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Changes in ZO-1 expression as an early indicator of treatment effectiveness in patients with chronic diabetic foot wounds

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Chronic diabetic foot wounds represent a persistent surgical challenge due to delayed healing, frequent complications, and high socioeconomic burden. Chronic hyperglycemia is known to impair epidermal barrier integrity, in part by altering the expression of tight junction proteins, including zonula occludens-1 (ZO-1).

Objective – to evaluate changes in ZO-1 expression in chronic diabetic foot wounds following combined local therapy and to assess the potential role of ZO-1 as an early molecular marker of epithelial barrier restoration.

Materials and methods. A prospective randomized study included 28 patients with chronic diabetic foot wounds. Patients were divided into an intervention group (n = 14) treated with a combined spray–and–gel regimen containing collagen, hyaluronate, amino acids, trace elements (Zn, Cu), and antiseptic components, and a control group (n = 14), receiving standard chlorhexidine dressings. Epidermal biopsy samples were obtained at baseline (Day 0) and after 10 days of treatment. ZO-1 expression was assessed using Western blot analysis, followed by densitometric quantification.

Results. Patients receiving combined local therapy demonstrated a marked increase in ZO-1 expression by Day 10 compared with baseline values, indicating restoration of intercellular junction integrity. No comparable changes were observed in the control group.

Conclusions. Combined local therapy promotes molecular recovery of the epidermal barrier in chronic diabetic foot wounds, as evidenced by increased ZO-1 expression. These findings support the clinical relevance of ZO-1 as an objective biomarker for treatment response in the surgical management of chronic diabetic wounds.

Keywords
diabetic foot, chronic wounds, tight junctions, zonula occludens-1, wound healing, local therapy.

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Scope and impact of chronic wounds

Chronic wounds are defined as defects of the skin and underlying tissues that fail to progress through the normal stages of healing within 3–4 weeks and may persist for months or longer [2, 9]. Unlike acute wounds, chronic wounds are characterized by prolonged inflammation, impaired tissue regeneration, and frequent recurrence. Clinically, chronic wounds represent a multifactorial surgical problem resulting from the interaction of local tissue damage and systemic disorders [1, 6].

Among chronic wounds, diabetic foot ulcers are associated with particularly high morbidity and mortality and lead to a substantial reduction in patient quality of life [10, 11]. In addition to clinical consequences, the economic impact of chronic wound care is considerable, placing a sustained burden on healthcare systems worldwide, with treatment costs reaching tens of billions of dollars annually [10]. The most prevalent categories of hard-to-heal wounds include diabetic foot ulcers, venous leg ulcers, and pressure ulcers [10, 11].

Effective management of chronic wounds requires a structured and systematic approach [4, 9]. Adequate wound bed preparation is fundamental in contemporary surgical practice. This process includes the removal of non-viable tissue, infection control, maintenance of optimal moisture balance, and stimulation of wound edge activity [2, 6, 9]. Proper wound bed preparation is widely regarded

as a prerequisite for successful surgical and conservative treatment of chronic wounds [2, 3, 9].

Principles of modern wound management

Current surgical strategies emphasize that successful healing of chronic wounds, particularly in patients with diabetes mellitus, cannot rely solely on correction of systemic metabolic or vascular abnormalities [1, 11]. Increasing attention is directed toward local therapeutic interventions aimed at modifying the wound microenvironment [1].

Clinical evidence indicates that hydrogel-based dressings improve healing outcomes by supporting epithelialization, maintaining adequate hydration, and facilitating the controlled release of bioactive substances [4]. Advanced hydrogel systems integrate biocompatibility with antimicrobial and anti-inflammatory properties, attributes that are especially important for treating diabetic wounds with impaired local defense mechanisms [4]. Adjunctive modalities, such as hyperbaric oxygen therapy, have demonstrated benefit in selected patient populations by reducing wound size and lowering the risk of major amputations [11].

Molecular pathology of diabetic wounds: the role of ZO-1

Diabetic foot ulcers develop in the setting of chronic hyperglycemia-induced metabolic disturbances affecting multiple organ systems [7, 11]. One of the key pathological mechanisms underlying delayed healing is dysfunction of the epidermal and microvascular barriers [5, 7].

Tight junctions play a critical role in maintaining epidermal integrity. Zonula occludens-1 (ZO-1) is a cytoskeleton-associated protein essential for tight junction assembly and regulation of paracellular permeability [10]. Experimental and clinical studies have shown that hyperglycemia reduces ZO-1 expression and alters subcellular localization in keratinocytes and endothelial cells [5, 7, 8]. These changes result in compromised barrier function, increased transepidermal water loss, and impaired re-epithelialization [5, 7].

During physiological wound healing, ZO-1 expression increases as epithelial continuity is restored, highlighting its role in intercellular junction repair [8]. Therefore, assessment of ZO-1 dynamics in chronic diabetic wounds may provide valuable insight into the molecular effectiveness of local therapeutic interventions [8, 11].

Rationale and objective

Restoration of epidermal barrier integrity is a fundamental goal in the surgical treatment of chronic wounds. Combining standard wound care with regenerative local agents may enhance epithelial repair by simultaneously improving the wound environment and supporting molecular mechanisms responsible for tight junction restoration [4, 8].

Objective – to evaluate changes in ZO-1 expression in chronic diabetic foot wounds following combined local therapy and to assess the potential role of ZO-1 as an early molecular marker of epithelial barrier restoration.

Materials and methods

Study population and design

This study employed a prospective, randomized design and enrolled 28 patients with chronic foot wounds secondary to diabetes mellitus. Inclusion required a wound duration of at least 3 months. Patients were assigned to two cohorts using systematic random sampling: the intervention group (n = 14) received combined local therapy, while the control group (n = 14) received standard dressings.

Intervention Protocols

Experimental group treatment

Patients in the experimental group received a two-step combined local therapeutic regimen:

- **Spray application.** The wounds were initially treated with a spray formulation containing decamethoxin (an antiseptic), low-molecular-weight hydrolyzed collagen, and the amino acids glycine, arginine, and aspartic acid, suspended in purified water.
- **Gel application.** Subsequently, a wound care gel was applied over the treated area. The gel contained low-molecular-weight hydrolyzed collagen, high-molecular-weight sodium hyaluronate, silver sulfadiazine (an antimicrobial agent), decamethoxin, colloidal anhydrous silicon dioxide (Aerosil, a structural agent), poloxamer, hypromellose (hydrogel matrix agents), and purified water.

This formulation was designed to address multiple facets of chronic wound pathology, providing structural support (collagen), maintaining hydration (hyaluronate, poloxamer), controlling infection (decamethoxin, silver sulfadiazine), and supplying cellular precursors and regulators (amino acids and trace elements, specifically Zn and Cu as discussed later).

Control group treatment

The control group received standard care, consisting solely of wound treatment with a chlorhexidine solution, followed by the application of an atraumatic sterile dressing soaked in the same solution. It is important to note that iodine-based preparations were explicitly excluded from the treatment protocols for both groups.

Sample collection and timing

To evaluate the molecular dynamics of skin intercellular contacts, ZO-1 protein expression was analyzed.1 Paired epidermis tissue samples were surgically collected from patients in both the intervention and control groups at two crucial time points:

- **Day 0.** Collected prior to the initiation of any local therapeutic intervention.
- **Day 10.** Collected immediately following the 10-day course of prescribed treatment.

Molecular expression analysis

The level of ZO-1 expression was determined using western blot analysis. The standard procedure involved separating tissue proteins by electrophoresis, transferring them onto a membrane, incubating the membrane with specific primary and secondary antibodies targeting ZO-1, and finally visualizing the resulting complexes using the chemiluminescent method. The ZO-1 protein band was successfully identified at an approximate molecular weight of 220 kDa.

Quantitative assessment of protein content was performed by densitometry, an accurate technique for determining relative protein amounts based on signal intensity on the blot. The widely available software ImageJ was used for this quantification, providing a precise means to measure the area and intensity of the signal bands. Densitometric measurements were reported as relative intensity units (i.u.), normalized to the background signal, allowing for comparative analysis between baseline (Day 0) and post-treatment (Day 10) samples within each patient.

Results

Initial qualitative findings (western blot)

Analysis of ZO-1 protein expression commenced with qualitative assessment via western blotting. At the initial baseline stage (Day 0), significant variability in ZO-1 band intensity was observed across patient samples, a finding characteristic of the diverse pathology of chronic wounds. After 10 days of combined local treatment, the analysis revealed a marked and consistent increase in ZO-1 signal intensity. Specifically, a pronounced intensification of the signal was observed in 13 of the 14 participants in the intervention group, indicating molecular activation of processes aimed at restoring tight intercellular contacts.

Following systematic organization and processing, the generalized blotograms (Fig. 1, as described below) confirmed this trend. There was a visible increase in ZO-1 expression (the protein band at 220 kDa) in the Day 10 (even) lanes compared to the corresponding baseline (odd) lanes. Notably, samples from Patient 4 showed particularly high enhancement, which correlated positively with observed clinical progress in wound healing dynamics.

Quantitative densitometric analysis

For objective quantification, the intensity of the ZO-1 bands was measured by densitometry in ImageJ. The data for the intervention group are summarized in Table 1.

The data in Table 1 reveal a substantial and consistent positive dynamic in ZO-1 expression across the majority of patients following 10 days of the combined local therapy. Several cases demonstrated exponential increases. For instance, Patient 4 exhibited an approximately 742 % increase (from 0.8683 to 6.4538 i.u.). This magnitude of molecular change suggests that the therapeutic complex initiates a powerful, targeted stimulus for cellular repair and tight junction protein synthesis, demonstrating a highly effective pro-reparative signal rapidly induced by the treatment.

Treatment response dynamics

The quantitative data were visually represented using bar charts to clearly illustrate the treatment effects in both cohorts (Fig. 2, 3).

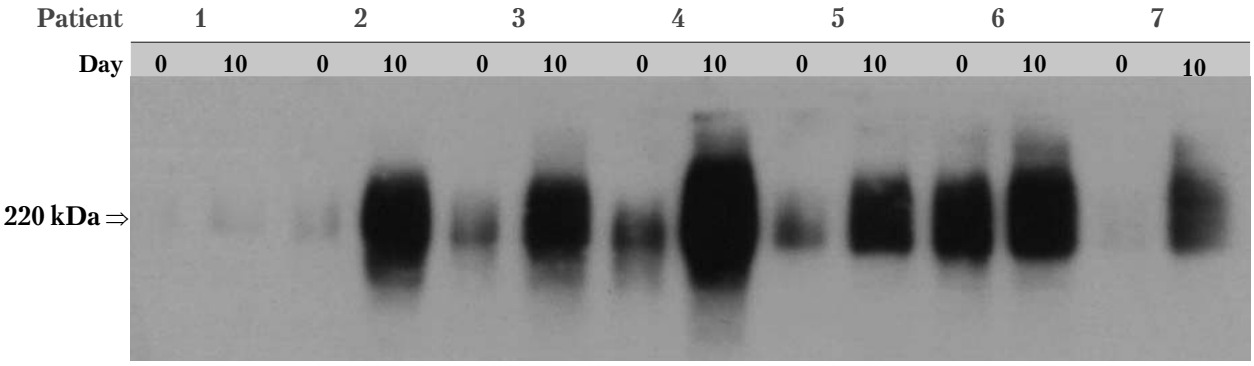

Figure 1. **Western blot expression of ZO-1 in patients with chronic foot wounds on days 0 and 10 of treatment. The arrow indicates the ZO-1 protein band at 220 kDa**

Table 1. **Densitometric values of ZO-1 band intensity in intervention group patients on days 0 and 10 of treatment, i.u.**

Patient	Day 0	Day 10
1	0.0622	0.0669
2	0.1285	2.5019
3	0.4640	2.1926
4	0.8683	6.4538
5	0.3272	1.7172
6	2.0024	3.5765
7	0.1757	1.2642
8	0.7149	3.8429
9	1.3824	4.7561
10	0.2593	0.4678
11	0.9475	1.3982
12	0.0368	5.2279
13	1.7249	3.1425
14	0.5031	2.9041

Fig. 2 shows the densitometry results for the intervention group, demonstrating a marked increase in ZO-1 band intensity after treatment. This visible upregulation confirms the therapeutic complex's ability to enhance ZO-1 expression. The sole exception was Patient 1, whose baseline expression was minimal (0.0622 i.u.) and whose subsequent post-treatment growth was marginal. This low response may indicate a subgroup of wounds with an exceptionally profound or non-responsive underlying barrier dysfunction.

In contrast, Fig. 3, representing the control group that received standard chlorhexidine dressings, showed no substantial increase in ZO-1 band intensity over the 10-day period. This clear differentiation underscores that the observed molecular restoration of the epithelial barrier is a specific therapeutic effect of the combined spray-and-gel regimen, rather than a non-specific response to basic antiseptic care.

Discussion

The role of combined therapy in tight junction restoration

Chronic diabetic wounds are characterized by decreased ZO-1 protein levels, a condition directly associated with impaired epithelial barrier function and delayed wound healing. The primary finding of this study–a significant increase in ZO-1 expression in the intervention group by Day 10–confirms that the combined local therapeutic approach successfully counters and reverses this fundamental molecular deficit.

The increased intensity observed on the western blot indicates successful molecular restoration of tight intercellular junctions, resulting in a demonstrable improvement in epithelial structural integrity. The specificity of this therapeutic response, entirely absent in the control group treated with chlorhexidine, highlights the unique biological activity of the bioactive components within the spray and gel. These components include collagen and hyaluronate, which provide a scaffold and maintain moisture, as well as crucial trace elements such as Zn and Cu. Zinc is indispensable for critical processes such as keratinocyte proliferation and immune system modulation, while copper is vital for promoting angiogenesis and stabilizing the extracellular matrix constituents,

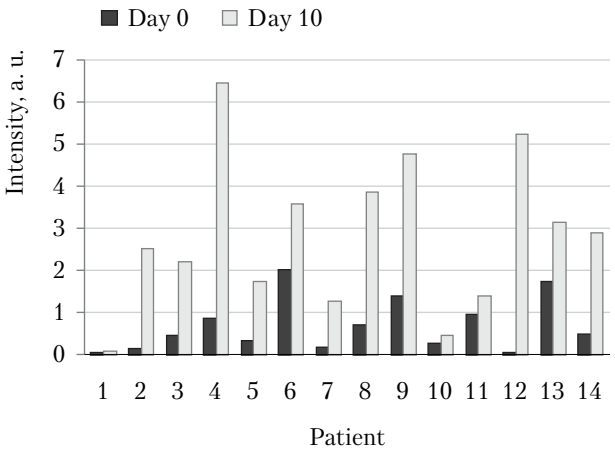

Figure 2. **Column chart of ZO-1 protein densitometry in 14 patients of the intervention group on day 0 and day 10 of treatment**

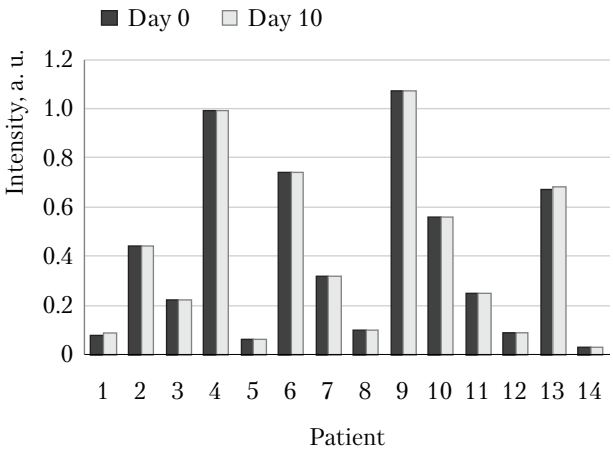

Figure 3. **Column chart of ZO-1 protein densitometry in 14 patients of the control group on day 0 and day 10 of treatment**

including collagen and elastin. The collective action of these components likely initiates and accelerates the cellular signaling cascades required for the rapid synthesis and accurate localization of ZO-1, thereby rapidly pushing the chronic wound environment toward an acute, pro-reparative state.

Correlation with clinical improvements

Molecular evidence of barrier recovery, as manifested by ZO-1 upregulation, aligns with favorable clinical observations in the experimental group. Patients receiving combined local therapy demonstrated faster wound cleansing, a marked reduction in local inflammation, and more robust granulation tissue formation compared to the control group. The rapid biochemical shift, captured by the Day 10 biopsies, signifies that the molecular foundation for successful healing – the restoration of the epithelial barrier – is established early in the treatment phase. This rapid commencement of molecular repair mechanisms precedes and predicts subsequent macroscopic clinical improvement, indicating that the complex formulation is designed to drive biological regeneration beyond simple wound coverage or antisepsis.

ZO-1 as a validated early biomarker

The findings provide strong evidence that ZO-1 expression dynamics represent a reliable, objective, and early tool for assessing therapeutic success in chronic wounds. The exclusive increase in ZO-1 observed in the cohort treated with the specialized spray and gel indicates that this protein is a sensitive indicator of molecular response to regenerative local therapy.

This objective molecular assessment provides significant advantages over traditional subjective clinical evaluations. By quantifying the protein level via densitometry within a short 10-day period, clinicians can obtain definitive evidence of whether the wound's underlying cellular machinery is responding to the regenerative agents. This objective validation capability allows for a faster assessment of treatment efficacy, enabling prompt clinical decisions regarding the continuation of a successful treatment path or the necessary modification of care for patients who show minimal molecular response, such as Patient 1. This advancement contributes to a more personalized and evidence-based approach to chronic wound management.

Perspectives

While the current study successfully confirms the molecular efficacy of the combined therapy in restoring ZO-1 expression, future research should focus on establishing a direct, long-term correlation between the magnitude of the Day 10 ZO-1 increase and definitive clinical endpoints, such as rates of complete healing and risk of wound recurrence. Further investigations are also required to fully elucidate the specific synergistic contributions of individual complex components (e.g., zinc versus collagen or hyaluronate) to optimize future wound care product development. Additionally, the case of the patient who exhibited minimal response highlights the need for dedicated research into the mechanisms of treatment resistance, potentially involving genetic factors or localized pathologies that may override the pro-reparative effects of the topical agents.

Clinical significance

The findings of this research establish several points of clinical importance derived from the molecular evidence:

- **Restoration of barrier integrity.** The significant increase in ZO-1 expression following treatment confirms the therapeutic regimen's ability to actively restore intercellular contacts and reinforce epithelial barrier function, a crucial step often stalled in diabetic pathology.
- **Regenerative efficacy.** The wound care spray and gel formulated with trace elements (Zn, Cu) should be considered effective therapeutic agents for actively stimulating molecular regeneration in chronic diabetic wounds.
- **Novel monitoring strategy.** ZO-1 expression analysis offers a quantifiable and valuable early laboratory marker for evaluating patient response to modern regenerative local therapies, providing objective data for guiding clinical management decisions.

Conclusions

The study conclusively demonstrated that the implementation of a comprehensive local therapy, incorporating standard antiseptic treatment with a mineral wound spray and gel containing trace elements (Zn, Cu), significantly restores impaired epithelial barrier properties in chronic diabetic foot wounds.

Densitometric quantification of ZO-1 protein showed a substantial increase in expression by the 10th day of treatment compared with baseline. This positive molecular dynamic was recorded across the majority of the treated patient cohort, clearly indicating improved intercellular contacts and accelerated tissue repair.

The totality of the data supports the conclusion that the application of trace element-based wound agents plays an important role in restoring the structural integrity of the epithelial barrier and normalizing tight junction function, specifically by increasing the level of ZO-1. This targeted molecular approach is recommended as an effective supplement to standard treatment protocols for chronic diabetic wounds.

Declaration of interests

The authors declare no conflict of interest.

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Authors contributions

D.Yakymiv: data processing, statistical analysis, and interpretation of results, and prepared the manuscript text; M. Prystupiuk: conceptualization, research design development, data collection.

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Зміни експресії ZO-1 як ранній показник ефективності лікування у пацієнтів з хронічними ранами стопи при цукровому діабеті

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Хронічні рани стопи при цукровому діабеті становлять значну проблему для хірургії через повільне загоєння, часті ускладнення та велике соціально-економічне навантаження. Відомо, що хронічна гіперглікемія порушує цілісність епідермального бар'єра, зокрема через зміну експресії білків щільних міжклітинних контактів, серед яких важливе місце посідає zonula occludens-1 (ZO-1).

Мета — оцінити зміни експресії ZO-1 у хронічних ранах діабетичної стопи після застосування комбінованої місцевої терапії та визначити потенційну роль ZO-1 як раннього молекулярного маркера відновлення епітеліального бар'єра.

Матеріали та методи. У проспективне рандомізоване дослідження було залучено 28 пацієнтів із хронічними ранами діабетичної стопи. Пацієнтів розподілили на групу дослідження (n=14), якій призначали комбіновану місцеву терапію у вигляді спрею та гелю, що містили колаген, гіалуронат, амінокислоти, мікроелементи (Zn, Cu) й антисептичні компоненти, та контрольну групу (n=14), в якій застосовували стандартні пов'язки з хлоргексидином. Біоптати епідермісу отримували на початку дослідження (0-й день) та через 10 днів лікування. Експресію ZO-1 визначали методом Western blot із подальшим денситометричним аналізом.

Результати. У пацієнтів, які отримували комбіновану місцеву терапію, на 10-й день спостерігали вірогідне підвищення експресії ZO-1 порівняно з вихідними показниками, що свідчило про відновлення цілісності міжклітинних контактів. У контрольній групі такі зміни не виявлено.

Висновки. Застосування комбінованої місцевої терапії сприяє молекулярному відновленню епідермального бар'єра при хронічних ранах діабетичної стопи, що підтверджується підвищенням експресії ZO-1. Отримані результати свідчать про клінічну значущість ZO-1 як об'єктивного біомаркера відповіді на терапію при хірургічному лікуванні хронічних діабетичних ран.

Ключові слова: хронічні рани, діабетична стопа, ранова репарація, щільні міжклітинні контакти, ZO-1 (zonula occludens-1), денситометрія, місцева терапія.

FOR CITATION

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