Acta Scientific MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 9 Issue 7 July 2025

Research Article

Experimental Evaluation of the Effect of Low Molecular Weight Chitosan on the Aseptic Wound Process

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DOI: 10.31080/ASMS.2025.09.2107

Received: April 28, 2025

Published: June 10, 2025

Abstract

The work provides a comparative experimental evaluation of the effectiveness of low-molecular-weight chitosan in an aseptic wound process. The study was conducted on an *in vivo* model simulating the acute phase of the aseptic wound process (1st day post-injury) and the phase of regenerative-plastic processes (7th day post-injury). Both models were performed on mice and simulate aseptic wounds in plastic surgery, injection cosmetology, and contour plastic surgery. The effectiveness of low-molecular-weight chitosan on the acute phase of the wound process and the regenerative-plastic processes of healing is demonstrated in comparison with Traumeel Cosmo Gel, which contains a composition of medicinal plant extracts and is used in professional cosmetology during the healing and recovery period of the skin after aesthetic procedures (facial cleansing, mesotherapy, bio revitalization, injection procedures). The obtained results showed that low molecular weight chitosan possesses not only high wound healing activity but also powerful anti-inflammatory and anti-edema effects. At the same time, its influence on the aseptic wound process is accompanied by the full regeneration of all skin structures without excessive fibrillogenesis. When using Traumeel Cosmo Gel, on the contrary, there is a combination of active collagen synthesis against the background of an inflammatory reaction, which is the pathophysiological basis for the formation of scar complications.

Keywords: Low Molecular Weight Chitosan; Wound Process; Skin Regeneration; Plastic Surgery

Introduction

Currently, the pharmaceutical market offers a vast array of various wound healing agents, ranging from synthetic compounds, such as Dex panthenol, to various biologically active substances of plant and animal origin. All these agents are positioned as means to reduce wound healing times, which additionally possess anti-inflammatory and antimicrobial properties. However, this approach does not correspond to the pathophysiological mechanisms of the wound process, especially when it comes to "clean" wounds without the development of purulent-inflammatory complications. In purulent-inflammatory processes, the mechanism of wound healing is closely related to the suppression of pathogenic microflora, which delays reparative-regenerative processes in the wound. After suppressing such microflora, wound healing occurs by secondary intention. In "clean" wounds, for example, after

surgical interventions, healing occurs by primary intention. It is this type of wound healing that is crucial in injection cosmetology, contour plastic surgery, and plastic surgery. The dominant arsenal of existing wound healing agents is aimed at treating infected wounds, while there are very few agents for treating aseptic wounds resulting from surgical interventions, especially in injection cosmetology, contour plastic surgery, and plastic surgery. This is because, in the case of superficial skin damage under aseptic conditions, the main criteria for biological activity are the morphological aspects of healing. In particular, the stimulation of wound surface contraction in such wounds may be associated with excessive collagen synthesis due to fibroblast activation, which leads to the formation of scar tissue and inadequate regeneration. Moreover, in aseptic injuries, such as in injection cosmetology, contour plastic surgery, and plastic surgery, the anti-inflammatory action, which reduces tissue swelling and pain response, is no less significant than the stimulation of regenerative-plastic processes. Existing homeopathic and herbal remedies, such as Traumeel Cosmo Gel, although widely used in injection cosmetology, contour plastic surgery, and plastic surgery, do not have a definitive evidence base confirming their positive effect on the key pathophysiological mechanisms of the wound process in aseptic wounds [1-7]. Among the promising wound healing agents that theoretically can affect all key pathophysiological links of the wound process are chitosan and its derivatives; however, they also lack objective morphological data proving their positive impact on regenerative-plastic processes. Currently, chitosan derivatives are primarily used as wound healing agents in traumatology, gynaecology, general surgery, and in the production of dressings [8-15]. Their effectiveness is evident; however, there are currently no comparative studies with agents used in injection cosmetology, contour plastic surgery, and plastic surgery.

The Aim of the Study

A comparative morphological assessment of the effect of a hydrophilic gel with low molecular weight chitosan and Traumeel Cosmo Gel on the aseptic wound process in the acute phase of inflammation (1st day post-injury) and the regenerative-plastic processes of healing (7th day post-injury).

Materials and Methods

Chitosan with an average molecular weight of 20 kDa was used in the work, which was obtained by acid hydrolysis from high-molecular crab chitosan. The degree of chitosan deacetylation was 95%. The average molecular weight of chitosan was determined by HPLC. Shimadzu LC-20 chromatographic complex (Japan), sample volume of 2% low molecular weight chitosan solution in eluent of 20 µl, BioSep-SEC-S 3000 Phenomenex penetrating chromatography column (USA) 75 x 7.80 mm, eluent 0.1 M acetate buffer with pH 5.5, eluent feed rate 1 ml/min, pressure 7.1 MPa, spectrophotometric detector SPD-M20A, detection wavelength 220 nm. The tested low molecular weight chitosan gel contained 0.1% low molecular weight chitosan and 2% hydroxypropyl cellulose as a gelling component dissolved in deionized water.

I Series of experiments. Comparative assessment of the impact of tested agents on regenerative-plastic processes (7th day post-injury).

The experiment was conducted on 20 male ICR line mice. At the beginning of the experiment, the mice were divided into two groups: the experimental group (E) and the control group (C), with 10 individuals in each group. The mice in the lumbar-sacral region had their fur shaved and a full-thickness skin section with subcutaneous tissue was excised, with an area of no more than 50 mm^2. Immediately after the injury was inflicted, the wound area of each animal was measured using Corel Draw 13, and the test substances were applied: in the experimental group, a hydrogel with low molecular weight chitosan, and in the control group, Traumeel Cosmo Gel. Then the test substances were applied daily for 7 days, measuring the wound area on days 2 and 7. After measuring the wound area on the 7th day, all animals were euthanized with an overdose of ether anaesthesia, the skin in the wound area was excised, and the samples were fixed for subsequent morphological assessment.

II Series of Experiments. Comparative Assessment of the Impact of Tested Agents on the Aseptic Wound Process in the Acute Phase of Inflammation (1st Day Post-Injury).

The experiment was conducted on 10 male ICR strain mice. At the beginning of the experiment, the mice were divided into two groups: the experimental group (E) and the control group (C), with 5 individuals in each group. The mice in the lumbar-sacral area had their fur shaved and a linear incision was made in the skin with subcutaneous tissue, 1 cm in length, and fascia separation around the wound. Immediately after the injury and after 6 hours, the test substances were applied; in the experimental group, a hydrogel with low molecular weight chitosan, and in the control group,

Traumeel Cosmo Gel. After 24 hours, all animals were euthanized with an overdose of ether anaesthesia, the skin in the wound area was excised, and the samples were fixed in 10% formalin for subsequent morphological assessment.

Statistical processing of the results was carried out using the Statistica 12.0 statistical software package (StatSoft, USA). The results are presented as the average value of the indicator and its standard error (M±SE).

Research Results

The results of the study are presented in Tables 1-3 and Figures 1-3.

Time after injury	Experimental		Control	
	Average Wound Area, мм², (M ± SE)	Wound Area, %, (M ± SE)	Average Wound Area, мм², (M ± SE)	Wound Area, %, (M ± SE)
1 day	27,5458 ± 7,7	100	43,84719 ± 11,9	100
3 day	11,52599 ± 3,4	44,2 ± 19	16,38431 ± 3,6	38,8 ± 9,3
7 day	5,12977 ± 1,5	19,2 ± 5,8	5,28551 ± 1,7	13,11 ± 7,9

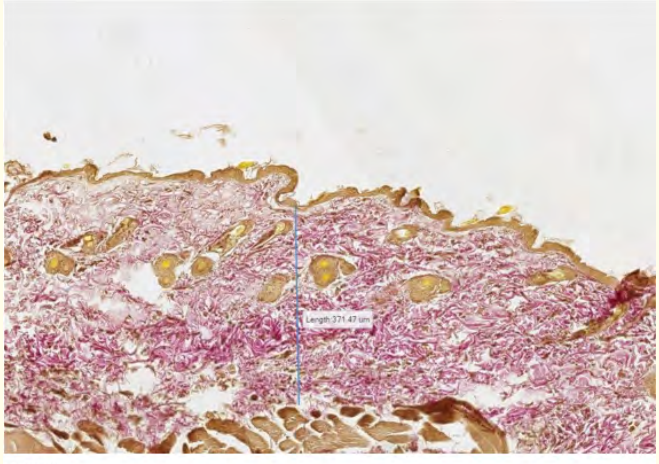
Table 1: Evaluation of the Effect of Low-Molecular-Weight Chitosan Gel (Experimental) and Traumeel Cosmo Gel (Control) on Wound Area Dynamics.

Indicator, (M ± SE)	Control	Experimental
Epidermal thickness at the wound edges, μm	76,82 ± 5,13	120,91 ± 5,41
Fibroblasts, numerical density (per 100 μm²)	24,20 ± 1,36	13,80 ± 1,08
Reticulin fibers, Vv	28,30 ± 2,09	17,40 ± 1,54
Neutrophils, numerical density (per 100 μm²)	46,10 ± 3,48	0
Macrophages, numerical density (per 100 μm²)	27,50 ± 2,05	40,70 ± 3,88
Type I collagen, Vv	58,00 ± 3,87	25,80 ± 2,16
Volume of hair follicles at the wound periphery	235,00 ± 12,95	293,82 ± 25,61

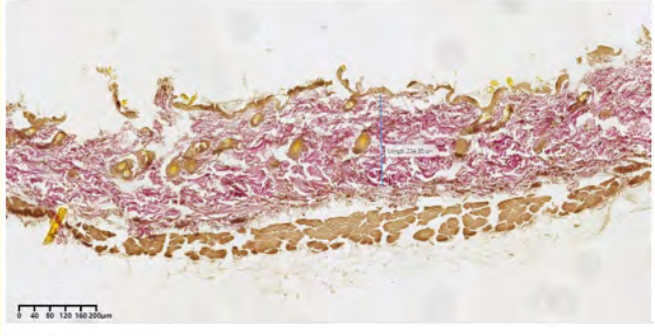
Table 2: Comparative Evaluation of the Effect of Low-Molecular-Weight Chitosan Gel (Experimental) and Traumeel Cosmo Gel (Control) on Acute Phase Indicators of Wound Healing.

Indicator, (M±SE)	Control	Experimental
Spongiosis, Vv	32,92 ± 1,50	22,27 ± 1,30
Leukocytic infiltration, Vv	72,00 ± 3,93	50,07 ± 3,32
Erythrocyte extravasation, Vv	37,33 ± 2,00	22,73 ± 2,55

Table 3: Comparative Assessment of the Effect of Low Molecular Weight Chitosan Gel (Experimental Group) and Traumeel Cosmo Gel (Control Group) on Reparative-Regenerative Processes in Aseptic Injury.

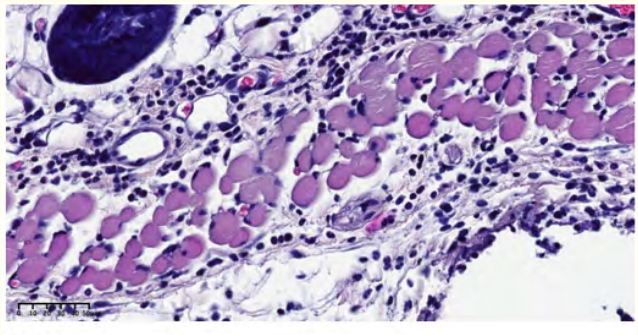


Control

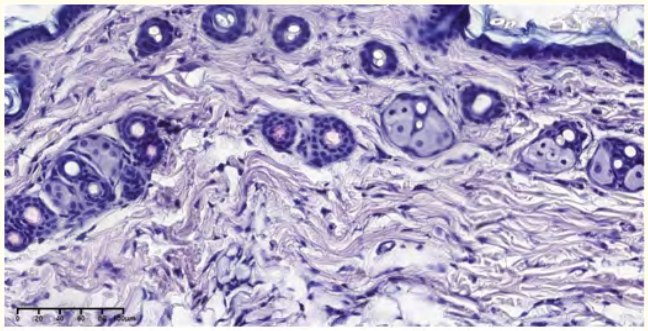


Experience

Figure 1: Painting according to Van Gieson. 7 days after injury.

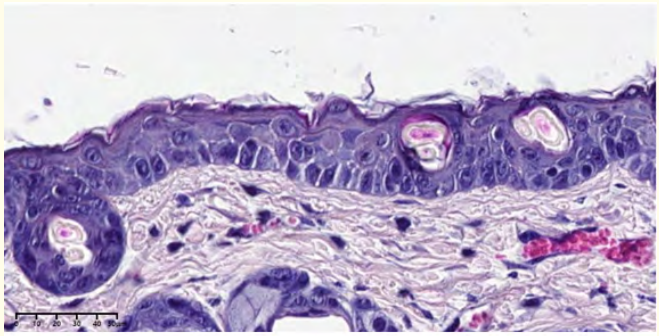


Control

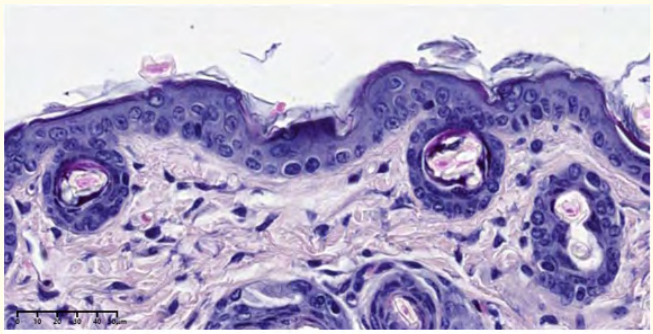


Experience

Figure 2: Coloration: hematoxylin, eosin. 7 days after injury.



Control



Experience

Figure 3: Coloration: hematoxylin, eosin. 24 hours after injury.

The volumetric density of the edema zone between keratinocytes (spongiosis) was higher in the control group, and leukocyte infiltration and erythrocyte extravasation were higher in the control group.

In the skin samples from the control group, compared to the experimental group, the thickness of the epidermis at the wound edges was significantly reduced. In the control group skin, the granular layer was either completely absent or poorly developed. The stratum corneum was either absent or contained areas of parakeratosis.

In the wound area of the control group, compared to the experimental group, there was a significant increase in the number of fibroblasts, indicating active inflammation and resulting in the production of a large amount of disorganized collagen.

In the control group skin, compared to the experimental group, a large amount of granulation tissue was observed in the wound area, with fibers being short, fragmented, and arranged chaotically. Thickened, hyalinized collagen bundles were also noted. Some animals showed signs of forming chronic wounds.

In the experimental group, the overall volume of granulation tissue was smaller, the fibers were arranged in an orderly, parallel manner, and their thickness was uniform.

In the wound area of the control group, compared to the experimental group, a large number of neutrophils were found, indicating an acute inflammatory process. Tissue debris was also detected on the wound surface. In the experimental group, all animals exhibited wound epithelialization, and neutrophils were absent.

In the wound area of the control group, compared to the experimental group, a large number of chaotically arranged collagen fibers were observed, which may subsequently lead to the formation of a keloid scar.

At the periphery of the wound in the experimental group, the hair follicles showed a larger volume of the cambial (growth) zone.

Discussion

Thus, comparative morphological studies have shown that the gel with low molecular weight chitosan exhibits pronounced anti-inflammatory action in the acute phase of the wound process and surpasses Traumeel Cosmo Gel in anti-inflammatory action. The anti-inflammatory action of low molecular weight chitosan is mediated through the stabilization of vascular permeability in

skin injury, reduction of leukocyte infiltration, and reduction of interstitial edema. All these parameters unequivocally indicate the promise of gel forms of low molecular weight chitosan in injection cosmetology, contour plastic surgery, and plastic surgery for alleviating post-procedural acute inflammatory reactions and effectively mitigating pain and edema components. It was also established that the gel with low molecular weight chitosan has an effect on the dynamics of changes in the area of an aseptic wound that is almost equal to that of Traumeel Cosmo Gel. However, the use of Traumeel Cosmo Gel, according to morphometric assessment results, is associated with an inadequate process of reparative regeneration, as excessive stimulation of fibrillogenesis, disorganization of granulation tissue, and neutrophilic infiltration indicate the activation of the inflammatory process and fibrous dysplasia of connective tissue, which ultimately creates conditions for scar complications despite the apparent reduction in wound area. When using the gel with low molecular weight chitosan, a full process of reparative regeneration is observed in the skin wound with the involvement of tissue macrophages, and the absence of neutrophil infiltration indicates complete suppression of the inflammatory process. The number of fibroblasts, collagen, and reticular fibers is synchronized with the activation of the wound healing process and effectively eliminates the risk of developing fibrous scar complications after skin injury. An additional factor confirming this is the greater thickness of the epidermis and cambial zone of hair follicles at the edges of the wound when using low molecular weight chitosan gel compared to Traumeel Cosmo Gel.

Conclusion

Overall, the obtained data allow us to evaluate the gel with low molecular weight chitosan as a highly promising agent for the treatment of aseptic wounds both in the acute phase and during regenerative-plastic healing processes. This effect is particularly valuable in injection cosmetology, contour plastic surgery, and plastic surgery for alleviating post-procedural edema, relieving pain, mitigating acute inflammation, and preventing scar complications due to the activation of physiological mechanisms of reparative regeneration, which are triggered by the activation of tissue macrophages.

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