinogen to fibrin polymer, increasing platelet contact with fibrin clots to coagulate blood. The resulting fibrin is highly resistant to plasmin action, increasing the quality and quantity of collagen fibrils under it. As a consequence, hemocoagulase stimulates wound healing by shortening the bleeding time, increasing cell division and capillary network formation, and acting as an effective hemostatic agent. It is commercially available in a variety of forms including injections and topical agents. 6 It is being studied widely for its wound healing properties and has been proven to be beneficial in secondary wound healing. 6-8 Hemocoagulase also functions similarly to thrombin, with the exception that it is not inhibited by antithrombin and can coexist in the bloodstream with antithrombin. Furthermore, unlike thrombin, hemocoagulase is not absorbed by fibrin clots and thus is not neutralized by that mechanism. 9 But, its role in primary wound healing (surgical wounds) has not been assessed in any of the previous studies. Botroclot, botropase, and reptilase are commercially available hemocoagulase formulations. 10 Our team has extensive knowledge and research experience that has translated into high-quality publications. 11-25

This in-vitro study aimed to evaluate the efficacy of topical hemocoagulase in improving the healing of surgical wounds. The null hypothesis proposed is that there is no difference in the histological parameters of wound healing between topical hemocoagulase and control groups.

2. Materials and Methods

This in-vitro study was conducted at the Department of BRULAC and Department of Oral and Maxillofacial Surgery, Saveetha Dental College and hospital. The approval of the study was given by the "Institutional Ethical committee, SIMATS Review Board" [IHEC/SDC/OSURG-1902/21/346]. The animal ethical committee approval was also obtained for this study [BRULAC/SDCH/SIMATS/IAEC/04-2022/104].

The study included 6 healthy male or female (total of 12 sites) rodents weighing at least 160 grams. Topical hemocoagulase (test) was used on the right side of the rodent while the left side served as control. The outcome assessed were histological wound healing parameters with the wound healing scale proposed by Sulthana et al. The scale parameters include the following: the amount of granulation tissue, inflammatory infiltrate, collagen fiber orientation, the pattern of collagen, amount of early collagen, and amount of mature collagen. The outcomes were assessed on postoperative Day 3 and Day 7. The topical hemocoagulase solution used in this study was from Jagat Pharma under the trade name Botroclot. Each ml of Botroclot topical solution contains (a) 0.2 Cu/ml aqueous solution of hemocoagulase isolated from Bothrops atrox or Bothrops jararaca. (b) 0.1 percent v/v chlorhexidine (as a preservative) and (c) IPq.s water for injection. About 0.5ml of the solution was used at each test site in this study.

Preparation of site

Surgical procedures were performed under sterile conditions in a sterile animal laboratory surgical room. Rats were anesthetized with ketamine hydrochloride (i.p) at the dosage of 70mg/kg body weight and xylazine (i.m.) at the dosage of 10mg/kg body weight. The lower half of the dorsal part of the trunk was shaved thoroughly and aseptically prepared with a solution of Betadine (Figure 1). 1 cm length vertical skin incision was made on the right and left side of the vertebral column, exposing the fascia and muscles underneath. The left-side incision area was considered the Control side and was untreated. On the right side, topical hemocoagulase (botroclot) solution was injected into the exposed tissue area and kept for 10 minutes for the solution to get absorbed by the underlying tissues (Figure 2). Care was taken during the surgery not to damage the vessels. Then the tissue flaps of both control and test sides were sutured with resorbable suture threads (Vicryl 5/0, Ethicon®, Somerville, NJ, USA) and betadine ointment is applied to the sutured area and then the rats were isolated in separate cages (Figure 3). The rats were examined daily for any inflammation or infection in the surgical site. Post experimental period days 3 and 7, one set of animals were sacrificed respectively by euthanizing the animals in a CO_2 chamber and the surgical wound site was incised and fixed in neutral buffered formalin for histopathological analysis (Figures 4 and 5).

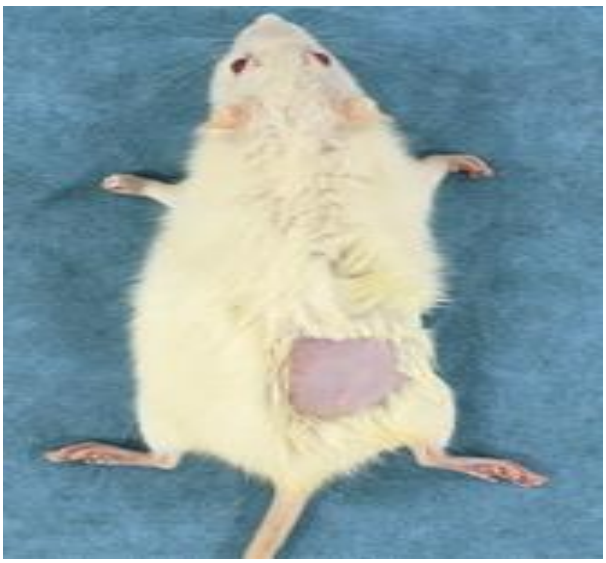

Figure 1: Surgical site prepared

Figure 2: 3-4 drops of topical hemocoagulase poured over the surgical site

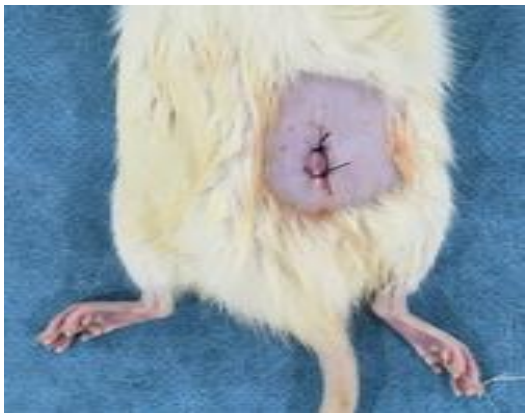

Figure 3: Closure of surgical site

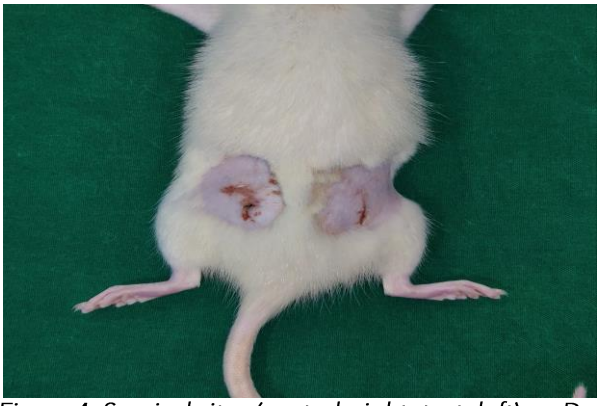

Figure 4: Surgical sites (control- right, test- left) on Day 3

Figure 5: Surgical sites (control- right, test- left) on Day 7

Histology

The tissue was sent for histological staining using the permanent paraffin section to the Department of Oral and Maxillofacial Pathology at Saveetha Dental College. The tissue was fixed in 10% neutral buffered formalin for 24 hours and in grades of alcohol for dehydration followed by acetone and xylene for 1 each. Paraffin wax impregnation was done for 24 hours, and further, the paraffin-embedded wax was sectioned using Leica microtome to 3 to 10-micron thickness, hematoxylin, and eosin stained and seen under the microscope to assess wound healing (Figures 6-9).

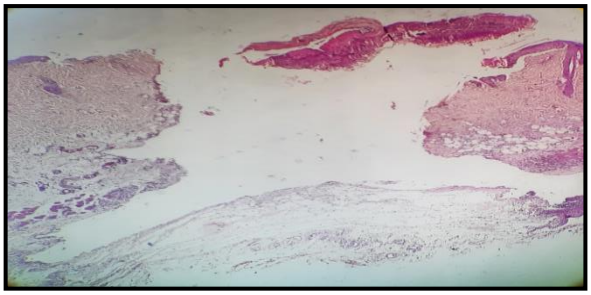

Figure 6: Permanent paraffin section of the control group on day 3

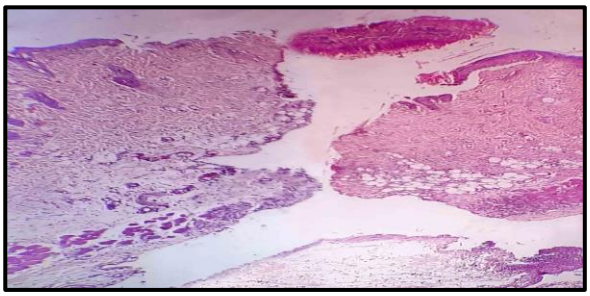

Figure 7: Permanent paraffin section of test group on day 3

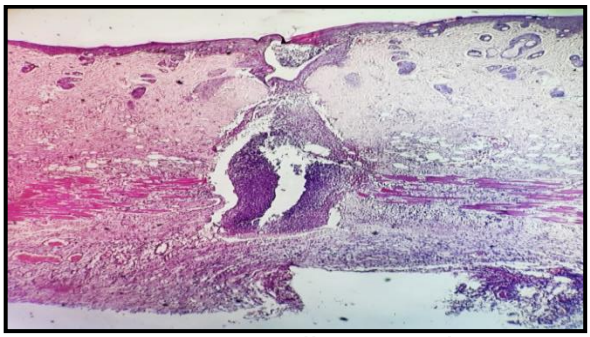

Figure 8: Permanent paraffin section of the control group on day 7

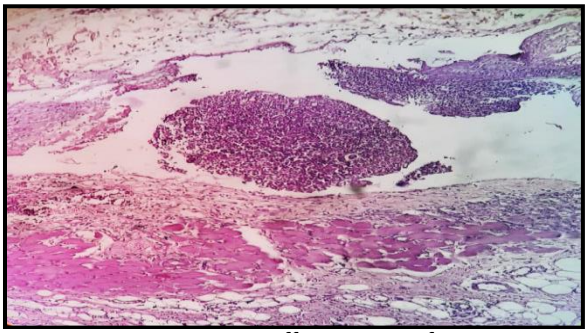

Figure 9: Permanent paraffin section of test group on day 7

3. Statistical Analysis

The collected data were analyzed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY:

IBM Corp. To describe data descriptive statistics, frequency analysis, and percentage analysis were used for categorical variables, and the mean and S.D were used for continuous variables.

4. Results

The objective of our study was to assess the efficacy of hemocoagulase on histological wound healing parameters among rodents subjected to surgical wounds. Our study results showed no difference in the wound healing parameters between the test and control groups (Figure 10 and Figure 11).

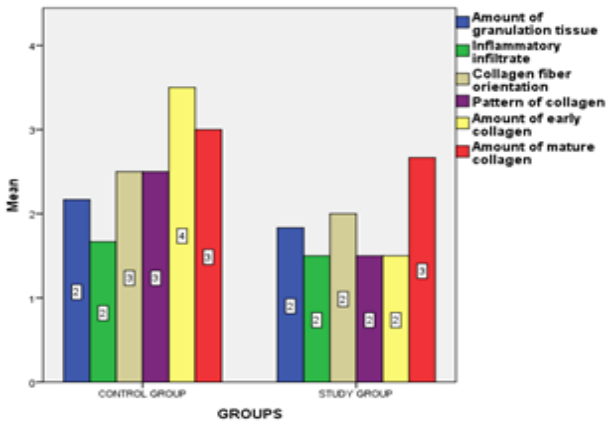

Figure 10: Graphical representation of histological wound healing parameters as assessed on Day 3

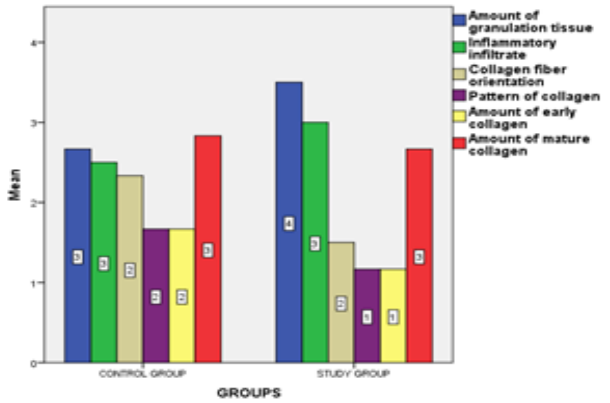

Figure 11: Graphical representation of histological wound healing parameters as assessed on Day 7

5. Discussion

Surgical wound healing may occur by primary, secondary, and tertiary intention depending on the nature, duration, and extent of the injury. Wounds that are well approximated and clean heal by primary intention with minimal scarring. 26 non-approximated or incorrectly approximated wounds heal by secondary intention with granulation tissue formation and have a higher proclivity to scar. 27

Though there is reasonable evidence with randomized controlled trials to claim the effectiveness of topical hemocoagulase in improving secondary wound healing, no study has been done, to the best of our knowledge, to assess the role of topical hemocoagulase in primary wound healing.

The study by Madhu et al compared wound healing properties of topical hemocoagulase to placebo in diabetic patients with non-healing/ healing ulcers. They found that there was a significant difference in wound healing between the two groups in favor of topical hemocoagulase. 6

The study by Shenoy et al who evaluated the effectiveness of topical hemocoagulase in the healing of extraction sockets showed that the difference between the placebo and the hemocoagulase group was significant. 7 Similarly, Aslam et al showed statistically significant differences between placebo and the hemocoagulase group in the healing of extraction sockets. 8

This study differs from the aforementioned studies as we aimed to assess the role of topical hemocoagulase in primary wound healing and the study design is in-vitro in nature. As expected, there was no difference in the primary wound healing properties of topical hemocoagulase and control. Hence, the null hypothesis was not broken in this study.

We acknowledge our choice of study design (in-vitro) could be a limitation. However, histological assessment of primary wound healing necessitates an unnecessary incisional biopsy of a healed site, which would be unethical. Since our objective was to evaluate the efficacy of topical hemocoagulase in improving the healing of surgical wounds, we chose an in-vitro study design.

6. Conclusion

We conclude from this in vitro study that there is no difference in the histological wound healing parameters between hemocoagulase and the control group. Topical hemocoagulase, though proven to play a role in improving secondary wound healing, has no significant role in primary wound healing among the rodent species.

7. Conflict Of Interest

The authors declare no conflicts of interest.

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