



Carbomer 940 Hydrogel Enhances Capillary Blood Flow and Tissue Viability in a Skin Burn Wound

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Abstract

Objective- To evaluate the application of Carbomer 940 hydrogel on saving zone of stasis in skin burn wound.

Design- Experimental study

Animals- Twenty rats.

Procedures- After shaving the backs, a 'comb burn' was contacted bilaterally on the dorsum of the rats 0.5 cm lateral and parallel to the midline by using a brass probe consisting of four rows (10×20 mm) and three interspaces (5×20 mm). All rats randomly separated into experimental and control groups. After burn induction, Carbomer 940 hydrogel was administered topically to the experimental group while 0.9% saline was administered through the same route in the control group. Dressings were changed every day. Doppler flowmetry measurements and histopathology were used for evaluation of perfusion and viability in the zone of stasis. Blood flow measurements repeated every 24 hours and skin specimens were taken at day 3 for histopathology evaluation.

Results- Our results show that blood flow in zone of stasis increased 24 hours post-burning in experimental group and the differences between the results obtained from the experimental and the control groups were found to be statistically significant ($p < 0.05$) at 24, 48 and 72 hours after burn creation. Histopathologically, epithelial tissue in zone of stasis in experimental group statistically more than the control group and sebaceous glands and hair follicles remained viable in group treated by hydrogel.

Conclusion and Clinical Relevance- Our experimental study revealed that Carbomer 940 hydrogel improved tissue perfusion and decreased the area of skin necrosis in the zone of stasis in rats. Application of this hydrogel in treatment of burn injury has positive effects and causes better burn wound healing in rats.

Keywords- Carbomer 940, Hydrogel, Rat, Zone of stasis, Burn wound healing.

Introduction

Disruption of the skin usually results in increased fluid loss, hypothermia, infection, scarring, compromised immunity and causes physical and psychological problems. All these factors together are very important; on the top of those, large skin damage can cause mortality.¹

In 1953, Jackson proposed a burn model, representing an initial pattern of thermal injury that could be divided into several zones.² Based on the severity of skin destruction and blood-flow alterations, three distinct zones of tissue injury can be recognized in his burn model. Centrally, the zone of necrosis is characterized by coagulation necrosis with thrombosed blood vessels, representing the irreversible tissue damage by direct contact of thermal energy. The intermediate zone, around the central zone is the zone of stasis, yet vital but with stagnant blood supply, resulting in an ischemic insult. The outermost layer is the zone of hyperemia with increased blood flow, representing an inflammatory response to tissue injury. Immediately after burn this pattern of tissue damage appears and remains for at least 24–48 h or longer in more severe burns. Investigations on the pathobiology of the zone of stasis revealed that irreversible tissue

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necrosis ensues with progression of hypoxia and ischemia in 1–48 h, leading to total loss of this intermediate zone. The reversible nature of damaged tissue in zone of stasis at the early stage of the burns suggested that this zone could possibly be salvaged when tissue necrosis associated with progressive ischemic insult was prevented.³

Hydrogels are ideal biopolymeric biomaterials for dressing of skin wounds. They have low interfacial tension, oxygen permeability and good moisturizing and mechanical properties that resemble physiological soft tissue.⁴ Therefore, extensive attention has been drawn on developing hydrogel-based wound dressings from biomaterials.⁵ Currently, two groups of hydrogels are recognized: a) preformed gels such as: Carbomers, celluloses, hyaluronic acid, polyvinyl alcohol and b) in situ forming gels such as: poloxamer, gellan gum, cellulose, acetate phthalate latex.

Carbopol is widely used as a main component of drug delivery gel systems for transdermal, buccal, ocular, rectal, and nasal applications. Some advantages of applying aqueous Carbomer hydrogels are high viscosity at low concentration, wide viscosity interval and characteristic flow behavior, compatibility with many active ingredients, bioadhesive properties, good thermal stability and excellent organoleptic characteristics and good patient acceptance.⁷ Numerous materials have been studied for saving the zone of stasis in skin burn wounds with reports of positive effects.⁸ There have been studies showing that some hydrogels accelerated different stages of skin wound healing and favorable effects on the zone of stasis. Hence, we examined the effects of Carbomer hydrogel alone, with no additional growth factors, cytokines, or cells on saving zone of stasis in burn wound in rats.

Materials and Methods

Animals

This study was performed at the Faculty of Veterinary Medicine, University of Tehran. Twenty adult Wistar rats weighing 300–350 g were used. The animals were kept two by two in individual propylene cages under standard laboratory conditions and fed with standard rat diet and water ad libitum. Rats were maintained on a 12 hour light/dark cycle at 22±2°C and 50±5% humidity.

Pharmaceutical ingredients and hydrogel preparation procedure

Carbopol® 940 Polymer purchased from Lubrizol Corporation, US. Carbomer 940 (1%) was added slowly to a Krebs-Henselitt buffer solution (pH 7.4), under constant stirring by a magnet stirrer. Then NaOH was added for achieving neutral pH and clearing of the gels. After addition of the full amount of solid material, the gels were allowed to swell under room temperature.

Burn Induction

Before the burn induction, the rats were anaesthetised intramuscularly with ketamine (10%, Alfasan, Holland), 75 mg/kg, and xylazine (2%, Alfasan, Holland) 10 mg/kg. The dosage was repeated when necessary. The entire dorsum of each rat was shaved to create a burn comb model. Burn wounds were created as in ‘burn comb model’, which was described by Regars and Ehrlich.⁹ The comb consisted of four rows (1 cm × 2 cm) and three interspaces (0.5 cm × 2 cm). The metal comb was immersed in boiling water for 5 minutes, until thermal equilibrium was achieved between the comb and the water. The hot comb was placed on the back of the rat 0.5 cm lateral and parallel to midline and held for 20 seconds without pressure (Fig.1) and this procedure was repeated on the other side of midline. All rats were randomly divided into experimental and control groups (n=10 rats/group). All rats were euthanized 72 hours after burn induction.

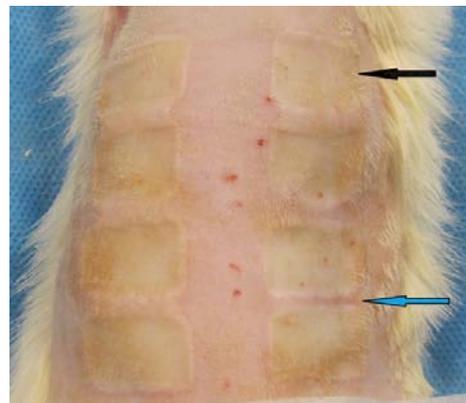


Figure 1. Burn comb model. Black arrow represents zone of necrosis which contact directly to the comb. Blue arrow represents zone of stasis.

Treatment

The experimental and control wounds were dressed with Carbomer hydrogel and 0.9 % saline, respectively. All wounds were covered with TegaDerm (3M, Minneapolis, MN). Dressing changes were made every day. The old dressings were removed and the wounds were cleaned with cotton swabs to remove any fluid, clots, fibrins, residual hydrogels, and any tissue debris. Everyday Laser Doppler flowmetry were performed, new dressings were applied, and the wounds were covered again with TegaDerm. This procedure was performed until euthanasia time.

Laser Doppler Flowmetry

The full thickness burns were confirmed by measuring the blood flow in the burned rows of the back skin using Laser Doppler flowmeter (MBF3D, Moore Instruments,

U.K.) 30 minutes after burn creation. The blood flows in the interspaces and unburned areas (caudally and cranially at least 2 cm distant from the burned areas) were measured 30 minutes after induction of burn injury just before the treatment with Carbomer hydrogel or 0.9 % saline. Measurements were repeated every day after burn creation before euthanasia. The blood flow values obtained from three different parts, burned rows (coagulation zone) (n=8), interspaces (zone of stasis) (n=6) and unburned areas (n=4), were recorded separately for each rat, and the means calculated were considered as the average blood flow value for each specific area.

Histopathological examination

All rats were sacrificed by inhalation of CO₂, 72 hours after burn induction. The burned and interspaces tissues with piece of peripheral unburned skin were harvested from all rats, pinned on a plastic plate (to keep the tissue flat) and fixed in 10% buffered formalin and processed to 5 µm paraffin sections and stained with hematoxylin and eosin (H & E) and Masson's trichrome stain (MT). All specimens were evaluated individually in 4 different zones (two burned zones and two interspaces zones). The main histologic outcome measures included the amount of inflammatory infiltrates for extent and severity, fibroplasia, collagen deposition, neovascularization (angiogenesis), re-epithelialization or complete repair. The Abramov's histologic scoring system was used for this study.¹⁰ Abramov's system assessed each parameter independently and assigned a score of 0–3. inflammatory infiltrates or extent, collagen deposition and complete repair were graded as: 0 (none), 1 (scant), 2 (moderate), 3 (abundant). and also, severity of inflammation was scored as follows: 0 – no inflammation; 1– presence of giant cells, occasional lymphocytes and plasma cells; 2 – presence of giant cells, plasma cells, eosinophils and neutrophils; and 3 – presence of many inflammatory cells and microabscesses. The amount of fibrosis was scored as: 0 = no fibrosis; 1 = minimal, loose; 2 = moderate; and 3 = florid dense. The fibroblast maturation or fibroplasia of granulation tissue was graded as: 0 (immature), 1 (mild maturation), 2 (moderate maturation), 3 (fully matured). Neovascularization was graded as: 0 (none), 1 (up to two vessels per high-power field [HPF]), 2 (3–4 vessels per HPF), 3 (more than 4 vessels per HPF).

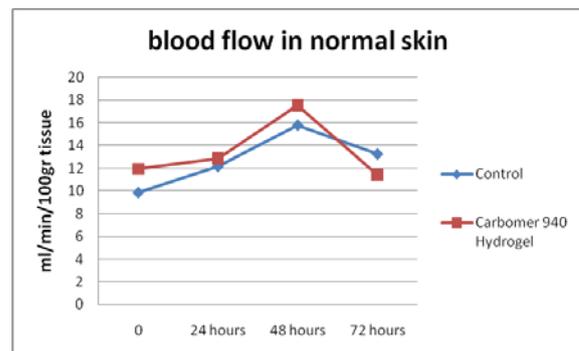
Data Analysis

Statistical analysis was performed using the SPSS version 16 (SPSS, Chicago, IL). Results were compared using Mann-Whitney U-test. Differences were statistically significant when $P < 0.05$.

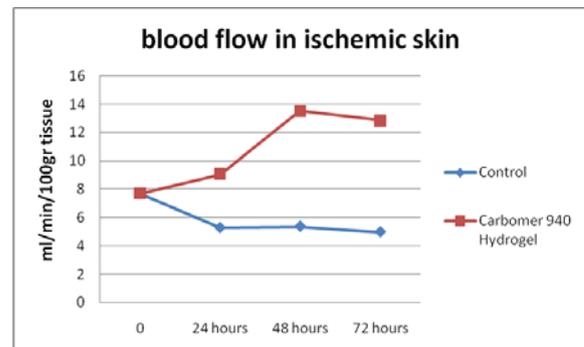
Results

Laser Doppler flowmetry

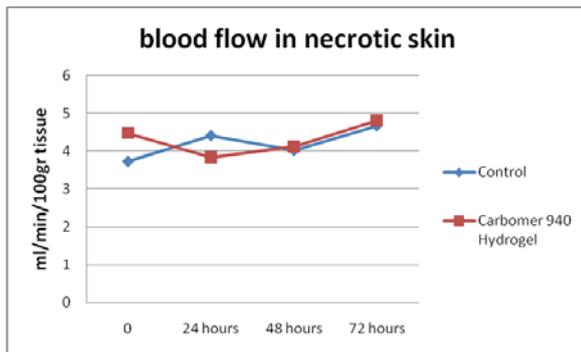
All rats survived the study and there were no visual side effects of the hydrogel or normal saline application. 30 minutes after burn induction blood flow measurement was performed in 3 different zones. Normal skin blood flow was measured as 9.83 and 11.93 ml/min/100 g tissue in control and experimental groups, respectively (Graph 1). Our results showed application of Carbomer hydrogel did not induce significant differences in normal skin blood flow compared with that of burned animals treated by 0.9 % saline during the study. Twenty four hours after burn, the mean blood-flow measurement in the zone of stasis was 9.06 ml/min/100 g tissue in Carbomer hydrogel-treated group, whereas 5.21 ml/min/100 g tissue in the control group. The differences between the experimental and control groups at post-burn hours 24, 48 and 72 were found to be statistically significant ($P < 0.05$) (Graph 2). There were no statistically significant differences between control and Carbomer hydrogel treatment groups at the zone of necrosis during the mentioned specific time of study (Graph 3).



Graph 1. Blood flow in four distinct times of study in normal skin.



Graph 2. Blood flow on ischemic zones. Blood flow increased in Carbomer 940 hydrogel rats 24 hours post-burning.



Graph 3. Laser Doppler flowmetry results showed no differences in blood flow of necrotic zones of both groups of study.

Histopathological findings

In the control burn wounds necrotic epithelium was seen histologically at the burned and interspaces areas. But in experimental group a thin epithelium was observed at the interspace areas. Diffuse collagen injury and necrosis of the hair follicle and sebaceous glands in the control interspace with preserved hair follicles and sebaceous glands in the hydrogel-treated interspace could be observed. The degree of re-epithelialization showed significant difference between control and experimental groups ($P < 0.05$). None of the other histological parameters were significantly different between two groups of this study (Table 1, Fig.2).

Discussion

Burn wound healing regarded as a specific process related to the general phenomenon of growth and regeneration of tissue.¹¹ Jackson's burn model described three concentric zones of a burn wound: the central zone of coagulation, the intermediate zone of stasis, and the outer zone of hyperemia.¹² Several biomaterials have been used to save the zone of stasis in skin burn wounds.

The main purpose of applying a wound dressing is to provide a moist environment to encourage the establishment of the best milieu for rapid healing.¹³ Hydrogels are tridimensional polymeric networks that can preserve a significant amount of water within their structures and swell without dissolving.¹⁴ This means that they can absorb wound exudates, which maintains moisture on the wound surface. Besides, hydrogels have high water vapor and oxygen permeability, as well as mechanical properties that resemble physiological soft tissues.¹⁵ Carbomer 940 hydrogel have a potential wide range of applicability in the pharmaceutical and dermocosmetic fields.⁷ In light of the above, we aimed to examine the effects of Carbomer 940 hydrogel on saving the zone of stasis.

Capillary blood flow is a key indicator that ensures nutrient delivery and oxygen supply to tissue. Previous studies have pointed that various events could result in

blood flow reduction in zone of stasis, including blood coagulation, increased vascular permeability, neutrophil recruitment and plugging of capillaries, and damage to the endothelium mediated by locally released oxygen radicals.¹⁶ Hence, dynamic studies of blood flow measurements after burns enable us to unravel mechanisms of progressive ischemia in the zone of stasis. So far, various strategies, such as increasing tissue perfusion, increasing the tolerance of the tissue to ischemia, and antithrombotic and antioxidant agents have been suggested to save the zone of stasis.¹⁷ In this study, we used laser Doppler flowmetry to access dynamic changes in blood flow following skin burn injury. Laser Doppler flowmetry is non-invasive and convenient for obtaining real-time information on hemodynamics. It can also detect marked alterations in the vascularization of skin wound healing. Laser Doppler flowmetry has been used extensively in experimental and clinical applications.¹⁸ Our results showed that blood flow increased in zone of stasis 24 hours following burn induction in rats treated by Carbomer 940 hydrogel.

Previously published papers have indicated that hydrogels enhance skin burn wound healing by affecting different stages of repair. Chitosan hydrogel improves the functions of polymorphonuclear leukocytes, macrophages and fibroblasts, and then it promotes granulation tissue formation and can be used in large open wounds.¹ It accelerates the infiltration of inflammatory cells such as neutrophils during inflammatory stage of wound repair; therefore, improves the debridement of wound area. Chitosan also accelerates re-epithelialization and will not leave wide scar.¹⁹ Our results in day 3 showed no statistical difference in inflammatory cell infiltration in necrotic and ischemic zones in both group of study (table 1). Sun and his colleagues indicated that dextran hydrogel accelerates the recruitment of endothelial progenitors and cells to the burn wound area, enabling rapid neovascularization after a week of treatment.²⁰ Although angiogenesis in ischemic and necrotic zones in Carbomer 940 treated wounds is more than the control group, it is not statistically different (table 1). They also showed that the dextran hydrogel promotes significant skin maturation; hydrogel-treated wounds have a mature epithelial structure with hair follicles and sebaceous glands.²⁰ Our histopathological results proved that epithelial layer, hair follicle and sebaceous glands remained viable in zone of stasis in Carbomer hydrogel treated rats. Poloxamer-188 is a nonionic block copolymer surfactant of polyoxyethylene and polyoxypropylene, it improves capillary blood flow in zone of stasis.¹⁶ It enhances fibrin network permeability and inhibits neutrophil chemotaxis; therefore, it can keep capillaries patent. It also binds to blood platelets and prevents the formation of occlusive thrombus and maintains blood flow in zone of ischemia in burn wounds.¹⁶ Our laser Doppler flowmetry measurements revealed that blood flow in zone of stasis in experimental group improved during the first 24 hours

and continued till 72 hours post-burning, the same as Poloxamer-188 effects.

Our study had several limitations. The most significant is the fact that the study was limited to the first 72 hours after burn injury. Therefore, we cannot comment on the ultimate effects on time to reepithelialization and scar formation. Second, the study was limited to rats and may not generalize to humans or other animals. Third, Masson trichrome staining is not suitable to differentiate normal and denaturated collagen fibers and better methods need for evaluation. Forth, the study is limited to contact burns from a heated brass comb and may not be representative of other forms of burn injury. However,

previous studies and wide clinical application of Carbomer 940 suggest absence of cytotoxicity, more experiments of skin cell viability in contact of Carbomer 940 is necessary.

In summary our results demonstrate the positive effects of Carbomer 940 on saving the zone of stasis in burn wounds. Our results also suggest that application of Carbomer 940 may reduce the progression of burn wound. Future studies should evaluate the long-term effect of Carbomer 940 on burn wound healing and possible mechanism of the hydrogel in preventing the conversion of zone of stasis to necrosis.

Table 1. Microscopic and histological results on ischemic and necrotic zones.

Day 3		Q1	Median	Q3
Necrotic zone	<i>Normal Saline</i>			
	Extent of Inflammation	0.5	0.5	1
	Severity of Inflammation	1	2	2
	Re-epithelialization	0	0	0
	Fibroplasia	0	0	0
	Fibrosis	3	3	3
	Angiogenesis	0.5	1.5	1.5
Ischemic zone	Extent of Inflammation	1	1	1
	Severity of Inflammation	2	2	2
	Re-epithelialization	0	0	0.5
	Fibroplasia	0	0	0
	Fibrosis	3	3	3
	Angiogenesis	0.5	1	1.5
Necrotic zone	<i>Carbomer Hydrogel</i>			
	Extent of Inflammation	1	1	1
	Severity of Inflammation	1	2	2
	Re-epithelialization	0	0	0
	Fibroplasia	0	0	0
	Fibrosis	3	3	3
	Angiogenesis	1	1.5	2
Ischemic zone	Extent of Inflammation	0.5	0.5	0.5
	Severity of Inflammation	1	1	1
	Re-epithelialization	1.5	1.5	1.5
	Fibroplasia	0	0.5	0.5
	Fibrosis	3	3	3
	Angiogenesis	1	1.5	1.5

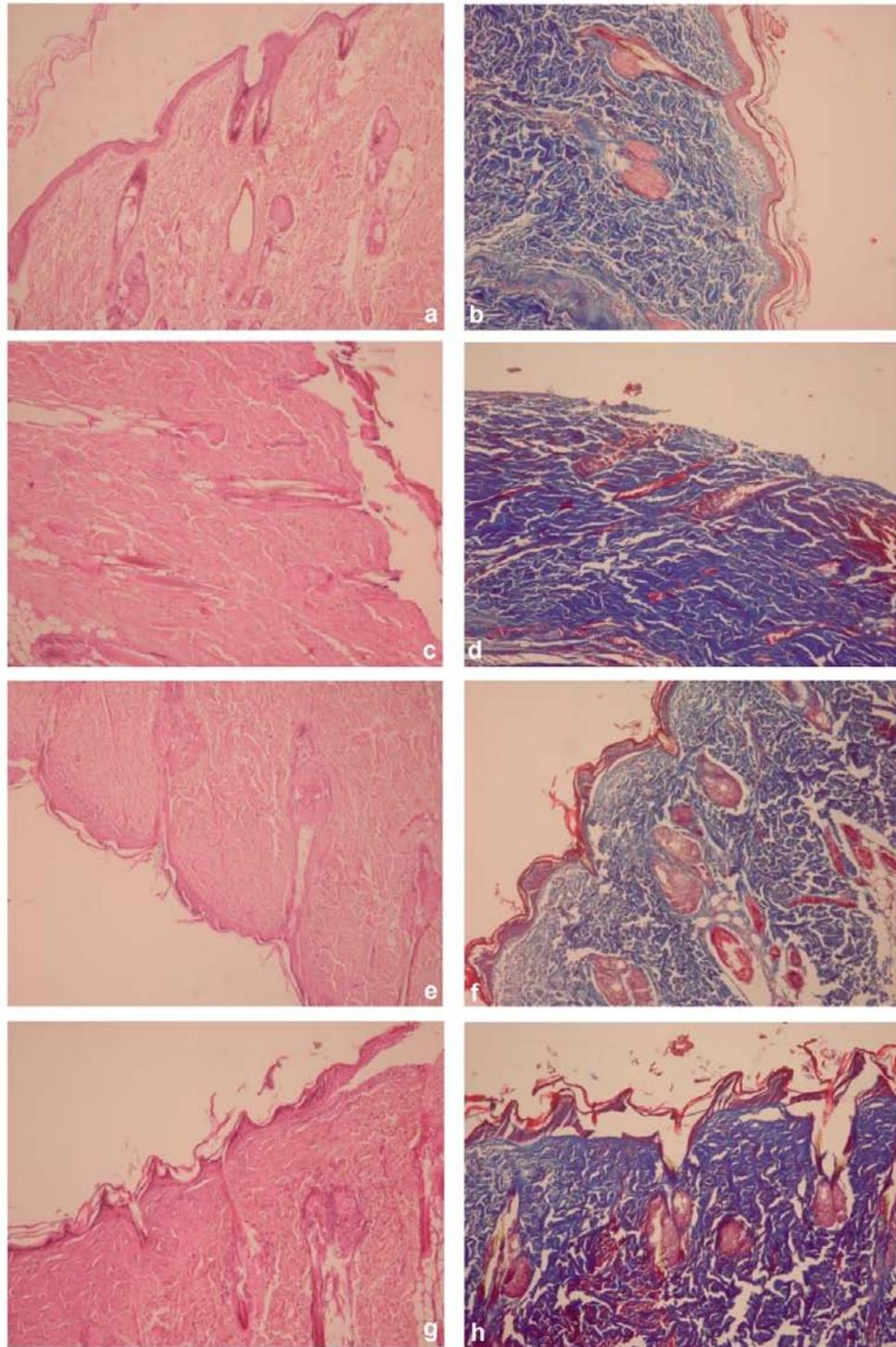


Figure 2. H&E staining (left column) and Masson trichrome staining (right column) of specimens **a,b**) Zone of stasis in Carbomer 940 hydrogel treated rat. Epithelial layer, hair follicles and sebaceous glands remained viable. **c,d**) Necrotic zones in experimental group, denaturation of collagen fibers visible. **e,f**) Necrosis of epithelium, hair follicles and sebaceous glands in zone of ischemia in control group. **g,h**) Zone of necrosis in control group

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چکیده

تسهیل جریان خون موبرگی و افزایش بقا بافتی در زخم سوختگی پوست توسط هیدروژل کربومر ۹۴۰

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هدف- ارزیابی کاربرد هیدروژل کربومر ۹۴۰ در حفظ ناحیه سکون در زخم سوختگی پوست.

طرح مطالعه- مطالعه تجربی در حیوانات زنده

حیوانات- ۲۰ سر رت

روش کار- بعد از تراشیدن مو در قسمت پشتی حیوان، با استفاده از مدل سوختگی شانهای که شامل چهار ردیف مستطیل شکل با ابعاد (۱۰×۲۰ میلیمتر) و سه فضای بینابینی با ابعاد (۵×۲۰ میلیمتر) میباشد، دو ردیف سوختگی به فاصله ۵/۰ سانتیمتر از ستون فقرات و به طور قرینه ایجاد گردید. تمامی رتها به صورت تصادفی به دو گروه آزمایش و کنترل تقسیم شدند. بعد از القا سوختگی، زخمهای گروه آزمایش توسط هیدروژل کربومر ۹۴۰ و زخمهای گروه کنترل توسط سالیین ۰/۹٪ به صورت موضعی درمان شدند. پانسمان و درمان توسط ترکیبات ذکر شده به صورت روزانه تعویض شد. جریان خون نواحی مختلف سوختگی توسط لیزر داپلر و به صورت روزانه اندازه گیری شد. در روز سوم مطالعه مقاطع هیستوپاتولوژیک تهیه گردید.

نتایج- نتایج حاصل از اندازهگیری توسط لیزر داپلر نشان میدهد جریان خون ۲۴، ۴۸، ۷۲ ساعت بعد از القا سوختگی در نواحی سکون در رتهای گروه آزمایش در مقایسه با گروه کنترل بهطور معنیداری افزایش یافته است. همچنین نتایج حاصل از مطالعه هیستوپاتولوژیک نشان میدهد بافت اپیتلیال در نواحی سکون در گروه آزمایش به طور معنیداری بیشتر از گروه کنترل میباشد، همچنین غدد سباسه و فولیکولهای مو در نواحی سکون در رتهای گروه آزمایش زنده باقی ماندند.

نتیجهگیری و کاربرد بالینی - مطالعه تجربی ما نشان میدهد که هیدروژل کربومر ۹۴۰ باعث بهبود جریان خون بافتی و کاهش مساحت نکروز در نواحی سکون میگردد. استفاده از این هیدروژل در درمان آسیب های سوختگی تاثیر مثبت داشته و باعث التیام بهتر زخمهای سوختگی در پوست رت میشود.

کلمات کلیدی: کربومر ۹۴۰، هیدروژل، رت، ناحیه سکون، التیام زخم سوختگی.