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Optimizing Skin Wound Healing Outcomes: A Comparative Analysis of Cytokine Response and Tissue Remodeling at treatment with Poysilicone-siloxane, Silicone, Betamethasone and Heparin-based preparations

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Abstract

Skin wound healing is a complex, dynamic process crucial for preserving bodily integrity, encompassing stages of hemostasis, inflammation, proliferation, and remodeling, involving cytokines like IL-1 and IL-6, fibroblasts, collagen, and elastic fibers. This study evaluates the effects of various treatments (poysilicone-siloxane based (PS), silicone (Sil), betamethasone (BMt) and heparin-based (Hep) preparations) on cytokine levels and histological markers during wound healing in laboratory rats with full-thickness skin wounds. PS, Hep and Sil creams were applied 2-3 times daily, and BMt was administered subcutaneously weekly. The changes in scar appearance during healing and treatment were assessed using the Vancouver Scar Scale (VSS). ELISA was used to assess IL-1 and IL-6 levels, while histological markers were evaluated via immunohistochemistry, Masson's trichrome, Van Gieson, Weigert's, and H&E staining. Results demonstrated that IL-1 levels significantly increased by day 7 in all groups, but only normalized by day 21 in PS and Sil-treated animals, while BMt-treated animals required until day 28. Control and Hep-treated groups showed persistent IL-1 elevation beyond day 28. Similarly, IL-6 levels rose on day 14, normalizing by day 28 in PS, Sil and BMt-treated groups, yet remaining elevated in Hep-treated and control groups. Histological analysis revealed that vascularization normalized by day 14 in PS-treated animals, by day 21 in Sil-treated animals, and persisted until day 28 in BMt and Hep-treated groups. Collagen deposition and elastic fiber normalization occurred earliest in PS-treated animals, followed by Sil and BMt, while Hep-treated animals exhibited delayed collagen deposition only by day 28. PS was the most effective treatment for promoting optimal scar characteristics, including reduced vascularity, proper pigmentation, pliability, and scar flattening. Sil and BMt groups showed moderate improvements in wound healing and scar characteristics, while Hep was less effective in accelerating wound healing and scar normalization. Conclusion: The findings highlight IL-1 and IL-6 as pivotal inflammatory mediators in wound healing and suggest that PS, Sil, and BMt effectively modulate cytokine responses and promote vascularization, collagen, and elastic fiber normalization. PS group exhibited the most rapid wound healing response, followed by Sil and BMt, while Hep had limited efficacy. These results underscore the importance of targeted therapeutic interventions in wound management and offer potential for optimizing treatment strategies for improved healing outcomes. Further research is warranted to elucidate precise mechanisms and to explore additional biomarkers

associated with tissue repair and inflammation resolution.

Key words: Skin wounds, IL-1, IL-6, Poysilicone-siloxane, Silicone, Betamethasone, Heparin.

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Introduction.

The skin, as the body's largest organ, serves as a crucial barrier protecting against pathogens, UV radiation, and mechanical injuries, while regulating water, electrolytes, and temperature [1-3].

Various factors may result in different types of skin injuries. Skin wounds, ranging from minor cuts to severe injuries, are common occurrences in everyday life. The healing process could be influenced by numerous factors, including age, nutrition, infection, and chronic conditions such as diabetes or vascular disease. The wound healing is vital to restore integrity and prevent complications [4]. Skin wound healing is a complex and intricate biological process essential for the restoration of tissue integrity and functionality. The ability of the skin to efficiently heal wounds is critical for maintaining homeostasis and protecting the body from infections and other external threats. Therefore, understanding the mechanisms underlying this process is of paramount importance, allowing for the development of effective therapeutic strategies to enhance wound healing and the improvement of patient outcomes [38].

This intricate process involves four stages: hemostasis, inflammation, proliferation, and remodeling. Hemostasis initiates clot formation to stop bleeding, inflammation recruits immune cells like neutrophils and macrophages, proliferation drives tissue rebuilding with collagen synthesis and angiogenesis, and remodeling strengthens the tissue through collagen maturation [5-9].

Cytokines like interleukin-1 (IL-1) and interleukin-6 (IL-6) play central roles in this process. IL-1, produced by immune and skin cells, enhances inflammation, immune cell recruitment, and keratinocyte proliferation, aiding re-epithelialization. IL-6, also involved in early inflammation, recruits immune cells and promotes the release of acute-phase proteins, which systemically respond to injury. Fibroblasts are essential, migrating to the wound site to synthesize collagen and ECM components critical for structural integrity and tissue elasticity. Collagen provides tensile strength, while elastic fibers restore flexibility. However, dysregulation in these stages can result in chronic wounds or excessive scarring, such as hypertrophic scars or keloids, impacting function and aesthetics [10-12].

Effective wound care, essential for successful healing, includes debridement, infection control, maintaining a moist environment, and supporting tissue regeneration through appropriate dressing types [13-15]. Advanced therapies like negative pressure wound therapy, hyperbaric oxygen therapy, and bioengineered skin substitutes are used for complex wounds [16-22]. Despite these options, facial wounds present additional challenges due to the potential for hypertrophic scarring and psychological effects, underscoring the need for effective interventions.

This study aims to evaluate the effects of various treatments (PS, Sil, BMt and Hep-based preparations) on immune parameters (IL-1 and IL-6) and the skin wound healing process to optimize outcomes, reduce scarring, and enhance patient quality of life.

Material and methods.

Experiments were carried out on male white lab. Rats with the body weight range 200-250 g. The animals were purchased from the vivarium of Aleksandre Natishvili *Institute of Morphology, Tbilisi, Georgia (*https://www.tsu.ge/en*).*

All animals were allowed to become acclimatized to laboratory conditions for one week before the experiment. During this period, the animals were kept under constant environmental conditions with a light-dark cycle of 12/12 at a temperature of 23±2°C. They were fed a standard laboratory chow and given free access to water.

For modeling of skin wounds the rats were anesthetized with nembutal (50 ml/kg). After shaving and cleaning with 70% alcohol, excisional, full-thickness skin wounds were aseptically made on the dorsal skin. After, a surgical suture of 5 cm was placed on the skin at 1 cm interval.

All animals were placed in the groups. Each group involved 10 rats. The group I – Healthy, intact rats; The group II control, untreated rats; The group III - rats treated with PS; The group IV - rats treated with Sil; The group V - rats treated with BMt; The group VI - rats treated with Hep;

PS (polysilicones, cyclic and polymeric siloxane + **Vitamin C Ester**), Sil (**Silicon, Hydrochloridum acid (**HOCL) **+ Na Hydrochlori** (NaOCL) and Hep (onion extract, heparin, allantoin) creams were applied to the wound surface as a thin layer 2-3 times a day for 4 weeks in the corresponding group animals. BMt (betamethasone 0.2 ml) was injected subcutaneously in the wound area once a week during 4 weeks.

Pro-inflammatory cytokines (IL-1, IL-6) were studied by ELISA. Blood samples for immunological investigations were collected and studied by the 7^{th}, 14^{th}, 21^{st} and 28^{th} days of experiment.

For assessment of the changes in scar appearance during healing and treatment the Vancouver Scar Scale (VSS) was used. It is one of the most frequently used outcome measures for scar assessment in both, clinical practice and research [23]. The scar characteristics included: vascularity (range 0-3), pigmentation (range 0-2), pliability (range 0-5), and height (mm, range 0-3). Each characteristic is given a score, which are added together to give an overall score between 0 and 13 [24]. The impacts of scarring can be multifaceted, including movement and function limitations, long term pain or psycho-social effects, and therefore use of an outcome measure is beneficial to monitor the scars' progress [25].

Histological assessments of healing stages.

To evaluate vascularization, collagen deposition, myofibroblast activity, and elastic fiber status at different stages of healing, the following techniques were employed: immunohistochemistry, Masson's trichrome staining, Van Gieson's staining, Weigert's staining, and hematoxylin and eosin (H&E) staining.

Immunohistochemistry for fibroblast detection. Tissue samples were collected, fixed in 10% neutral buffered formalin, and embedded in paraffin. Formalin-fixed and paraffin-embedded (FFPE) sections were sliced to a thickness of 4–5 µm. To block nonspecific binding, the sections were treated with a blocking agent, such as 5% bovine serum albumin (BSA). A primary antibody specific to a fibroblast marker was applied and incubated following the manufacturer's protocol (e.g., 1–2 hours at room temperature or overnight at 4°C). After incubation, the slides were washed with phosphate-buffered saline (PBS) to remove unbound antibody. A secondary antibody conjugated to a detectable marker was applied and incubated as per the protocol (e.g., 30–60 minutes at room temperature). Following a PBS wash, the sections were counterstained with hematoxylin to visualize nuclei and examined under a microscope.

Masson's Trichrome staining for collagen assessment. FFPE sections were deparaffinized by immersion in xylene (2–3 changes, 5 minutes each) and rehydrated through graded alcohols (100%, 95%, 70%) to distilled water (2–3 minutes per step). Slides were fixed in Bouin's solution at 56°C for 1 hour or at room temperature overnight, then rinsed under running tap water until the yellow color was mostly removed (5–10 minutes). Nuclei were stained with Weigert's iron hematoxylin for 10 minutes, followed by rinsing in running tap water for 10 minutes. Cytoplasm was stained with Biebrich scarlet-acid fuchsin solution for 5–10 minutes, then briefly rinsed in distilled water. Sections were differentiated in phosphomolybdic-phosphotungstic acid for 10–15 minutes. Collagen fibers were stained with aniline blue for 5–10 minutes, followed by a rinse in 1% acetic acid for 2–5 minutes. Slides were dehydrated through graded alcohols (95% and 100%), cleared in xylene (two changes, 5 minutes each), and mounted using a resinous medium with coverslips.

Van Gieson staining for collagen and muscle fiber differentiation. FFPE sections were deparaffinized (xylene, 2–3 changes, 5 minutes each) and rehydrated through graded alcohols to distilled water. Nuclei were stained with Weigert's iron hematoxylin for 5–10 minutes, followed by rinsing under running tap water for 10 minutes. Slides were immersed in Van Gieson solution (1% acid fuchsin mixed with saturated aqueous picric acid, typically 1 ml of acid fuchsin to 100 ml of picric acid solution) for 5 minutes. Excess stain was removed with a brief rinse in distilled water. Slides were dehydrated through graded alcohols (95% and 100%), cleared in xylene (two changes, 5 minutes each), and mounted using a resinous medium.

Weigert's Resorcin-Fuchsin staining for elastic fibers. FFPE sections were deparaffinized in xylene (2–3 changes, 5 minutes each) and rehydrated through graded alcohols to distilled water.

Slides were immersed in Weigert's Resorcin-Fuchsin solution for 10–30 minutes. Excess stain was removed by rinsing the slides in distilled water. Sections were differentiated in 70% ethanol containing a few drops of ferric chloride until elastic fibers became distinctly visible. For contrast, sections were lightly counterstained with hematoxylin and briefly rinsed in distilled water. Slides were dehydrated through graded alcohols (95% and 100%), cleared in xylene (two changes, 5 minutes each), and mounted using a resinous medium.

Hematoxylin and Eosin (H&E) staining. FFPE sections were deparaffinized and rehydrated through graded alcohols to distilled water. Nuclei were stained with hematoxylin, followed by rinsing in water. Cytoplasm and other cellular components were stained with eosin. Slides were dehydrated, cleared in xylene, and mounted for microscopic examination.

Results were analyzed statistically. Statistical significance was evaluated by using ANOVA or Mann-Whitney's U test. $p<0.05$ was accepted as statistically significant.

Results and discussion.

The study investigated the wound healing effects of various treatments (PS, Sil, BMt, and Hep) compared to a control group on interleukin levels (IL-1 and IL-6), vascularization, myofibroblast and inflammatory cell presence, collagen deposition, and elastic fiber normalization across different days (7th, 14th, 21st, and 28th) of the wound healing process.

Interleukin-1 (IL-1) Dynamics: On the 7th day, IL-1 levels were significantly elevated across all groups. Increases were observed in the control (74%), Sil (107%), BMt (88%), and Hep (75%) and PS (101%) groups ($p<0.001$). By the 14th day, IL-1 levels remained elevated in all groups, with slight reductions observed in BMt, and Hepgroups. In PS and Sil-treated animals IL-1 was decreased by15% and 16% compared to control ($p<0.05$).

On the 21st day, IL-1 had normalized in PS and Sil groups, with notable reductions of 32% and 35% ($p<0.001$) compared to control. Meanwhile, BMt showed a modest decrease of 11% ($p<0.05$), and Hep-treated animals had no significant change. By the 28th day, IL-1 returned to baseline in Sil, PS, and BMt groups. In the Hep group, IL-1 was still elevated but had shown slight non-significant decreases.

Interleukin-6 (IL-6) Dynamics: IL-6 levels showed delayed changes compared to IL-1. On the 14th day, IL-6 was slightly increased in control (12%; $p<0.01$), Sil (30%; $p<0.001$), BMt (12%; $p<0.01$), and Hep (11%; $p<0.05$) groups. In Sil and PS-treated animals, IL-6 increased by 17% and 25% ($p<0.02$) on this day. By the 21st day, IL-6 began to normalize, especially in Sil, PS, and BMt-treated groups, showing decreases of 12% and 15% ($p<0.05$) in Sil and PS groups.

By the 28th day, IL-6 levels had fully normalized in PS and Sil-treated animals, while in the control and Hep groups, IL-6 levels remained elevated, suggesting delayed resolution of inflammation.

Histological Observations: Vascularization at the wound site was significantly increased on the 7th day across all groups. Control group animals exhibited elevated vascularization up to the 28th day. PS-treated animals normalized vascularization by the 14th day, Sil-treated animals by the 21st day, while BMt and Hep groups retained high vascularization up to the 28th day.

Myofibroblast and inflammatory cell presence was high on the 7th day in control and PS-treated animals. Control and Sil-treated animals maintained moderate levels of these cells throughout the healing process, while PS-treated animals normalized by the 21st day. By contrast, only moderate levels were observed in other groups.

Collagen and Elastic Fiber Formation: Initially, collagen fibers were scant across all groups. By the 14th day, moderate collagen presence was observed in PS-treated animals, maintained throughout the healing process. Sil and BMt-treated animals showed normal collagen levels by the 21st day, while Hep-treated animals displayed abundant collagen only by the 28th day. Elastic fibers were moderate across groups initially, normalizing by the 21st day in PS-treated animals and by the 28th day in Sil-treated animals.

Tab. N1 Blood vessels, Myofibroblasts, Inflammatory cells, Collagen and Elastic fibers during skin wound healing process at different methods of treatment.

Staining	H&E			Masson	Van Gieson	Weigert
Normal skin	Blood vessels	Myofibroblasts	Inflammatory cells	Collagen fibers	Collagen fibers	Elastic fibers
	Scanty/single	Scanty/single	Scanty/single	Moderate	Moderate	Scanty/single

Staining		H&E			Masson	Van Gieson	Weigert
Control		Blood vessels	Myofibroblasts	Inflammatory cells	Collagen fibers	Collagen fibers	Elastic fibers
Day of wound healing	7th	Abundant	Abundant	Abundant in epi-dermis, Dermis and subepidermal fat	Scanty/single	Scanty/single	Scanty/single
	14th	Abundant	Abundant	Moderate number of cr. inflammatory cells	Scanty/single	Scanty/single	Moderate number
	21st	Abundant	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number
	28th	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number

Staining		H&E			Masson	Van Gieson	Weigert
Sil		Blood vessels	Myofibroblasts	Inflammatory cells	Collagen fibers	Collagen fibers	Elastic fibers
Day of wound healing	7th	Abundant	Moderate number	Moderate number in epidermis and dermis	Single	Single	Single
	14th	Moderate number	Abundant	Moderate number	Scanty	Scanty	Moderate number
	21st	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number
	28th	Few amount	Moderate number	Moderate number	Moderate number	Moderate number	Single

Staining		H&E			Masson	Van Gieson	Weigert
PS		Blood vessels	Myofibroblasts	Inflammatory cells	Collagen fibers	Collagen fibers	Elastic fibers
Day of wound healing	7th	Moderate number	Abundant	Abundant	Scanty	Scanty	Scanty
	14th	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number
	21st	Scanty	Scanty	Single	Moderate number	Moderate number	Scanty
	28th	Single	Single	Single	Moderate number	Moderate number	Single

Staining		H&E			Masson	Van Gieson	Weigert
BMt		Blood vessels	Myofibroblasts	Inflammatory cells	Collagen fibers	Collagen fibers	Elastic fibers
Day of wound healing	7th	Moderate number	Single	Single	Single	Single	single
	14th	Abundant	Scanty	Moderate number	Single	Single	Single
	21st	Moderate number	Moderate number	Moderate number	Moderate number	Scanty	Moderate number
	28th	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number

Staining		H&E			Masson	Van Gieson	Weigert
Hep		Blood vessels	Myofibroblasts	Inflammatory cells	Collagen fibers	Collagen fibers	Elastic fibers
Day of wound healing	7th	Moderate number	Single	Single	-	-	-
	14th	Abundant	Scanty	Abundant	Single	Single	Single
	21st	Abundant	Moderate number	Moderate number	Moderate number	Moderate number	Moderate number
	28th	Moderate number	Abundant	Moderate number	Abundant	Abundant	Moderate number

The scar appearances for the different treatment groups can be characterized based on the Vancouver Scar Scale (VSS) data, which includes four criteria: vascularity, pigmentation, pliability, and height [24].

Here's an analysis of the scar characteristics and wound healing process at different time points (7th, 14th, 21st, and 28th days) for each treatment group:

1. Control Group

- **Vascularity:** Remained elevated throughout the healing process (2-3 on day 7 to 21, then decreased to 2 by day 28), indicating a persistent inflammatory response and incomplete resolution of inflammation.
- **Pigmentation:** Started with mild hypopigmentation (1), but remained at 1 or 2 during the 14th to 28th days, suggesting some irregular pigmentation development, with the possibility of hyperpigmentation.
- **Pliability:** Pliability worsened over time, ranging from firm (3) to contracture (5), indicating the formation of a more rigid and potentially hypertrophic scar.
- **Height:** The scar height started at 2 and remained in the moderate range (2-3), suggesting a raised scar that did not flatten adequately by day 28.

2. PS-treated Group

- **Vascularity:** Showed the best improvement, with vascularity decreasing from 2 (on day 7) to 0 (by day 28), indicating the most efficient resolution of inflammation and the least vascularized scar.

- **Pigmentation:** Pigmentation returned to normal early, with pigmentation levels of 0-1 across all time points, indicating proper and even skin color restoration.
- **Pliability:** Pliability remained good throughout, with the scar being supple (1) by day 14 and beyond, indicating healthy tissue without excessive stiffness or scarring.
- **Height:** Scar height was normalized by day 14, with a score of 0, indicating a flat scar with no significant elevation.

3. Sil-treated Group

- **Vascularity:** Vascularity decreased from 2 on day 7 to 1 on day 28, indicating a moderate resolution of the inflammatory response, but not as efficient as PS.
- **Pigmentation:** Pigmentation was mostly within the normal range (0-1), with a brief increase to hyperpigmentation (2) on day 14, but it normalized by day 28.
- **Pliability:** The pliability remained moderate, with a score of 3 on day 7, improving slightly by day 21 to 2, and showing mild firmness (1) by day 28. This suggests some stiffness and a more rigid scar compared to PS.
- **Height:** Scar height was somewhat reduced, showing a gradual decrease from 3 to 1, indicating a less raised scar but still not as flat as the PS-treated scars.

4. BMt-treated Group

- **Vascularity:** Vascularity stayed high (2-3) during the early phase (day 7 to day 21) but dropped to 2 by day 28, indicating a moderate resolution of inflammation.
- **Pigmentation:** Similar to Sil, pigmentation was within the normal range for most time points, with a slight increase in pigmentation on day 14 and normalization by day 28.
- **Pliability:** The pliability was firm (3) on day 7, but improved to yielding (2) by day 14, and then remained in the supple (1) range from day 21 to 28.
- **Height:** The scar height gradually improved from 2 to 1 by day 28, indicating partial flattening, but still elevated compared to the PSgroup.

5. Hep-treated Group

- **Vascularity:** Vascularity remained high (2-3) throughout the healing period, suggesting prolonged inflammation and slow normalization of vascularization.
- **Pigmentation:** Pigmentation remained in the normal to mild hypopigmentation range (1) at most time points, indicating some pigment restoration but with possible irregularities.
- **Pliability:** Pliability was firm (3) initially, decreasing to yielding (2) by day 14 and reaching supple (1) by day 28, indicating gradual improvement in scar flexibility.
- **Height:** The scar height decreased gradually from 3 to 1, suggesting some flattening over time, but it remained raised compared to the more effective treatments like PS.

Summary of the skin scar characteristics:

- **Vascularity:** PS showed the best reduction in vascularity (signifying faster inflammation resolution), followed by Sil and BMt with more moderate improvement. The control and Hep groups showed slower resolution of inflammation.
- **Pigmentation:** PS and Sil groups showed the best and most stable pigmentation restoration, while the control and Hep groups exhibited more irregular pigmentation.

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- **Pliability:** PS had the most supple and flexible scar, with Sil and BMt showing moderate firmness, while the control and Hep groups exhibited more stiffness.
- **Height:** PS led to a flat scar by day 14, with Sil and BMt showing moderate improvement in height. The control and Hep groups showed slower reductions in scar height.

Tab. N2 Scar appearances during the skin healing process in control and treated rats during the whole period of wound healing. (The Vancouver Scar Scale).

	Scar appearances	**Vascularity** (range 0-3)				**Pigmentation** (range 0-2)				**Pliability** (range 0-5)				**Height** – mm (range 0-3)			
	The Vancouver Scar Scale	Normal – 0 Pink – 1 Red – 2 Purple – 3				Normal – 0 Hypopigmentation – 1 Hyperpigmentation – 2				Normal – 0 Supple – 1 Yielding – 2 Firm – 3 Ropes – 4 Contracture – 5				Flat – 0 <2 – 1 2–5 – 2 >5 – 3			
	The day of skin wound healing	7th	14th	21st	28th	7th	14th	21st	28th	7th	14th	21st	28th	7th	14th	21st	28th
1	**Healthy**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	**Control**	2	3	2	2	1	1	1	2	3	3	4	3	2	2	2	1
3	**PS**	2	1	0	0	0	1	1	0	2	2	1	0	1	1	1	0
4	**Sil**	2	2	1	1	0	2	1	1	3	2	2	1	2	1	1	1
5	**BMt**	2	2	2	1	1	2	1	1	3	3	2	1	2	1	1	1
6	**Hep**	2	3	2	2	1	2	2	1	3	3	3	2	2	2	1	1

Discussion.

The process of wound healing is a complex sequence involving hemostasis, inflammation, proliferation, and remodeling [26, 27]. This study aimed to evaluate the effects of different treatments— PS, Sil, BMt, and Hep on wound healing, focusing on key parameters such as cytokine dynamics (IL-1 and IL-6), vascularization, fibroblast activity, collagen deposition, elastic fiber restoration and skin wound appearances.

Cytokine dynamics: IL-1 and IL-6 response. Interleukins (IL-1 and IL-6) are critical cytokines that play prominent roles during the inflammatory phase of wound healing. IL-1 is typically elevated in the early stages of inflammation and decreases as the inflammatory response resolves. IL-6, on the other hand, remains elevated during the proliferative phase, where it promotes fibroblast activity and tissue remodeling [28, 29].

In this study, the **IL-1** levels showed a significant increase on the 7th day in all treated groups, which is consistent with the early inflammatory response following injury. The PS and Sil-treated group exhibited a rapid decrease in IL-1 levels, normalizing by the 21st day, while BMt-treated animals showed normalization by day 28. In contrast, Hep-treated animals displayed slower normalization, indicating a prolonged inflammatory response. This suggests that PS, Sil and BMt help resolve inflammation more efficiently than Hep.

Similarly, **IL-6** concentrations were elevated on the 14th day in all treatment groups, with gradual normalization by day 28 in PS, Sil and BMt groups. The control and Hep groups, however, continued to show elevated IL-6 levels, suggesting ongoing inflammation and a potential delay in wound healing. This extended elevation of IL-6 in Hep-treated animals may be linked to its limited efficacy in modulating early inflammatory responses, whereas PS, Sil and BMt treatments appear to accelerate the resolution of both IL-1 and IL-6 levels.

Vascularization is crucial for wound healing as it provides necessary nutrients and oxygen to the site of injury [30, 31].

In control animals, increased vascularization was observed up to the 28th day, indicative of chronic inflammation, which can hinder the transition to the proliferative phase. Both **PS** and **Sil** demonstrated more rapid normalization of vascularization. Specifically, PS-treated animals showed normalization by the 14th day, and Sil-treated animals by day 21, suggesting a faster resolution of inflammation. In contrast, BMt and Hep-treated animals maintained increased vascularization, indicating delayed inflammation resolution.

PS, a silicone-based gel, has been shown to modulate cytokine responses and promote faster wound healing by reducing pro-inflammatory cytokine levels. Sil, containing hypochlorous acid, also exhibited antimicrobial and anti-inflammatory properties, contributing to the observed normalization of vascularization by controlling microbial load and inflammation.

Fibroblast activity and myofibroblasts. The presence of myofibroblasts and inflammatory cells is crucial for wound contraction and the early stages of healing **[**32, 33**]**.

On the 7th day, both control and PS-treated groups showed abundant myofibroblasts and inflammatory cells. By the 21st day, only the PS-treated group had returned to normal levels, indicating a more rapid transition from the inflammatory to the proliferative phase. Myofibroblasts play a key role in ECM production and wound contraction, and their prolonged presence can lead to excessive scarring. Sil-treated animals demonstrated moderate levels of myofibroblasts throughout healing, suggesting a balanced inflammatory response that supports healing without excessive scarring.

Flosteron and Hep-treated groups showed prolonged inflammation and myofibroblast activity, which may impede the effective resolution of the wound and lead to delayed re-epithelialization and ECM remodeling. This prolonged inflammatory state in the control and BMt-treated animals may hinder efficient tissue repair and contribute to excessive scar formation.

Collagen deposition. Collagen synthesis is critical for providing structural integrity to healing tissue [34]. Initially, all groups showed minimal collagen deposition on day 7. By the 14th day, PS-treated animals exhibited moderate collagen presence, which was maintained throughout the study, indicating accelerated collagen deposition and enhanced tissue repair. Sil and BMt treatments resulted in normalization of collagen deposition by day 21, suggesting effective collagen synthesis at a slower rate. Hep-treated animals exhibited abundant collagen by day 28, indicating delayed but robust collagen deposition, which might be beneficial for preventing hypertrophic scarring but less ideal for acute wound management.

The faster collagen deposition in PS-treated animals is consistent with previous studies demonstrating the effectiveness of silicone gels in enhancing collagen synthesis and reducing scar formation. Sil's anti-inflammatory properties likely contribute to balanced collagen synthesis without excessive scarring, while BMt's immunosuppressive effects may slow fibroblast recruitment and collagen synthesis.

Elastic fiber restoration. Elastic fibers provide resilience and flexibility to healing tissue. In this study, all groups showed moderate levels of elastic fibers initially, with PS-treated animals achieving normalization by day 21. Sil-treated animals showed normalization by day 28, indicating that it also supports the restoration of elastic fibers, though at a slower pace than PS. BMt andHeptreatments showed a slower rate of elastic fiber restoration, which may affect tissue flexibility and scar stiffness in the long term.

The faster normalization of elastic fibers in PS-treated animals suggests that this treatment supports not only collagen synthesis but also the restoration of other ECM components, enhancing tissue flexibility and strength. This restoration of elastic fibers is an important factor in reducing scar stiffness and improving skin elasticity.

Comparative efficacy of treatments.

Overall, **PS** demonstrated the most rapid and comprehensive effects on wound healing, including early normalization of vascularization, myofibroblast activity, collagen deposition, and elastic fiber restoration. This makes PSan excellent choice for acute wound management.

Sil also showed favorable results, particularly in balancing inflammation and collagen deposition. Its antimicrobial and anti-inflammatory properties make it suitable for wounds at risk of infection, and it supported moderate but effective tissue repair. However, its effects were not as rapid as those observed with PS.

BMt, while beneficial in controlling excessive inflammation, might delay the healing process due to its immunosuppressive effects. This makes it less suitable for acute wound healing but could be useful in cases where excessive inflammation and scarring are a concern.

Finally, **Hep**, with its delayed but robust collagen deposition, could be beneficial in preventing hypertrophic scars in the later stages of healing. However, its efficacy in early wound repair appears limited, as it showed prolonged inflammation and delayed vascularization normalization.

The alterations in cytokine levels, tissue morphology, and scar appearance observed in the different treatment groups reflect a combination of factors related to inflammation resolution, fibroblast activity, collagen deposition, and the restoration of extracellular matrix (ECM) components.

IL-1 and IL-6 are key pro-inflammatory cytokines involved in the immune response during wound healing. Their levels typically rise during the early inflammatory phase of wound healing to promote inflammatory cell infiltration and initiate the repair process [34, 35]. However, prolonged elevation of these cytokines can delay the transition to the proliferative and remodeling phases, resulting in chronic inflammation and abnormal scar formation.

IL-1 is known to be upregulated in response to tissue injury, as it promotes the activation of immune cells and enhances the inflammatory response by increasing the production of other cytokines, including IL-6 study. The rapid normalization of IL-1 levels in PS and Sil-treated groups suggests that these treatments may help resolve inflammation more efficiently. PS, which is a silicone-based gel, has been shown to modulate cytokine levels and promote faster wound healing by reducing pro-inflammatory cytokine levels. Sil (Hydrochloride acid) exhibits anti-inflammatory properties and has antimicrobial effects that may help resolve inflammation more rapidly [36].

IL-6 involved in the transition from the inflammatory to the proliferative phase of wound healing, promoting fibroblast activity and collagen production.Persistent elevation of IL-6 can lead to delayed resolution of inflammation and prolonged fibrosis. The PS and Sil showed a faster normalization of IL-6 levels, indicating that these treatments were more effective in controlling inflammation. The BMt normalized IL-6 levels only by the 28th day. In contrast, the control and Hep-treated groups showed delayed normalization, suggesting a prolonged inflammatory response, which may contribute to the delayed healing and scar formation observed in these groups.

Vascularization is essential for the delivery of nutrients and oxygen to the healing tissue, as well as the removal of waste products. It plays a critical role in the inflammatory and proliferative phases of wound healing [37]. Rapid normalization of vascularity indicates efficient inflammation resolution and successful transition to tissue remodeling.

Prolonged vascularization, as observed in the control group, suggests a persistent inflammatory state. Chronic inflammation can delay wound healing and promote the formation of hypertrophic scars. In contrast, effective treatments like PS and Sil showed faster normalization of vascularization, which is indicative of a more rapid resolution of the inflammatory phase and transition to the proliferative phase.

PS and Sil treatments were associated with a more rapid reduction in vascularity, indicating efficient inflammation resolution. PS, in particular, has been shown to improve wound healing by modulating

inflammatory cytokines and promoting tissue remodeling. Silicone gels like PS are known to reduce vascularity in scars by controlling excessive inflammation and stimulating collagen synthesis. Sil, with its hypochlorous acid content, reduce bacterial load and inflammation, leading to improved vascular normalization.

Myofibroblasts play a crucial role in wound contraction, ECM production, and the remodeling of collagen fibers. While their presence is necessary for wound healing, excessive or prolonged myofibroblast activity can lead to excessive scar formation, such as hypertrophic scars or contractures [32, 33].

Control and Hep-treated Groups: In the control group, the prolonged presence of myofibroblasts and inflammatory cells suggests delayed transition from the inflammatory to the proliferative phase. The slow resolution of inflammation and the persistence of myofibroblasts may contribute to excessive fibrosis and scar contracture. Hep, which contains heparin and allantoin, is known for its modulating effects, but it may not accelerate the transition from the inflammatory to the proliferative phase as efficiently as other treatments like PS.

PS and Sil: PS-treated animals normalized myofibroblasts and inflammatory cell levels by day 21, suggesting a faster resolution of inflammation and a more efficient transition to the proliferative phase. Sil, with its anti-inflammatory properties, showed moderate levels of myofibroblasts, indicating a balanced inflammatory response that supports healing without excessive scarring.

Collagen Deposition is essential for providing structure and strength to healing tissues. However, excessive collagen deposition can lead to the formation of hypertrophic scars or keloids.

PS: PS-treated animals exhibited early and sustained collagen deposition, which suggests accelerated tissue repair. Silicone-based treatments like PS have been shown to improve collagen synthesis and enhance wound healing by controlling inflammation and promoting fibroblast activity. Early normalization of collagen deposition is key to preventing excessive scar formation.

Sil and BMt: Sil and BMt also promoted collagen deposition, but at a slower pace compared to PS. This could be due to their more balanced approach to inflammation and collagen synthesis. BMt, which has anti-inflammatory and immunosuppressive properties, may delay collagen synthesis, but this could be beneficial in preventing excessive fibrosis. Sil's antimicrobial and anti-inflammatory properties support balanced collagen deposition, but its effect is not rapid compared to PS.

Elastic Fibers are critical for tissue flexibility and resilience. The elastic fiber restoration is important for minimizing scar stiffness and improving skin elasticity.

PS-treated animals showed rapid normalization of elastic fibers, indicating that this treatment not only accelerates collagen synthesis but also supports the restoration of other ECM components. This contributes to enhanced tissue flexibility and strength, which is crucial for minimizing scar formation and improving functional outcomes.

Sil and **BMt-**treated animals showed slower but steady restoration of elastic fibers, while BMt-treated had a slower rate of normalization. Both treatments support the restoration of elastic fibers, but their slower pace may affect the overall flexibility of the healing tissue, which could contribute to the formation of stiffer scars.

Scar Characteristics (vascularity, pigmentation, pliability and height) is influenced by multiple factors, including the balance between collagen deposition, inflammation and ECM remodeling.

The most rapid reduction in vascularity was observed in PS-treated animals, reflecting efficient inflammation resolution and rapid transition to tissue remodeling. The slower reduction in vascularity in the control and Hep-treated groups indicates persistent inflammation, which can contribute to hypertrophic scarring.

Proper pigmentation restoration, as seen in PS and Sil-treated groups, is essential for minimizing abnormal pigmentation such as hyperpigmentation or hypopigmentation. The more irregular pigmentation in the control and Hep-treated groups may be due to prolonged inflammation, which can disrupt melanocyte activity and skin pigmentation.

The pliability of the scar, as measured by the Vancouver Scar Scale, was greatest in PS-treated animals, indicating on of a flexible and supple scar. The control and Hep-treated groups exhibited increased scar stiffness, likely due to prolonged inflammation and myofibroblast activity, which can result in contractures and hypertrophic scarring.

Conclusion:

The results of this study underscore the importance of selecting appropriate treatments tailored to the specific needs of the wound. PS and Sil appear to be more effective treatments for accelerating wound healing based on both cytokine and histological data. They demonstrated the most rapid resolution of inflammatory markers (IL-1 and IL-6) and histological indicators, including vascularization and cellular presence. BMt showed moderate efficacy, while the Hep and control groups experienced delayed normalization, especially in inflammatory cytokine levels and collagen deposition. PS emerged as the most effective treatment for promoting rapid wound healing. It was the most effective treatment for promoting optimal scar characteristics, including reduced vascularity, proper pigmentation, pliability, and scar flattening, while Sil demonstrated a balanced approach to inflammation control and collagen synthesis. BMt and Hep showed potential but highlighted the trade-off between inflammation control and the speed of tissue repair. Sil and BMt showed moderate improvements in wound healing and scar characteristics, while Hep was less effective in accelerating wound healing and scar normalization.

In summary, the PS emerged as the most effective treatment for promoting rapid wound healing and optimal scar characteristics, while Sil showed balanced efficacy in controlling inflammation and supporting tissue repair. BMt and Hep, while beneficial for controlling inflammation and fibrosis, showed slower effects in promoting wound healing and normalizing scar characteristics. The differential effects of these treatments suggest that tailored therapeutic strategies are necessary to optimize wound healing outcomes.

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