

Received: 2022.03.20  
Accepted: 2022.06.16  
Available online: 2022.07.25  
Published: 2022.08.03

# Evaluation of Bromelain-Based Enzymatic Debridement Combined with Laser Doppler Imaging and Healing of Burn Wounds

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**Financial support:** This work was supported by research grants DS495 and GI10 of the Medical University of Lublin  
**Conflict of interest:** None declared

**Background:** Accurate estimation of burn depth is crucial for correct treatment decision making. Bromelain-based enzymatic debridement (ED) may improve clinical assessment of burn depth. Laser Doppler imaging (LDI) provides a valuable indicator of burn depth by analyzing microcirculation within tissue beds. This study aimed to evaluate bromelain-based enzymatic debridement combined with laser Doppler imaging and healing of 42 wounds in 19 patients with mixed second- and third-degree thermal burns.





**Material/Methods:** We included 42 wounds in 19 patients with mixed deep dermal and full-thickness thermal burns. All patients were treated with eschar-specific removal agent for ED. The perfusion of each wound after ED was assessed using LDI. Healing time was estimated by 2 experienced burn surgeons and marked by the observation of epithelization. The usefulness of the LDI performed after ED in predicting healing time was estimated. The findings were analyzed to determine a cut-off value for LDI that indicates if a burn will heal spontaneously.

**Results:** We observed that burn wounds with higher mean perfusion healed faster. The analysis showed a strong relationship between perfusion after ED and healing time (Spearman rank correlation coefficient=-0.803). A mean perfusion greater than 296.89 indicated that the wound could heal spontaneously and does not require skin grafting.

**Conclusions:** LDI examination of an already debrided wound allows for a reliable assessment of perfusion at an early stage of treatment. The use of a safe and effective debridement method in conjunction with a non-invasive diagnostic tool could improve burn management.

**Keywords:** Burns • Debridement • Laser-Doppler Flowmetry • Perfusion • Wound Healing

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/936713>

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## Background

Burns depth estimation is a challenging task that requires objective techniques to accomplish. Distinguishing between burn wounds that will heal spontaneously and those that will need surgery is critical to patient outcomes, providing better quality of care and keeping medical costs down. This requires a method that is effective in terms of accuracy and timely decision making [1,2].

Clinical assessment is the most common technique used to determine the burn depth. However, it is accurate in only 60 to 75% of cases, even when carried out by an experienced burn surgeon [3]. Biopsy with histological examination is considered the criterion standard and is regarded as an excellent method for experimental research. However, biopsy is an invasive method, leaves additional scars, and needs an experienced pathologist to interpret specimens. Its application therefore has limited practical application in the clinical setting [4].

The management plan and prognosis for superficial and deep burns are entirely different; therefore, an accurate burn depth diagnosis is crucial. Superficial burns usually heal in 3 weeks without pathological scars. On the contrary, deep burns are associated with burn scar pigmentation disorders, hypertrophic scarring, and even contracture formation [5]. An appropriate treatment strategy is selected based on the assessment of burn depth [6,7]. Clinically, burns can be divided into superficial, which are treated conservatively, and deep burn wounds requiring surgical therapy [8,9]. An overly aggressive strategy of treatment for superficial burns will cause unnecessary damage to healthy tissues. In contrast, conservative treatment of deep burns may lead to prolonged healing and significantly worse outcomes [10]. In addition, the choice of an adequate method of treatment is made more demanding by the fact that most of the burned surface is not uniform in depth. Tangential excision is insufficiently selective because both necrotic and healthy tissue are excised. The principal drawback of fascia excision is that the debridement inevitably involves removing some portion of healthy and viable subcutaneous tissue. Another disadvantage is that this method may create a considerable contour deformity [11].

Enzymatic debridement (ED) proved to be an effective method for selective eschar removal [12,13]. Nexobrid®, a form of bromelain-based debridement agent that is derived from pineapple stems, has gained popularity in recent years. Its benefits are mainly due to eschar removal without removing any viable tissue. ED is particularly useful in deep partial and full-thickness wounds and has been evaluated in several studies [14].

Clinical examination of debrided wound appears to be a promising technique. ED may improve clinical assessment of burn depth, increasing its specificity and sensitivity [15].

Various technologies have been developed to obtain a more precise estimation of burn depth: high-frequency ultrasound, magnetic resonance imaging, vital dyes, indocyanine green videoangiography, thermography, near-infrared spectroscopy and laser Doppler imaging (LDI). The latter seems to be the most advantageous technique for the evaluation of burn wound and thus for the determination of wound healing potential. LDI is a non-invasive, non-contact method of measuring blood flow in tissue. It works by analyzing microcirculation within tissue beds [16-19].

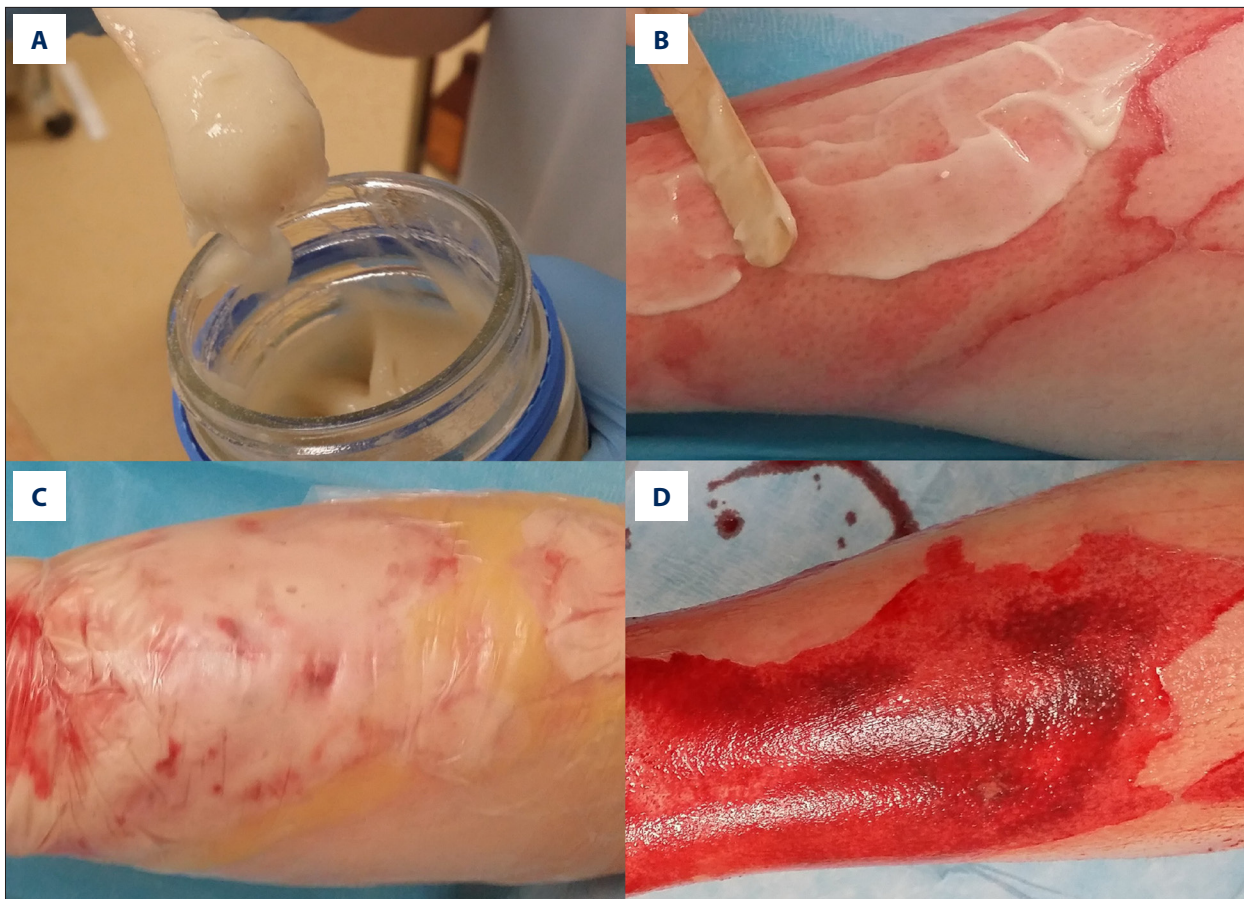
Therefore, this study aimed to evaluate bromelain-based enzymatic debridement combined with laser Doppler imaging and healing of 42 wounds in 19 patients with mixed second- and third-degree thermal burns.

## Material and Methods

The study protocol received approval from the Ethics Committee of the Medical University of Lublin (reference: KE-0254/249/2020). Informed consent was obtained from the patients, allowing the authors to include the pictures and treatment details in this study.

We included 42 burn wounds in 19 patients presenting in the East Centre of Burns Treatment and Reconstructive Surgery, District Hospital in Łęczna (Poland) in 2020. We enrolled patients with mixed deep dermal and full-thickness thermal burns who underwent ED and diagnosis with LDI. The procedure was done between days 1-3 of the burn injury. Of the 42 wounds included in the study, all were classified in visual assessment as mixed second- and third-degree burns.

ED was performed using Nexobrid (MediWound GmbH, Germany), which consists of proteolytic enzymes enriched in bromelain derived from stems of pineapple plants and is indicated for the removal of dead tissue in thermal burns [20]. ED is highly recommended in mixed mid-to-deep dermal or indeterminate burns to preserve as much viable dermis as possible for improved functional and esthetic outcome. Patients were treated following the protocol used in the Burn Center according to the manufacturer's instructions and European consensus guidelines [21-23]. The entire procedure of ED was performed either under general anesthesia in the operation room/intensive care unit for severe burns when additional procedures were required or bedside in the burn ward by applying analgo-sedation protocol in spontaneously breathing patients. First, the wound was prepared by removing blisters and necrotic epidermis. Then, the mixture of Nexobrid was prepared and applied to the wound along with an occlusive foil dressing for 4 hours (Figure 1).



**Figure 1.** Enzymatic debridement procedure (A – preparation of the Nexobrid, B – Nexobrid application, C – occlusive dressing, D – effect of enzymatic debridement).

The perfusion of each wound was assessed after enzymatic treatment. Each time, before the examination, the wound bed was thoroughly prepared by removing dissolved eschar, exudates, and the remnant of the preparation via scraping with a sterile tongue depressor and gauze.

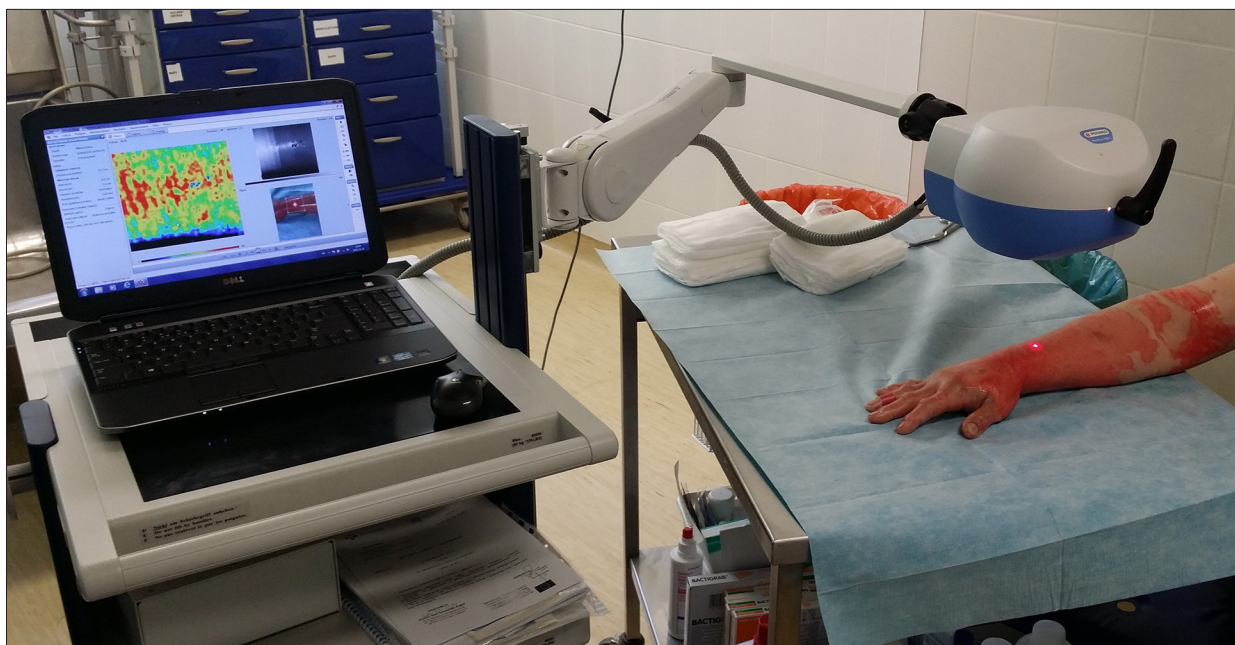
After the ED procedure and LDI examination, followed by soaking the wound for at least 2 hours, dressings were changed with polyhexanide gel and paraffin gauze every other day. If there was no significant progress in epithelization between 14-21 days after ED, autologous skin grafting was applied. In the postoperative period, care of the skin-grafted wounds in these patients was performed similarly to the patients treated conservatively, and the first dressing change was postponed to 3 days after skin grafting. Healing time was estimated by 2 physicians and marked by the observation of epithelization. Clinical assessment was performed by burn surgeons with several years of experience. Wound healing time was assessed as the time between injury and complete epithelialization, calculated in post-burn days.

LDI scans were done using PeriScan PIM 3 (Perimed AB, Stockholm, Sweden). The PeriScan PIM 3 System is a blood

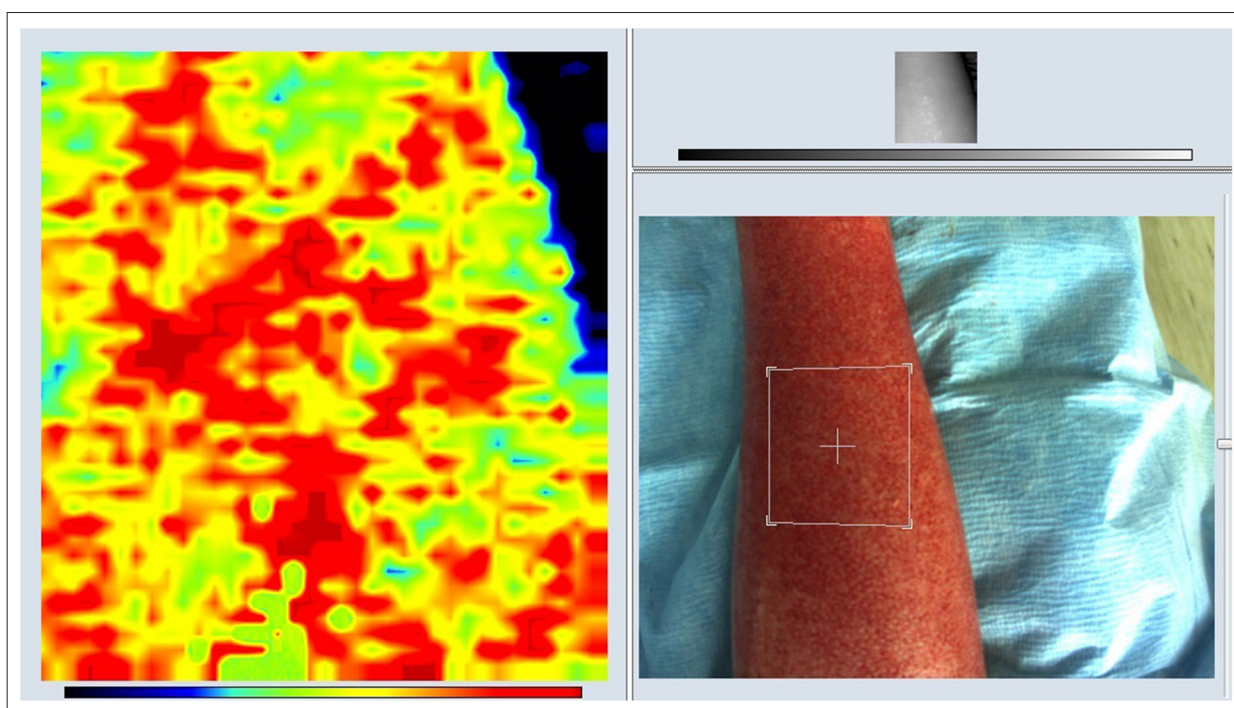
perfusion imager based on laser Doppler technology which uses a low-power, 670-nm, solid-state laser beam. The system can visualize spatial blood perfusion over time in selected measurement areas.

The experienced burn surgeons defined regions of interest (ROI) according to their clinical diagnosis at the time of the LDI scan. Determination of ROIs was based on clinical evaluation of intermediate depth. The mean surface area of the ROI ranged from 12 to 44 cm<sup>2</sup> (mean 20 cm<sup>2</sup>), with the head of the LDI unit placed perpendicularly to the wound. The immobility of the examined area was ensured as far as possible. The average distance from the scanner to the top of the wound was 14 cm (**Figure 2**).

Blood perfusion was presented with a color-coded palette ranging from dark blue to red, numerically corresponding to perfusion units (PU). Moreover, camera and greyscale photos were obtained (**Figure 3**). Mean PU for each ROI was calculated using the software of the LDI System. Mean PU reflects the average concentration and velocity of blood cells, which is proportional to tissue perfusion.



**Figure 2.** Laser Doppler examination (Periscan PIM 3, Perimed AB, Stockholm, Sweden).



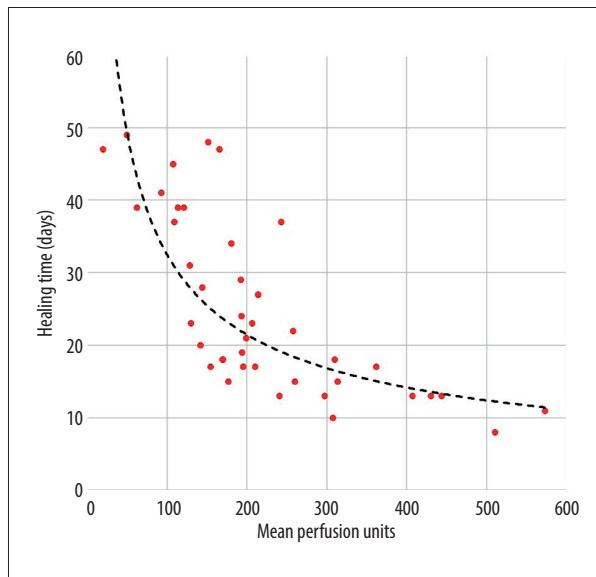
**Figure 3.** Laser Doppler image of the enzymatically debrided forearm burn wound and corresponding clinical photograph.

The potential of the LDI performed after ED was estimated. The findings were analyzed to determine a cut-off value for LDI that indicates if a burn will heal spontaneously and agreement accuracy between the LDI outcome/healing potential category and actual healing results.

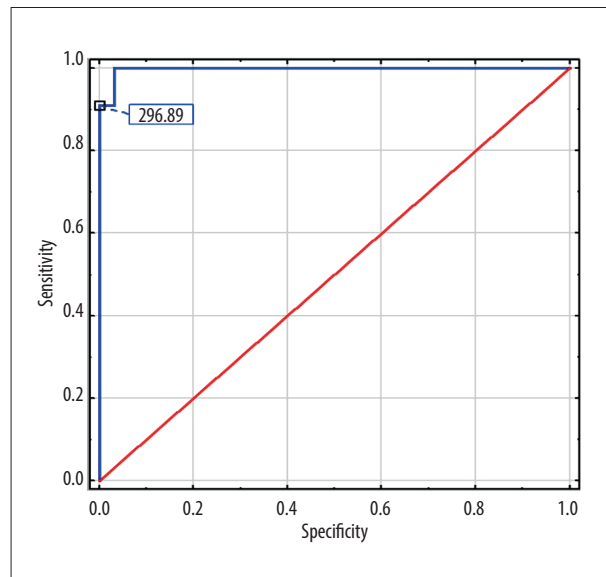
Data were summarized as  $\text{means} \pm \text{SD}$ . Differences were considered significant when the  $P$  value was less than 0.05. The relationship between perfusion and healing time was calculated using the Spearman correlation coefficient. The usefulness of LDI in assessing the potential for burn wound healing was evaluated using receiver operating characteristic (ROC) curve analysis.

**Table 1.** Characteristic of the study group.

Demographics			Burn size		
Mean age	35.0	(5-63)	Average % TBSA	18.3%	(3-48%)
Male	13	68.4%	<b>Region of ROI</b>		
Female	6	31.6%			
Burn cause			Hand	16	38.1%
			Forearm	12	28.6%
Flame	13	72.2%	Arm	5	11.9%
Scald	2	11.1%	Thigh	4	9.5%
Cinder	2	11.1%	Leg	3	7.1%
Asphalt	1	5.6%	Foot	1	2.4%
Electric arc	1	5.6%	Chest	1	2.4%



**Figure 4.** Relationship between mean PU (perfusion units) and healing time.



**Figure 5.** ROC (receiver operating characteristic) curve for mean PU (perfusion units) after enzymatic debridement in predicting the spontaneously healing.

## Results

A total of 42 burn wounds among 19 patients were selected as ROIs (1 to 4 ROIs/patient). The mean age of the patients was 35 years. Within the study group, the most common cause of injury was flame (72%). Scald burns accounted for 11% of total cases. The burn size ranged from 3% to 48% total body surface area (**Table 1**).

Eleven wounds (26.2%) healed spontaneously, while 31 wounds (73.8%) needed to be covered with skin grafts. The healing time ranged from 8 to 49 days (median, 21.5 days). We observed that burn wounds with higher mean PU healed faster. The healing time became shorter as the mean PU increased

(**Figure 4**). The statistical analysis showed a strong relationship, with Spearman rank correlation coefficient = -0.803 ( $P < 0.0001$ ).

The receiver operating characteristic curve (ROC) showed that the mean PU determined by LDI after ED could be an excellent objective tool in predicting the potential for burn wound healing. The area under the ROC curve was 0.997 (95% CI = 0.988-1.000). A mean PU greater than 296.89 could indicate that the wound would heal spontaneously and would not require skin grafting (**Figure 5**). The agreement accuracy between the LDI outcome/healing potential category and actual healing results was 97.62% (**Table 2**).

**Table 2.** Agreement/diagnostic accuracy between the laser doppler imaging outcome/healing potential category and spontaneous healing/skin grafting.

No	Flux value	Healing category	Skin grafts	Agreement
1	92.59	Low potential	Yes	Yes
2	49.29	Low potential	Yes	Yes
3	19.96	Low potential	Yes	Yes
4	296.89	High potential	No	Yes
5	195.46	Low potential	Yes	Yes
6	193.62	Low potential	Yes	Yes
7	510.4	High potential	No	Yes
8	307.37	High potential	No	Yes
9	120.57	Low potential	Yes	Yes
10	180.69	Low potential	Yes	Yes
11	151.28	Low potential	Yes	Yes
12	165.3	Low potential	Yes	Yes
13	242.98	Low potential	Yes	Yes
14	113.74	Low potential	Yes	Yes
15	107.51	Low potential	Yes	Yes
16	310.01	High potential	No	Yes
17	257.53	Low potential	No	No
18	443.67	High potential	No	Yes
19	407.35	High potential	No	Yes
20	572.98	High potential	No	Yes
21	176.73	Low potential	Yes	Yes
22	240.56	Low potential	Yes	No
23	154.69	Low potential	Yes	Yes
24	108.83	Low potential	Yes	Yes
25	62.25	Low potential	Yes	Yes
26	206.63	Low potential	Yes	Yes
27	193.02	Low potential	Yes	Yes
28	192.63	Low potential	Yes	Yes
29	214.26	Low potential	Yes	Yes
30	144.17	Low potential	Yes	Yes
31	141.94	Low potential	Yes	Yes
32	313.37	High potential	No	Yes
33	199.02	Low potential	Yes	Yes
34	361.59	High potential	Yes	Yes
35	169.89	Low potential	Yes	Yes
36	210.42	Low potential	Yes	Yes
37	430.56	High potential	No	Yes

**Table 2 continued.** Agreement/diagnostic accuracy between the laser doppler imaging outcome/healing potential category and spontaneous healing/skin grafting.

No	Flux value	Healing category	Skin grafts	Agreement
38	259.78	Low potential	Yes	Yes
39	127.98	Low potential	Yes	Yes
40	213.6	Low potential	Yes	Yes
41	169.61	Low potential	Yes	Yes
42	129.67	Low potential	Yes	Yes
<b>Agreement</b>				<b>97.62%</b>

## Discussion

Enzymatic debridement seems to be the method of choice in wounds of varying depth due to its selectivity. Nexobrid allows for the minimally invasive removal of necrotic tissue. When ED is applied to superficial wounds, it is not harmful to the vital layers of the skin [24].

Due to the ability to preserve vital dermis and reducing the need for subsequent grafting, this method is considered to be a minimally invasive modality. The benefits of ED are evident in published randomized and single-arm studies [14]. Randomized, multi-center studies show that the use of Nexobrid reduces the time required for complete debridement of burn wounds [18].

Nexobrid, in addition to its debridement features, is also a diagnostic tool [16,17]. Identifying the layer to which the wound was debrided is the key for determining the burn depth. Clinical examination is the most commonly method used for evaluating wound bed after enzymatic debridement. It is based on assessing wound bed color and bleeding patterns. The larger the diameter of the circular patterns in the dermis, the deeper the layer affected. The exposed subcutaneous tissue after the procedure is a clear indication for wound coverage with skin grafting [20]. The reliability of the clinical evaluation of burn depth is questionable. It is the same with the visual assessment of wound bed after using Nexobrid, because considerable variation exists between different clinicians. The most difficult task is to differentiate between superficial and deep partial-thickness burn. A high level of experience with enzymatic debridement is crucial.

The use of LDI to evaluate tissue perfusion after ED appears to be an effective method to reliably determine the burn depth. This study showed that the perfusion value of wounds after the use of Nexobrid has a strong correlation with healing time. In addition, the analysis showed that wounds with a mean PU greater than 296.89 did not require skin grafts. This may provide a cut-off value indicating of a wound's potential for spontaneous healing. Other researchers also determined cut-off

perfusion values, which varied between 200 PU and 300 PU. However, they used it to distinguish superficial partial-thickness from deep partial-thickness or full-thickness burns in the standard LDI diagnostic procedure for assessing an undebrided wound [25-30]. LDI is regarded as an effective aid to clinical judgment when considering the excision of burns with indeterminate depth [31,32]. Nonetheless, in this regard, the results of this study reveal a novel diagnostic and therapeutic option.

The use of both methods allows evaluation of the perfusion of wounds that have been debrided of dead tissue. Both techniques (ED and LDI) work well together, especially for burn wounds of indeterminate depth [14,22]. The ability to predict whether a burn wound will heal spontaneously or not would significantly improve patient outcomes and decrease the time to recovery, reducing the hospital stay, overall cost, and morbidity. Early and accurate burn depth diagnosis improves the efficacy and precision of burn care, but more uniform and research is needed on the use of the combined technique.

## Limitations

This is the first study investigating the use of a combination of ED and LDI in the assessment of burn depth, and it has some limitations. The combined technique may generate higher costs and be time-consuming. However, this novel approach can evaluate burn wound depth more precisely, predict outcome, and help make a decision for surgery in the early stages of care for patients with indeterminate burns. Some factors may affect the measurement, such as the presence or absence of burn shock, debridement, and examination of various parts of the body with different thickness or wound temperature. Notwithstanding these drawbacks, the ED/LDI combined technique proved more effective when compared to a single LDI method [33]. The optimal scan time is important, as the burn evolves over 48-72 hours. For that reason, it is recommended to perform the LDI at least 48 hours after injury [34-36]. Examination of an already debrided wound allows for a reliable assessment of perfusion at a very early stage of treatment.

## Conclusions

Laser Doppler imaging examination of an already enzymatically debrided wound allows for a reliable assessment of perfusion at a very early stage of treatment. The use of a safe and effective debridement method in conjunction with a non-invasive diagnostic tool may ultimately meet a high number of requirements for burn assessment in routine clinical use.

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