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Histological Markers of Wound Healing and Scar Quality: Evaluating Different Therapeutic Approaches in Full-Thickness Skin Wounds

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ABSTRACT

Background: The skin wound healing process is dynamic and involves multiple cellular and molecular pathways. Disruptions at any stage, hemostasis, inflammation, proliferation, or remodeling, can impair the healing process, leading to undesirable complications. Treating skin wounds is challenging and can be problematic due to the complexity of the healing process and the diverse factors influencing outcomes.

Objectives: This research examines the impacts of polysilicon-siloxane, silicone, heparin, and steroid-based preparations on histological indicators of wound healing in laboratory rats with full-thickness skin wounds.

Methods: Scar appearance and healing progression were assessed using the Vancouver Scar Scale (VSS). Histological evaluations were conducted using staining techniques such as H&E, Masson's trichrome, Van Gieson, and Weigert's, as well as immunohistochemistry.

Results: Investigations revealed that vascularization normalized by day 14 of wound healing in animals treated with the polysilicon-siloxane-based preparation (PS), by day 21 in those treated with silicone (Sil), and persisted until day 28 in the betamethasone (BMt) and heparin-treated (Hep) groups. Collagen deposition and normalization of elastic fibers occurred rapidly in the PS group, followed by the Sil and BMt groups. At the same time, Hep-treated animals showed delayed collagen deposition, which was evident only by day 28. PS was the most effective in achieving favorable scar features, including reduced vascularity, appropriate pigmentation, improved pliability, and flatter scars. Sil and BMt significantly improved wound healing and scar characteristics, whereas Hep demonstrated limited effectiveness in accelerating healing and in normalizing scars.

Conclusions: The results suggest that PS, Sil, and BMt-based preparations play a significant role in enhancing vascularization, collagen organization, and elastic fiber restoration during the wound-healing process. PS showed the fastest wound-healing outcomes, followed by Sil and BMt, while Hep showed minimal efficacy. These findings highlight the value of targeted therapeutic strategies in wound care and offer insights for optimizing treatment protocols to improve healing results. Further studies are recommended to investigate the precise mechanisms involved and to identify additional biomarkers related to tissue repair and inflammation resolution.

Keywords: Heparin; histological markers; polysilicon-siloxane; silicone; skin wounds; steroid preparations.

BACKGROUND

Skin wound healing is a complex and dynamic biological process that restores tissue continuity and function. This intricate process involves a highly coordinated sequence of cellular and molecular events divided into four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Each stage is critical to successful healing, with specific cellular and biochemical mediators orchestrating the repair process.[1]

The healing process begins immediately after injury with hemostasis, where platelets aggregate, release clotting factors, and form a clot to prevent further blood loss while forming a fibrin scaffold for cell migration. The inflammatory phase follows, involving the recruitment of immune cells such as neutrophils and macrophages, which clear debris and pathogens while releasing cytokines and growth factors. The proliferation phase is characterized by fibroblast activation, angiogenesis, and re-epithelialization. Finally, during the remodeling phase, collagen deposition matures, and extracellular matrix (ECM) components such as elastic fibers restore tissue tensile strength and elasticity.[2,3]

Key cellular and molecular players in the process of wound healing are:

- Fibroblasts: These mesenchymal cells play a pivotal role in the proliferation and remodeling phases by synthesizing ECM proteins, mainly collagen and elastic fibers, which provide structural integrity to the wound. Fibroblasts also release growth factors that promote angiogenesis (e.g., VEGF and TGF-β) and keratinocyte proliferation.
- Collagen: This major ECM protein is central to wound healing. Type III collagen is predominantly deposited early in the healing process, providing a temporary matrix that is later replaced by type I collagen during remodeling to restore strength.
- Elastic Fibers: Elastic fibers, composed of elastin and fibrillin, are essential for tissue flexibility and resilience. Their organization during the later stages of healing ensures the skin's functional recovery.
- Inflammatory Cells: Neutrophils, macrophages, and lymphocytes play crucial roles in the inflammatory phase by clearing pathogens and debris, releasing signaling

molecules, and transitioning the wound environment from inflammation to tissue repair.[4-6]

Advances in wound management have led to the development of topical agents, systemic therapies, and physical interventions to optimize healing. Some modern treatments include silicone-based creams and wound dressings to maintain moisture and promote epithelialization; growth factor-based treatments to enhance cell proliferation and migration; corticosteroid injections to reduce excessive inflammation; advanced wound dressings containing antimicrobial agents to reduce infection risk; and laser and light-based therapies to improve scar appearance and reduce fibrosis.[7-9]

Despite these advances, treatments have limitations and side effects. Corticosteroids may suppress fibroblast activity and delay wound closure. Growth factor treatments can be expensive and may carry a risk of overstimulation, leading to hypertrophic scarring. Silicone-based products are generally safe but may irritate sensitive individuals. Effective wound management requires balancing the benefits of these treatments with their potential adverse effects and tailoring interventions to the patient's specific needs.

Understanding the biological mechanisms underlying wound healing and the roles of fibroblasts, collagen, inflammatory cells, and elastic fibers is crucial for improving treatment outcomes. Continuous research into innovative therapies and biomaterials promises to overcome current challenges in wound management.

We aimed to evaluate the effects of various treatments (polysilicon-siloxane, silicone, heparin, and steroid-based preparations) on histological markers and the skin wound healing process to optimize outcomes, reduce scarring, and enhance patient quality of life.

METHODS

Experiments were conducted on male white lab rats weighing 200-250 g. The animals were purchased from the vivarium of Aleksandre Natishvili Institute of Morphology, Tbilisi, Georgia (https://www.tsu.ge/en). The research protocol was approved by the TSMU Animal Welfare and Use Ethics Committee.

All animals were allowed to acclimate to laboratory conditions for 1 week before the experiment. During this period, they were kept under constant environmental conditions with a 12/12 light-dark cycle at 23±2°C. They were fed standard laboratory chow and given free access to water.

For modeling 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. Then, a 5 cm surgical suture was placed on the skin at a 1 cm interval.

Treatment protocols: PS (polysilicon-siloxane), Sil (Silicon), and Hep (heparin) 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) was injected subcutaneously into the wound area once a week for 4 weeks.

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

Scar assessment: 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 clinical practice and research.[11] 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 is added together to give an overall score between 0 and 13.[10]

Histological assessments of healing stages: To evaluate vascularization, collagen deposition, myofibroblast activity, and elastic fiber status at different stages of wound healing, the following histological techniques were employed.

Immunohistochemistry (IHC): Tissue sections were fixed and blocked to minimize nonspecific binding. They were incubated with primary antibodies targeting specific antigens, followed by secondary antibodies conjugated to detection markers. The markers were visualized using a fluorescence microscope, enabling the precise localization of proteins involved in healing.

Masson's trichrome staining: This technique differentiated tissue components. Sections were treated with dyes: Biebrich scarlet stained cytoplasm and muscle, followed by phosphomolybdic acid to remove nonspecific staining, allowing collagen fibers to be counterstained with aniline blue or green.

Van Gieson's staining: This method highlighted collagen and differentiated it from other tissue types. Collagen was stained red with acid fuchsin, while muscle and cytoplasm appeared yellow due to picric acid.

Weigert's staining: Elastic fibers were identified using Weigert's resorcin-fuchsin solution, which binds specifically to elastin, rendering elastic fibers dark purple or black.

Hematoxylin and eosin (H&E) staining: This standard technique was used to evaluate tissue structure. Hematoxylin-stained nuclei are blue, while eosin-stained cytoplasm and extracellular components are pink.

These methods collectively provided detailed insights into the structural and cellular dynamics of wound healing, including vascularization, extracellular matrix composition, and elastic fiber integrity.

RESULTS

Vascularization: All treatment groups exhibited increased vascularization on the seventh day post-injury. In the control group, vascularization remained elevated up to the 28th day. In the PS group, vascularization normalized by the 14th day, while Sil-treated animals achieved normalization by the 21st day. Conversely, BMt and Hep-treated groups displayed persistent high vascularization levels until the 28th day.

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Myofibroblast and inflammatory cell activity: On the seventh day, the control and PS-treated groups showed heightened levels of myofibroblasts and inflammatory cells. While the control group and Sil-treated animals maintained moderate activity throughout the study, the PS group achieved normalization by the 21st day. Other treatment groups showed only moderate levels of these cells during the observation period.

Collagen and elastic fiber formation: Initially, collagen fibers were scant across all groups. By the 14th day, the PS-treated group displayed moderate collagen deposition, which remained throughout the healing process. Sil and BMt-treated groups reached normal collagen levels by the 21st day, whereas Hep-treated animals showed abundant collagen deposition only by the 28th day.

Elastic fibers were initially present in moderate amounts in all groups, with normalization achieved by the 21st day in the PS group and by the 28th day in the Sil-treated group.

Analysis of scar characteristics and wound healing at various time points

Control group

- Vascularity: Maintained elevated levels throughout the observation period, ranging from scores of 2-3 on days 7 to 21, with a slight decrease to 2 by day 28. This pattern reflects a prolonged inflammatory phase with delayed resolution.
- Pigmentation: Initially exhibited mild hypo-pigmentation (score 1), progressing to irregular pigmentation (scores of 1-2) between days 14 and 28, indicating uneven pigmentation with the possibility of hyperpigmentation.
- Pliability: Demonstrated a decline in scar pliability (score 3), suggesting the development of a stiff and potentially hypertrophic scar.
- Height: Scar height remained moderately raised (scores of 2-3) across the time points, with inadequate flattening observed by the end of the study.

PS (polysilicon-siloxane-treated) group

- Vascularity: Showed the most significant improvement, with scores reducing from 2 on day 7 to 0 by day 28. This indicates efficient inflammation resolution and minimal vascularization in the scar tissue.
- Pigmentation: Returned to normal quickly, consistently scoring 0-1, demonstrating uniform skin tone restoration.
- Pliability: Maintained excellent outcomes, reaching supple (score 1) by day 14 and sustaining this pliability through the study period, indicative of healthy tissue without stiffness.
- Height: Achieved normalization early, with scar height scoring 0 by day 14, resulting in flat and well-healed scars.

Sil (silicone-treated) group

- Vascularity: Gradually decreased from a score of 2 on day 7 to 1 by day 28, reflecting moderate improvement in inflammatory response resolution, but less efficient than in the PS group.
- Pigmentation: Generally, within the normal range (score 0-1) but temporarily showed hyperpigmentation (score 2) on day 14, which resolved by day 28.
- Pliability: Showed gradual improvement, moving from firmness (score 3) on day 7 to slight firmness (score 2) by day 21 and supple (score 1) by day 28, indicating moderate recovery of tissue flexibility.
- Height: Displayed a progressive reduction from raised scars (score 3) to a flatter appearance (score 1) by day 28, though not as effectively flattened as scars treated with PS.

BMt (Betamethasone-treated) group

- Vascularity: Persisted at higher levels (scores of 2-3) until day 21 before decreasing to 2 by day 28, suggesting moderate resolution of inflammation;
- Pigmentation: Similar to the Sil group, pigmentation was generally normal (score 0-1) but experienced a brief increase (score 2) on day 14 before normalizing by day 28;
- Pliability: Improved steadily, transitioning from firmness (score 3) on day 7 to yielding (score 2) by day 14 and supple (score 1) by day 21, indicating healthy scar flexibility by the end of the study;
- Height: Showed gradual improvement, with scores reducing from 2 on day 7 to 1 by day 28, reflecting partial flattening but less effective than in the PS group.

Hep (heparin-treated) group

- Vascularity: Remained high (scores of 2-3) throughout the study, indicating prolonged inflammation and delayed vascular normalization;
- Pigmentation: Stayed in the normal to mildly hypopigmented range (score 1), suggesting some degree of pigment restoration, though inconsistencies were observed;
- Pliability: Improved over time, starting with firmness (score 3) on day 7, progressing to yielding (score 2) by day 14, and achieving supple (score 1) by day 28, indicating a gradual recovery in scar flexibility;
- Weight: Gradually decreased from raised (score 3) to flatter scars (score 1) by day 28, though not as effectively flattened as those treated with PS.

In summary:

Vascularity:

- Normalized by day 14 in PS-treated animals;
- Normalized by day 21 in Sil-treated animals;
- Continued to persist until day 28 in BMt and Hep-treated animals.

Collagen deposition and elastic fiber normalization:

- PS showed the fastest collagen deposition and elastic fiber normalization, occurring earliest among all treatments;

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- Sil- and BMt-treated animals showed moderate improvements, with collagen deposition and elastic fiber normalization occurring later;
- The hep-treated group exhibited delayed collagen deposition, which only normalized by day 28.

Scar characteristics:

- PS was the most effective in promoting optimal scar characteristics, including reduced vascularity, proper pigmentation, pliability, and scar flattening;
- Sil- and BMt-treatments led to moderate wound healing and scar formation improvements;
- Hep was less effective in accelerating wound healing and scar normalization.

DISCUSSION

Vascularization is pivotal in wound healing, as it supplies nutrients and oxygen to the injury site and removes metabolic waste products. In the control group, increased vascularization persisted until day 28, suggesting chronic inflammation that may hinder the transition to the proliferative phase and delay healing.

In contrast, PS and Sil's treatments demonstrated quicker normalization of vascularization, indicating a more efficient resolution of inflammation. Specifically, PS was normalized by day 14, whereas Sil was normalized by day 21.

On the other hand, the BMt and Hep groups exhibited prolonged vascularization, suggesting a slower resolution of inflammation. PS has been documented to modulate cytokine responses, reducing pro-inflammatory cytokine levels and thereby promoting more rapid healing.

Similarly, a Sil-based preparation containing hypochlorous acid exerts both antimicrobial and anti-inflammatory effects, thereby accelerating vascular normalization by controlling microbial load and inflammation.[14]

Fibroblasts and myofibroblasts are essential for wound contraction and early healing. On day 7, the control and PS groups showed abundant myofibroblasts and inflammatory cells. However, by day 21, only the PS group had returned to normal levels, indicating a more rapid shift from the inflammatory to the proliferative phase. Myofibroblasts, which produce extracellular matrix (ECM) components, are critical for wound contraction, but prolonged activity may lead to excessive scarring.[15]

Sil-treated animals displayed moderate myofibroblast levels throughout the healing process, indicating a balanced inflammatory response that did not promote excessive scarring. Conversely, BMt and Hep-groups exhibited persistent inflammation and myofibroblast activity, potentially leading to delayed re-epithelialization and ECM remodeling.

Collagen Deposition is vital for providing structural integrity to the healing tissue.[16] On day 7, minimal collagen was observed across all groups. By day 14, PS-treated animals exhibited moderate collagen deposition, which continued throughout the study, suggesting accelerated tissue repair.

Sil and BMt treatments normalized collagen deposition by day 21, reflecting effective but slower collagen synthesis. The help group showed abundant collagen by day 28, indicating delayed but robust collagen deposition, which could be advantageous in preventing hypertrophic scarring but less effective for acute wound management.

Elastic Fiber Restoration contributes to the resilience and flexibility of healing tissue.[17,18] In this study, the PS group demonstrated rapid normalization of elastic fibers by day 21, while the Sil Group normalized by day 28, supporting a restoration process, albeit at a slower pace.

BMt and Hep groups showed slower rates of elastic fiber restoration, potentially affecting the tissue's long-term flexibility and contributing to stiffer scars. PS's prompt recovery of elastic fibers underscores its comprehensive effects on collagen and ECM components, thereby improving skin elasticity and reducing scar stiffness.

Comparative efficacy of treatments

PS group exhibited the fastest and most comprehensive effects, with early normalization of vascularization, myofibroblast activity, collagen deposition, and elastic fiber restoration. These attributes make it a prime choice for managing acute wounds.

The Sil group also showed favorable results, particularly in controlling inflammation and promoting balanced collagen deposition. Its antimicrobial properties make it a good option for wounds at risk of infection, though its effects were not as rapid as in the PS group.

BMt effectively controlled excessive inflammation but may have delayed healing due to its immunosuppressive properties. This makes it less suitable for acute wound healing but potentially beneficial in cases with excessive inflammation or concerns about scarring.

While promoting delayed but robust collagen deposition, Hep demonstrated prolonged inflammation and delayed vascular normalization, suggesting it may be more appropriate for late-stage scar prevention than for early wound healing.

The observed effects of these treatments reflect a complex interplay of inflammation resolution, fibroblast activity, collagen deposition, and ECM restoration.

PS and Sil's groups showed more rapid, effective healing, with PS particularly advantageous for acute wound management. Conversely, BMt and Hep groups had slower but beneficial effects, with Hep particularly useful for late-stage scar prevention.

Understanding the mechanisms underlying these treatments, such as cytokine modulation in PS and Sil's anti-inflammatory properties, helps explain their differing efficacy in promoting wound healing and improving scar characteristics.

CONCLUSIONS

PS- and Sil-treated groups demonstrated superior effectiveness in accelerating wound healing, as evidenced by

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improvements in histological markers and scar characteristics, including vascularity, pigmentation, pliability, and height. These treatments showed rapid improvements in key histological markers, such as vascularization and cellular infiltration, facilitating more efficient healing.

BMt showed moderate efficacy, while the Hep and control groups experienced delayed normalization, particularly in collagen deposition.

Among the treatments tested, PS emerged as the most effective in promoting rapid wound healing and optimal scar characteristics. It reduced vascularity, ensured proper pigmentation, improved pliability, and contributed to scar flattening.

Sil treatment, on the other hand, provided a balanced approach by controlling inflammation and promoting steady collagen synthesis. Though its effects were not as rapid as PS's, it still yielded positive results in wound healing and scar improvement.

In contrast, BMt showed moderate progress in enhancing wound healing and scar characteristics, making it a suitable option for cases requiring inflammation control.

While supporting collagen deposition, hep treatment was less effective at accelerating overall wound healing and scar normalization, suggesting a more suitable application in later stages of healing.

These differential outcomes suggest that personalized treatment strategies tailored to the wound's specific requirements are crucial for optimizing healing and minimizing scarring. They also highlight the significance of selecting appropriate treatments tailored to the wound's needs.

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