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ORIGINAL RESEARCH-CLINICAL SCIENCE

Topically administered purified clinoptilolite-tuff for the treatment of cutaneous wounds: A prospective, randomised phase I clinical trial

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Abstract

In an ageing society, chronic ulcers pose an increasingly relevant healthcare issue associated with significant morbidity and an increasing financial burden. Hence, there is an unmet medical need for novel, cost-effective therapies that improve healing of chronic cutaneous wounds. This prospective, randomised, open-label, phase I trial investigated the safety and tolerability of topically administered purified clinoptilolite-tuff (PCT), mainly consisting of the naturally occurring zeolite-mineral clinoptilolite, in artificial wounds in healthy male volunteers compared to the standard of care (SoC). We found that topically administered PCT was safe for therapeutic application in acute wounds in healthy male volunteers. No significant differences in wound healing or wound conditions were observed compared to SoC-treated wounds. However, we found a significantly higher proportion of CD68-positive cells and a significantly lower proportion of α-smooth muscle actin-positive cells in PCT-treated wounds. Scanning electron microscopy revealed PCT particles in the restored dermis in some cases. However, these did not impede wound healing or clinical symptoms. Hence, purified PCT could represent an attractive, cost-effective wound treatment promoting the process of healing.

KEYWORDS
chronic wounds, clinical trial, clinoptilolite, wound dressing zeolite

1 | INTRODUCTION

Chronic wounds represent a major healthcare issue worldwide, imposing substantial financial and social burden.[1,2] Chronic ulcers are among the most commonly encountered healthcare problems.[3] Their prevalence is

Abbreviations: αSMA, α-smooth muscle actin; EDS, energy-dispersive X-ray spectroscopy; H&E, hematoxylin and eosin; IHC, immunohistochemistry; PCT, purified clinoptilolite-tuff; SoC, standard of care.

estimated to be 0.18%–2% in the overall population and up to 5% in patients aged over 65 years.[4] Leg ulcers, the predominant type of chronic wound, can often be attributed to vascular diseases, such as chronic venous insufficiency, arterial occlusive disease, diabetes, and prolonged pressure.[5] Although leg ulcers are common, treatment represents a major challenge,[6] as many wounds fail to heal.[7–9] Risk factors for healing failure include a larger wound area, longer wound duration, and wound infection.[10] There is also a clear association with age, as chronic wounds predominantly affect elderly patients.[6,11] Therefore, in an ageing society, chronic ulcers pose an increasingly relevant healthcare issue associated with significant morbidity and an increasing financial burden.[3,6] In developed countries, the cost of treatment for chronic wounds is estimated to account for 3% of healthcare budgets.[1] Hence, there is an unmet medical need for novel, cost-effective therapies that improve healing of chronic cutaneous wounds.[12]

Topically administered purified clinoptilolite-tuff (PCT), mainly consisting of the naturally occurring zeolite-mineral clinoptilolite, may represent an attractive, cost-effective therapeutic substance for the treatment of cutaneous wounds.[13,14] PCT was produced passing a thoroughly quality-controlled and validated purification process with terminal micronization- and heating-steps and sterilisation using gamma-irradiation. This procedure guarantees a heavy-metal reduced, fined-grained and sterile product that can be safely and efficiently applied for medical purposes.[15] The medical utility of clinoptilolite results from its physicochemical properties that makes it suitable for medical use.[16–18] Clinoptilolite is strongly hygrophilic, possesses a unique ability for reversible cation exchange and a high adsorption capacity for diverse biomolecules.[15–24] Because of the resulting potential detoxifying effects, clinoptilolite-based products have been proposed in many medical applications.[16,18,23,25,26] PCT is currently marketed for oral use aimed at preventing the absorption of toxins, heavy metals and dietary cholesterol.[15,25] Recently, an increasing number of clinoptilolite-based products have shown high potential in wound healing, skin inflammation and irritation, adsorption of exudate and prevention of microorganism penetration.[18–20,27–35] A major advantage of PCT application in wound healing is its ability to adsorb wound exudate and irreversibly bind bacteria. In vitro studies on the adsorption capacity of zeolite have shown that adsorption is not limited to microorganisms, as clinoptilolite can also adsorb bacterial toxins.[15,27,36,37] In addition, clinoptilolite has also been shown to efficiently adsorb biogenic amines that contribute to foul wound odours and pruritus.[38,39] This represents new potential area of application, as the treatment of malodorous wounds still represents a largely unsolved medical need, especially in the field of chronic wounds associated with neoplastic skin manifestations.[40,41]

In vivo studies in rodents have shown promising results for the use of clinoptilolite-based products in wound management.[29,42,43] However, so far, the clinical safety of topical PCT for the treatment of cutaneous wounds has not yet been examined in prospective, controlled clinical trial. Therefore, the present prospective phase I clinical trial evaluated the safety and tolerability of topically administered PCT in artificial wounds in healthy male volunteers compared to the standard of care (SoC).

2 | MATERIALS AND METHODS

This study was conducted in compliance with Good Clinical Practice and the Declaration of Helsinki[44] and received approval from the Ethics Committee of the Medical University of Vienna (EK-1056/2020). All volunteers gave their informed consent. The trial is registered at clinicaltrials.gov (NCT04417647). This trial was reported following the CONsolidated Standards of Reporting Trials guideline.[45]

The study was designed as a prospective, randomised, open-label, controlled, explorative trial to investigate the safety and tolerability of topically administered PCT in healthy male volunteers with artificial full-thickness wounds. The investigational product was compared to SoC, which consisted of rinsing with 0.9% saline solution and application of wound dressing. Due to the nature of PCT and SoC, the study was open label. The study was performed at a single investigational site, the Department of Clinical Pharmacology of the Medical University Vienna, Austria, between June and August 2020. The skin punch biopsy procedure and histological evaluation of biopsy specimens were performed by the Department of Dermatology. The histopathological analysis was performed between August and December 2020.

2.1 | Skin punch biopsies and treatment

After an initial screening visit, 12 healthy volunteers were scheduled for the first intervention (Visit V2, Day 0), where all participants received two full-thickness 4-mm punch biopsies with a minimum distance of 10 cm on the inner side of the proximal and distal parts of the nondominant upper arm. After performing the two biopsies, both wounds were cleansed with 0.9% saline solution. The application of verum (PCT) and control (SoC) to the proximal and distal full-thickness wounds was randomised 1:1, resulting in six participants per group (Group 1: SoC treatment on the proximal wound and PCT application on the distal wound and Group 2: PCT application on the proximal wound and SoC treatment on the distal wound). A uniform layer of PCT powder was applied directly to the open surface of the wound to cover the entire wound surface. To prevent treatment crossover, the wound receiving SoC was covered first. Subsequently, a nonadhesive wound dressing (Adaptic™; Johnson & Johnson Medical, Inc.) was applied to both wounds, conventional Mepore® (Mölnlycke Health Care GmbH) wound dressing was placed on top to further protect the wound, and the dressing was secured in place by a bandage. Wound cleansing and dressing change were performed every second day from V3 to V8 (Days 2–12). The local tolerability was assessed by the investigator using an erythema score and by the subjective assessment of local pain. At V9 (end of treatment, Day 14 ± 1), rebiopsies (6-mm punch biopsy) were performed at the same locations overlapping the previous biopsy sites. The resulting wounds were closed with surgical sutures, which were removed at the end of study visit (Day 28 ± 1) (Figure 1).

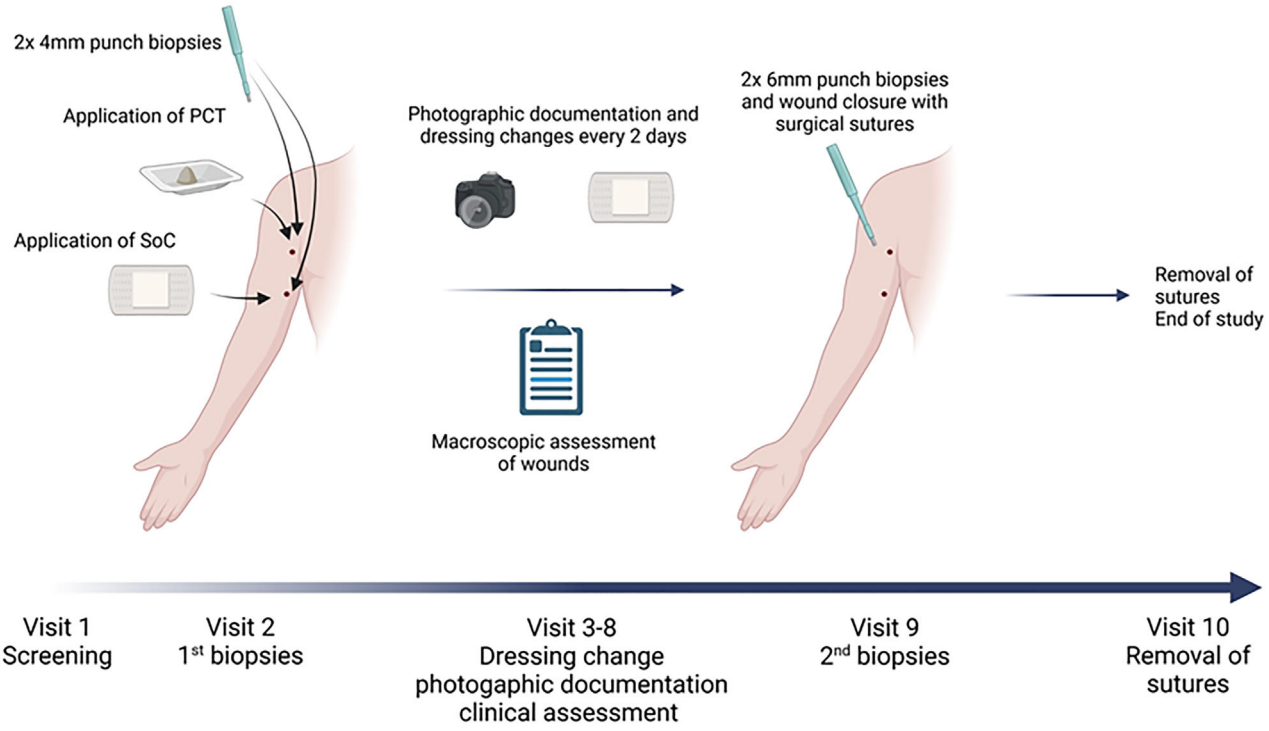

FIGURE 1 Study design. Participants attended a screening visit prior to the study start (Visit 1, Day −14 to −1). At Visit 2 (Day 0), all subjects received two full-thickness 4-mm punch biopsies (first biopsy) on the inner side of the nondominant upper arm. Purified clinoptilolite-tuff and standard of care were applied in a randomised manner to the proximal and distal full-thickness wounds. Wound cleansing and dressing change, as well as assessment of the wound condition, were performed every second day during Visits 3–8 (Day 2 and then every second day). In addition, the respective treatment was reapplied during each visit. At Visit 9 (Day 14 ± 1), end-of-treatment (EoT), 6-mm full-thickness punch rebiopsies (second biopsy) were performed covering the first biopsy, and the wound site was closed with a surgical suture. All sutures were removed at the end-of-study visit (EoS, Visit 10, Day 28 ± 1) [Color figure can be viewed at wileyonlinelibrary.com]

2.2 | Macroscopic observation of wound healing and wound condition

Wound healing, including wound closure, cavity formation, and wound condition (presence of infection and exudate), were assessed as dichotomous outcomes over 14 days of treatment. In addition, the erythema severity in PCT- and SoC-treated wounds was assessed using a 5-point erythema severity grade ranging from 0 (no visible reaction) to 4 (severe erythema with induration, vesicles, bullae, or pustules and/or erosion or ulceration). Assessments were performed during V3 to V9 (Day 2–14 ± 1).

2.3 | Photographic documentation

Standard clinical wound images were recorded with a digital camera at V2 after the first biopsy and every second day during V3–V8, until the end of treatment (V9; Day 14 ± 1). A disposable paper ruler was placed directly on the skin and included in the photographic frame as an internal standard to allow for subsequent image calibration. The maximum diameter and wound surface area were determined by two independent operators for each time point using IC measure (The Imaging Source Europe GmbH). The measurements were compared to those measured at V2 (first intervention day) to document the decrease in the wound size over time. Upon study completion, processed image files and collected data were transferred to the statistician via a secured web transfer (Figure S1).

2.4 | Preparation of biopsy specimens

Each biopsy specimen (both the proximal and distal 4- and 6-mm biopsies) was prepared such that one half was fixed in 4% formalin for subsequent paraffin embedding and the other half was cut into two equal pieces, one of which was embedded in a formaldehydein/glutaraldehyde cosolution for further analysis using electron microscopy.

2.5 | Histological analysis

All specimens were stained with hematoxylin and eosin (H&E), Elastica van Gieson, von Kossa, and Masson-Trichrome (CoverStainer for H&E and ArtisanLink for other stains; Dako). In addition, immunohistochemical staining was performed using the following antibodies: anti-CD68 (Clone KP1; Dako), anti-CD10 (Clone 56C6; Novocastra), anti-CD31 (Clone JC70A; Dako), and anti-α-smooth muscle actin

(Clone 1A4; Dako). Stained slides were scanned using Aperio Scan Scope and imaged with Aperio Image Scope (Leica Biosystems). H&E-stained slides were prepared to obtain a general overview over the tissue and to evaluate the thickness of the epidermis and dermis and re-epithelialization of the wound bed. Von Kossa-stained slides were used to evaluate the presence of calcification. Elastica van Gieson-stained slides were used to evaluate the presence of elastic fibres and collagen. Masson trichrome staining was performed for the histological visualisation of collagen and identification of collagen subtypes. The amount of mature collagen within the wound area was assessed in a semi-quantitatively manner by scoring for a substantial amount of collagen, a moderate amount of collagen, a low amount of collagen, or no collagen. The biopsies were analysed in a paired fashion: PCT-treated wounds were compared to SoC-treated wounds at V9 and to initial skin biopsies taken at V2 (Figure S2).

2.6 | Quantitative analysis of immunohistochemistry stained tissue sections

Scanned slides were uploaded to the image analysis platform *HALO* (Indicalab®). The 'Multiplex IHC v2.3.4' program was chosen to measure the total cell count and the number of positively stained cells. Annotation layers were drawn to include the dermis and subcutis in the analysis and to exclude the epidermis and a potential superficial wound crust. The settings were checked in real-time tuning and adjusted to achieve optimal results (Figure S3).

2.7 | Scanning electron microscopy

Unlabeled PCT can barely be detected by light microscopy due to its small particle size. However, due to the higher density, the particles clearly contrast with the surrounding tissue in the backscatter (BSE) mode in scanning electron microscopy (SEM).

Biopsy specimens reserved for SEM were incubated in a fixative solution containing 4% depolymerized paraformaldehyde (Science Services) and 2.5% glutaraldehyde (Science Services) in 0.1 M HEPES (Sigma-Aldrich), pH adjusted to 7.5 with NaOH at room temperature for 3 h and thereafter at 4°C for 1 week. The samples were then post-fixed in 1% osmium tetroxide at 4°C for 2 h in the dark. After three washes with MilliQ-purified water, samples were dehydrated in an ascending acetone series (VWR) and embedded in an epoxy resin following the manufacturer's instructions (Epoxy Embedding Medium kit; Sigma-Aldrich). The resin blocks were sectioned using an EM UC6 ultramicrotome (Leica) until the sections showed wound tissue. At this point, the sample surface was sputter-coated with platinum and examined under a Clara SEM in BSE mode at 15 kV (Tescan). Elementary analysis was used to formally identify the embedded particles as aluminosilicates using a 30-mm^2 XFlash detector (Bruker).

TABLE 1 Macroscopic observation of wound healing and wound condition in purified clinoptilolite-tuff-treated and standard of care-treated wounds

Purified clinoptilolite-tuff		V3	V4	V5	V6	V7	V8	V9
Presence of epithelium (macroscopically)	Yes	7	12	12	12	10	11	11
	No	5	0	0	0	1	0	0
Wound area fully closed	Yes	0	0	0	0	0	0	5
	No	12	12	12	12	11	11	6
Presence of undermining and tunnelling	Yes	0	0	0	0	0	0	0
	No	12	12	12	12	11	11	11
Presence of exudate on the dressing	Yes	1	1	1	0	2	0	0
	No	11	11	11	12	9	11	11
Presence of wound infection	Yes	0	0	0	0	0	0	0
	No	12	12	12	12	11	11	11
Standard of care		V3	V4	V5	V6	V7	V8	V9
Presence of re-epithelialization	Yes	5	11	12	12	11	11	10
	No	7	1	0	0	0	0	1
Wound area fully covered with epithelium	Yes	0	0	0	0	0	1	2
	No	12	11	12	12	11	10	9
Presence of undermining and tunnelling	Yes	0	0	0	0	0	0	0
	No	12	12	12	12	11	11	11
Presence of exudate on the dressing	Yes	1	0	1	0	0	0	0
	No	11	12	11	12	11	11	11
Presence of wound infection	Yes	0	0	0	0	0	0	0
	No	12	12	12	12	11	11	11

Note: The investigator evaluated wound healing and wound condition as dichotomous outcomes. Assessment was performed during Visits 3–9 (V3–V9).

TABLE 2 Clinician's assessment of erythema severity in purified clinoptilolite-tuff-treated and standard of care-treated wounds in healthy male volunteers at the different time points

Purified clinoptilolite-tuff	V3	V4	V5	V6	V7	V8	V9
Mean (*SD*)	0.50 (0.52)	0.75 (0.62)	1.08 (0.67)	0.75 (0.45)	0.55 (0.52)	0.73 (0.65)	0.73 (0.65)
Standard of care	V3	V4	V5	V6	V7	V8	V9
Mean (*SD*)	0.50 (0.67)	0.67 (0.65)	0.92 (0.67)	0.67 (0.49)	0.55 (0.52)	0.55 (0.52)	0.73 (0.47)

Note: The 5-point erythema severity grading ranged from 0 (no visible reaction) to 4 (severe erythema with induration, vesicles, bullae, or pustules and/or erosion or ulceration). Assessment was performed during Visits 3–9 (V3–V9).
Abbreviation: *SD*, standard deviation.

An overview image of the sample surface was captured at a low magnification (×30–×50) and, if highly contrasted particles were detected, they were imaged and analysed by energy-dispersive X-ray spectroscopy (EDS) at a higher magnification (×1270).

2.8 | Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 27 (IBM, Inc.) and GraphPad 8.0.1 (Graphpad Software, Inc.). Statistical comparisons were performed using the Fisher's exact test for binary variables, Wilcoxon signed-rank test for ordinal variables, and paired *t*-test for continuous variables. $p < 0.05$ were considered statistically significant. Results of continuous variables are displayed as mean (±standard deviation) if not indicated otherwise. An electronic laboratory notebook was not used.

3 | RESULTS

Twelve healthy volunteers were recruited. One dropped out after Visit 6 due to an incident unrelated to the study; therefore, 12 participants were eligible for analysis until V6 (Day 8) and 11 participants from V7 (Day 10). Eleven participants and 44 histological samples were included in the histological analysis.

At end of treatment (V9, Day 14 ± 1), more PCT-treated wounds than SoC-treated wounds were macroscopically fully closed. However, the difference was not significant (5/11 and 2/11, respectively; $p = 0.36$). Similar frequencies between the two treatment conditions were observed for all other tested parameters, including the presence/absence of epithelium, cavity formation, exudate, and infection (Table 1). The erythema severity was minimal over the entire 14-day treatment period (Table 2), and did not differ between PCT- and SoC-treated wounds.

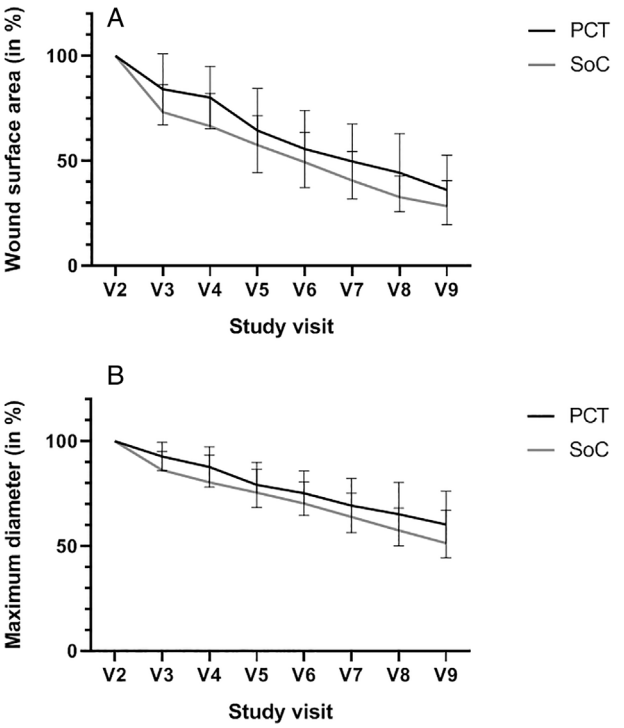

FIGURE 2 Assessment of wound size parameters. The maximum diameter and wound surface area of both the proximal and distal wounds were determined by two independent operators for each time point (visit [V] 2–9) using IC measure. All measurements were set in relation to the parameters measured at V2 (first intervention day) to document the decrease in the wound size over time. (A) Wound surface area in percentage of that of V2. (B) Maximum diameter of the wound in percentage of that of V2. Curves depict mean ± standard deviation. PCT, purified clinoptilolite-tuff; SoC, standard of care

3.1 | Wound surface area

The average remaining wound surface areas at V9 were 7.1 mm^2 (±2.8 mm^2) in PCT-treated wounds and 5.8 mm^2 (±2.6 mm^2) in SoC-treated wounds. The remaining wound surface areas at V9 compared to V2 were 36.1% (±16.6%) and 28.5% (±12.1%) in PCT and SoC-treated wounds, respectively. The difference between PCT- and SoC-treated groups was not statistically significant ($p = 0.07$, Figure 2A).

3.2 | Maximum diameter

The remaining maximum diameters at V9 averaged 3.3 mm (±0.8 mm) in PCT-treated wounds and 2.9 mm (±0.8 mm) in SoC-treated wounds. The remaining maximum diameters at V9 compared to V2 were 60.3% (±15.9%) and 51.5% (±15.7%) in PCT and SoC-treated

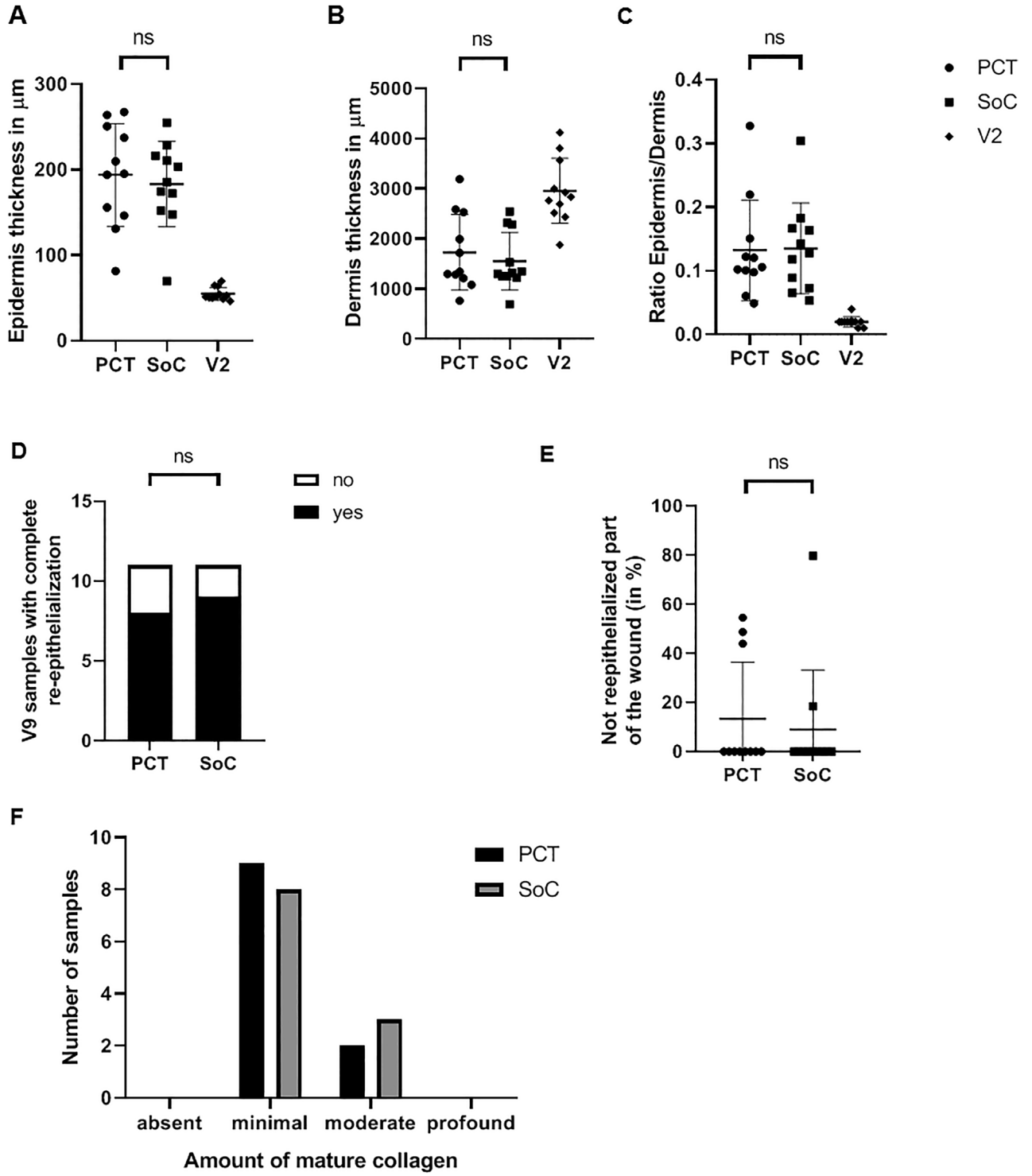

FIGURE 3 Histological analysis. H&E-stained slides were prepared to evaluate the thickness of the epidermis and dermis, ratio of the epidermis to the dermis, full re-epithelialization of the wound bed, and relative proportion of the nonfully re-epithelialized part of the wound. Masson trichrome staining was performed for the histological visualisation and characterisation of collagen. The amount of mature collagen within the wound area was analysed in a semi-quantitative manner to identify a profound, moderate, or minimal amount of mature collagen or no collagen. PCT-treated wounds were compared to SoC-treated wounds. ns, not statistically significant. (A) Thickness of the epidermis in micrometres. (B) Thickness of the dermis in micrometres. (C) Epidermis/dermis ratio. (D) Number of V9 samples with complete re-epithelialization. (E) Non-re-epithelialized proportion of the wound in percentage. (F) Percent of samples with no collagen or a minimal, moderate, or profound amount of mature collagen. H&E, hematoxylin and eosin; PCT, purified clinoptilolite-tuff; SoC, standard of care

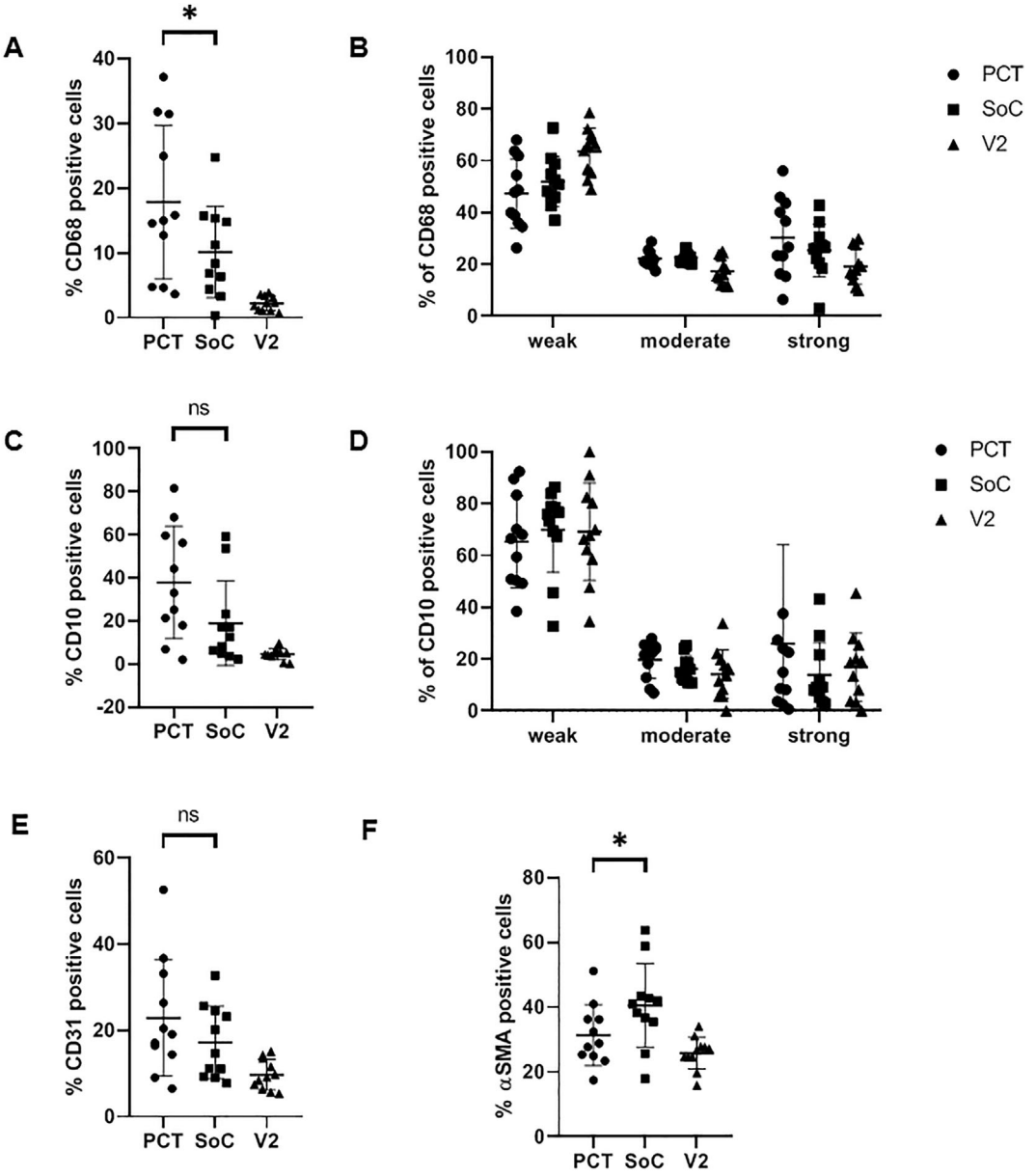

FIGURE 4 Quantitative analysis of IHC-stained tissue sections. The proportion of positive cells was determined for the respective stainings. A distinction was made between weakly, moderately, and strongly positive cells. ns, not statistically significant; *, statistically significant. (A) CD68-positive cells. (B) CD68 weakly, moderately, and strong positive cells. (C) CD10-positive cells. (D) CD10 weakly, moderately, and strongly positive cells. (E) CD31-positive cells. (F) αSMA-positive cells. IHC, immunohistochemistry; PCT, purified clinoptilolite-tuff; SoC, standard of care

wounds, respectively. Although there was a trend towards a smaller maximum diameter in SoC-treated wounds, the results did not differ significantly between the two groups ($p = 0.05$, Figure 2B).

3.3 | Histological assessment

3.3.1 | Epidermis and dermis

The average thickness of the epidermis of PCT-treated wounds was 194.0 μm (±60.1 μm), whereas that of SoC-treated wounds was 183.3 μm (±49.8 μm) ($p = 0.60$, Figure 3A). Compared to the epidermis thickness at V2 (54.8 ± 7.6 μm), epidermis thickness more than tripled during wound healing. The average thickness of the dermis of PCT-treated wounds was 1727.4 μm (±756.4 μm), whereas that of SoC-treated wounds was 1551.4 μm (±574.4 μm, $p = 0.38$, Figure 3B). Compared to the dermis thickness at V2 (2957.3 ± 647.9 μm), dermis thickness decreased significantly upon wound healing. Consequently, the mean epidermis/dermis ratios were significantly higher at V9 compared to V2: 0.13 (±0.08), 0.14 (±0.07) and 0.02 (±0.01) for PCT-treated wounds at V9, SoC-treated wounds at V9 and V2 samples, respectively. No significant difference could be

observed between both treatment groups at V9 ($p = 0.92$, Figure 3C).

3.3.2 | Re-epithelialization

Full re-epithelialization occurred in eight PCT-treated wounds and nine SoC-treated wounds. Three and two wounds were not fully re-epithelialized in the PCT- and SoC-treated groups, respectively ($p > 0.99$, Figure 3D). The not re-epithelialized gaps measured 1469 μm (±297 μm) in PCT-treated, non-re-epithelialized wounds and 1759 μm (±1820 μm) in SoC-treated, non-re-epithelialized wounds ($p = 0.79$). In relation to the size of the original wound, the non-re-epithelialized gap accounted for 49.1% (±5.3%) and 49.1% (±43.4%) of the wounds in the PCT-treated and SoC-treated groups, respectively. The difference between the PCT and the SoC groups was not statistically significant ($p = 0.99$, Figure 3E).

3.3.3 | Calcification

None of the samples showed signs of calcification.

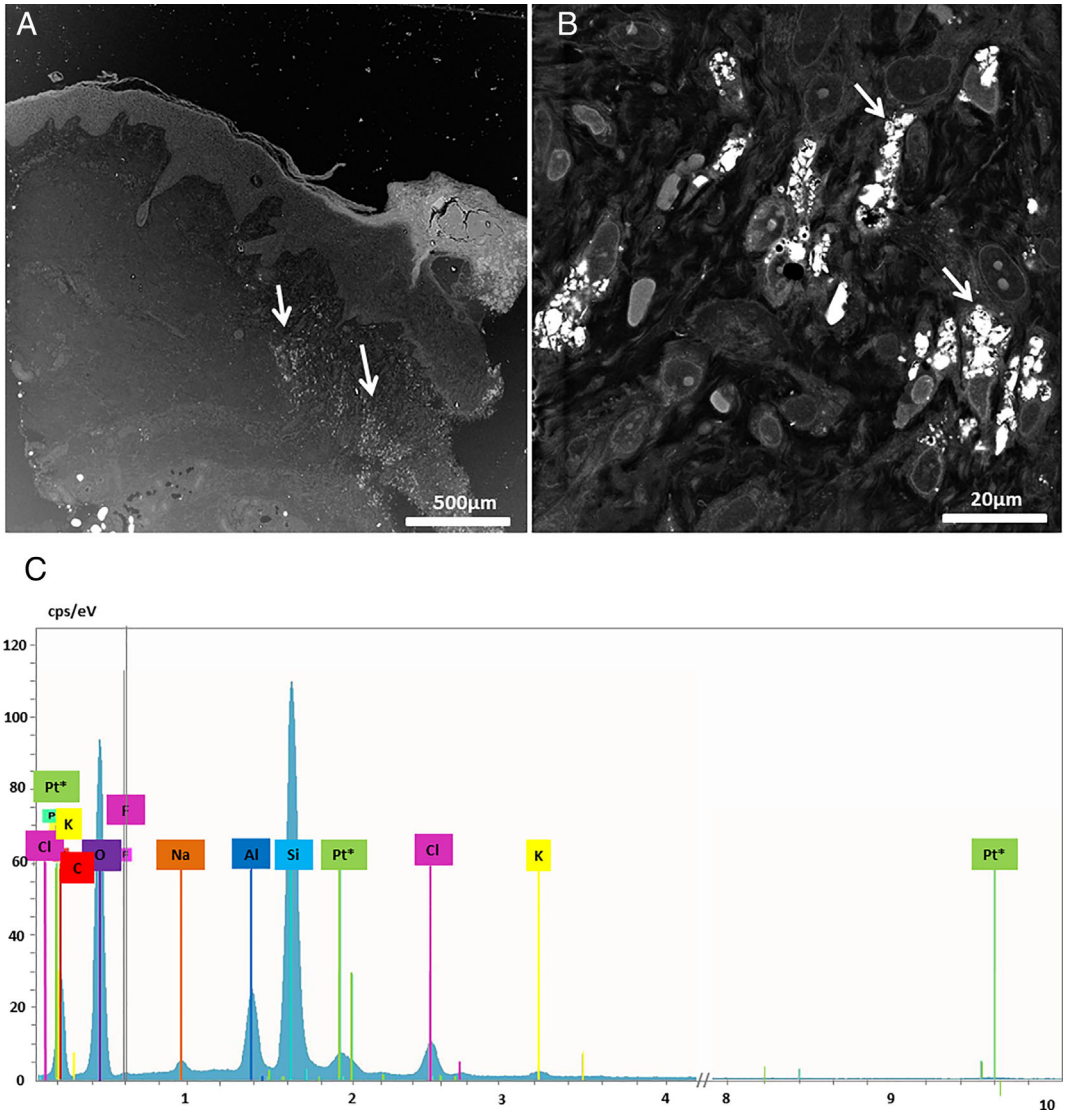

FIGURE 5 Detection and analysis of the distribution of purified clinoptilolite-tuff (PCT) particles inside the healed skin tissue by SEM. Biopsy specimens collected at the end-of-treatment visit were embedded in epoxy resin, and the presence and distribution of PCT particles were analysed by SEM/EDS. (A) Representative SEM image taken at ×47 magnification of a transversal section surface of a biopsy, showing high concentrations of PCT particles in the wound scab and limited clouds of particles inside the dermis. No particles were detected in the epidermis. Arrow heads point towards aggregated particles. (B) Representative SEM image taken at ×1270 magnification showing highly contrasted PCT particles seemingly internalised in dermal cells. The particles do not form extracellular agglomerates. Arrow heads point towards aggregated particles. (C) Typical EDS spectrum of one particle selected from panel (C), demonstrating the particles consist of alumosilicate. EDS, energy-dispersive X-ray spectroscopy; SEM, scanning electron microscopy [Color figure can be viewed at wileyonlinelibrary.com]

3.3.4 | Elastic and collagen fibres

The quantity of elastic fibres and collagen within the wound area was markedly decreased compared to the area surrounding the wound in all samples treated with PCT or SoC (11/11 samples each). In all V2 biopsies (11/11), elastic fibres and collagen were evenly distributed. However, no difference was detected between PCT- and SoC-treated wounds.

Low and moderate amounts of mature collagen were detected in nine and two PCT-treated wounds and in eight and three SoC-treated wounds, respectively. The difference between the PCT- and the SoC-treated wounds was not significant ($p = 0.63$, Figure 3F).

3.4 | Quantitative analysis of immunohistochemistry stained tissue sections

3.4.1 | CD68-positive cells

The proportions of CD68-positive cells in PCT-treated wounds, SoC-treated wounds and V2 samples were 17.9% (±11.9%), 10.1% (±7.1%) and 2.2% (±1.2%), respectively. PCT-treated wounds had a significantly higher proportion of CD68-positive cells than SoC-treated wounds ($p = 0.04$, Figure 4A).

We further distinguished between weakly, moderately, and strongly positive cells. In PCT-treated wounds at V9, the proportions of weakly, moderately and strongly CD68-positive cells were 47.3% (±13.3%), 22.3% (±3.1%), and 30.4% (±15.3%), respectively. The corresponding proportions in SoC-treated wounds were 52.0% (±9.8%), 22.6% (±2.2%) and 25.5% (±10.2%), respectively. In V2 samples, the corresponding proportions were 63.6% (±9.1%), 17.3% (±5.0%) and 19.2% (±6.8%), respectively (Figure 4B). The presence of foreign-body giant cells was observed in three PCT-treated wounds and four SoC-treated wounds ($p > 0.99$).

3.4.2 | CD10-positive cells

The proportions of CD10-positive cells in PCT-treated wounds, SoC-treated wounds and V2 samples were 37.9% (±26.1%), 18.9% (±19.7%) and 4.5% (±2.6%), respectively. The difference between PCT- and SoC-treated wounds was not statistically significant ($p = 0.07$, Figure 4C). In PCT-treated wounds, 65.3% (±17.7%), 19.8% (±7.2%) and 26.0% (±38.1%) were weakly, moderately, and strongly positive for CD10, respectively. The corresponding proportions in SoC-treated wounds were 69.9% (±16.4%), 16.3% (±4.9%) and 13.9% (±12.5%), respectively. In V2 samples, the corresponding proportions were 69.2% (±18.9%), 14.1% (±9.4%) and 16.8% (±13.3%), respectively Figure 4D.

3.4.3 | CD31-positive cells

The proportions of CD31-positive cells in PCT-treated wounds, SoC-treated wounds and V2 samples were 22.9% (±13.5%), 17.2% (±8.5%) and 9.7% (±3.5%), respectively. The difference between PCT- and SoC-treated wounds was not significant ($p = 0.22$, Figure 4E).

3.4.4 | α-Smooth muscle actin-positive cells

The proportion of α-smooth muscle actin (αSMA)-positive cells in PCT-treated wounds, SoC-treated wounds and V2 samples were 31.4% (±9.4%), 40.5% (±12.9%) and 25.8% (±4.9%), respectively. PCT-treated wounds had a significantly lower proportion of αSMA-positive cells than SoC-treated wounds ($p = 0.03$, Figure 4F).

3.5 | Scanning electron microscopy

Nine PCT-treated wounds had PCT particles in the scab and healed skin tissue (Figure 5A). The particles were mainly observed in loosely distributed aggregates in the dermis, no particles were detected in the epidermis. Visual assessment at high magnification and elemental spot analysis via EDS revealed shape and size as well as chemical composition clearly consistent with PCT particles (Figure 5B,C).

4 | DISCUSSION

The primary focus of this clinical study was the investigation of the safety and tolerability of topically administered PCT compared to SoC in acute wounds induced in healthy subjects. We further examined wound healing, wound condition and wound size by macroscopic observation, and characterised biopsy specimens by histopathological and immunohistochemical investigations and SEM.

Our data showed that treatment with PCT was generally well-tolerated. It did neither induce pain nor result in exacerbated erythema or swelling.

Over the 14 days of treatment, the outcome was similar when scored for macroscopic aspects of wound healing and wound condition, including wound closure, cavity formation, exudate and infection. These findings suggest that PCT did not alter wound healing or the wound condition in healthy volunteers. In addition, the presence of erythema in PCT-treated wounds was comparable to that in SoC-treated wounds, and erythema severity was minimal over the entire 14-day treatment period. PCT-treated wounds showed a tendency for a larger wound surface area and maximum diameter at the end of study visit. However, these differences were not statistically significant. Furthermore, we did not detect any statistically significant difference in the epidermal healing response, based on measurements of the epidermis and the dermis, and full re-epithelialization of the wound bed between the treatment groups. Taken together, these results indicate that PCT treatment did not change the natural course of re-epithelialization in this acute wound healing setting. Similarly, we did not observe any difference in the quantity of elastic fibres and collagen or in the proportion of CD10-positive activated dermal fibroblastic cells between the two treatment groups.

PCT-treated wounds showed a significantly higher proportion of CD68-positive cells, indicative of macrophages, than SoC-treated wounds. This observation suggests that PCT treatment induces a macrophage-dominated inflammatory response. This may be due to the presence of PCT particles in the wounds, which is recognised as a foreign material. This interpretation is in line with findings obtained by the SEM, which documented the presence of PCT particles in the treated wounds at the end of the study period. However, foreign-body giant cells were found in only a minority of either PCT- or SoC-treated wounds.

PCT-treated wounds had a significantly lower proportion of αSMA-positive cells than SoC-treated wounds. Thus, PCT treatment may lead to a less contractile wound phenotype, which would be compatible with the slightly, but not significantly, smaller decrease in wound size over time in PCT- versus SoC-treated wounds.

Angiogenesis represents a central part of the proliferative phase of cutaneous wound healing. Therefore, CD31 was used to assess the amount of blood vessels found within the histological sections. The difference in the proportions of CD31-positive cells in the PCT- and SoC-treated wounds was not statistically significant, revealing that PCT treatment did not have any major impact on wound angiogenesis in this setting.

Because of their detoxifying effects, clinoptilolite-based products have been proposed in many medical applications.[16,18,23] However, progress in serious evidence-based research on the efficacy of clinoptilolites is sometimes hampered by hyperbolic and unreasonable promotion as panacea, for example, in the treatment of immunodeficiency[46] and of cancers.[16] In vivo studies in rodents have shown promising results regarding the use of clinoptilolite-based products in wound management.[29,42,43] However, the clinical safety of topical application of clinoptilolite (nonpurified and purified) has not yet been examined in any clinical trial for the treatment of cutaneous wounds. The present results indicate that topical PCT application is safe for treating acute wounds in healthy male volunteers, as no adverse events were reported throughout this phase I clinical trial. Clearly, these preliminary results obtained with a limited number of samples need to be confirmed with a larger sample to definitively conclude about the safety of clinoptilolite application in wound healing in a more complex population.

A major advantage of PCT application in wound healing is its ability to adsorb wound exudate and irreversibly bind bacteria. In vitro studies on the adsorption capacity of zeolite show that this adsorption is not limited to microorganisms, as clinoptilolite can also adsorb bacterial toxins.[15,27,36,37] Previous studies have hypothesised that zeolites may improve the wound healing process and accelerate wound closure.[28,29] These effects were beyond the scope of this phase I clinical trial. However, we observed no statistically significant difference in the re-epithelialization rate or the wound size between the study groups. Importantly, the present study was aimed at evaluating the safety of PCT on local cutaneous wounds and hence lacked assay sensitivity for detecting efficacy in wound healing. In addition, all observations are limited to the setting of artificially created, acute wounds. The implications for larger chronic wounds with superinfection remain unclear.

Previous reports also emphasised the fact that the mechanisms operating in acute wound closure differ substantially from those required for chronic wound healing; thus, results obtained in an acute wound setting are of limited value to understand how a chronic situation can be remedied.[47–49]

In addition to its effects on wound closure, clinoptilolite has also been shown to efficiently adsorb biogenic amines that contribute to foul wound odours and pruritus.[38,39] This represents new potential area of application, as the treatment of malodorous wounds still represents a largely unsolved medical need, especially in the field of chronic wounds associated with neoplastic skin manifestations.[40,41]

Limitations of the study include the use of the acute wound setting (with limited translational value for other types of wounds), the limited observation time of 2 weeks, the rather small size of the artificially generated wounds, and the location of the wounds on the upper extremity.

5 | CONCLUSION

The results of this phase I clinical trial indicate that topical use of purified, heavy-metal reduced, micronised and sterilised, clinoptilolite-tuff in acute cutaneous wounds is safe and well-tolerated in healthy male volunteers for the duration of 2 weeks. However, future studies including a larger sample size are needed to evaluate the safety and efficacy of PCT in chronic wounds in a more complex setting.

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CONFLICT OF INTERESTS

Benedikt Weber and Michael Freissmuth serve as scientific advisors to Glock Health, Science and Research GmbH, Austria. Stephane Nizet, Claudia Meisslitzer and Cornelius Tschegg are employees of Glock Health, Science and Research GmbH, Austria. Julia Deinsberger, Elias Marquart, Kateryna Uspenska, Ghazaleh Gouya, Jan Niederdöckl and Michael Wolzt have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Julia Deinsberger: Investigation, visualisation, writing—original draft. **Elias Marquart:** Investigation, writing—review and editing. **Stephane Nizet:** Methodology, investigation, writing—review and editing. **Claudia Meisslitzer:** Funding acquisition, investigation, writing—review and editing. **Cornelius Tschegg:** Funding acquisition, writing—review and editing. **Kateryna Uspenska:** Conceptualization, project administration. **Ghazaleh Gouya:** Conceptualization, project administration. **Jan Niederdöckl:** Investigation, writing—review and editing. **Michael Freissmuth:** Investigation, writing—review and editing. **Michael Wolzt:** Conceptualization, methodology, writing—review and

editing, project administration, supervision. **Benedikt Weber:** Conceptualization, methodology, investigation, writing—original draft, project administration, supervision.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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