BMJ Open Diabetes Research & Care

# A randomized, placebo-controlled study of chitosan gel for the treatment of chronic diabetic foot ulcers (the CHITOWOUND study)

Matevž Slivnik ,[1,2] Maja Navodnik Preložnik,[3] Mojca Fir,[1] Janja Jazbar,[2] Nanča Čebron Lipovec,[2] Igor Locatelli ,[2] Hélène Liette Lauzon,[4] Vilma Urbančič Rovan[5,6]

**To cite:** Slivnik M, Navodnik Preložnik M, Fir M, *et al*. A randomized, placebo-controlled study of chitosan gel for the treatment of chronic diabetic foot ulcers (the CHITOWOUND study). *BMJ Open Diab Res Care* 2024;**12**:e004195. doi:10.1136/bmjdrc-2024-004195

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjdrc-2024-004195).

Received 15 March 2024
Accepted 7 June 2024



[1]Vizera d.o.o, Ljubljana, Slovenia
[2]University of Ljubljana Faculty of Pharmacy, Ljubljana, Slovenia
[3]General Hospital Celje, Celje, Slovenia
[4]Primex ehf, Siglufjörður, Iceland
[5]University Medical Centre Ljubljana, Ljubljana, Slovenia
[6]University of Ljubljana Faculty of Medicine, Ljubljana, Slovenia

**Correspondence to**
Matevž Slivnik;
matevz.slivnik@vizera.eu

## ABSTRACT

**Introduction** To assess the efficacy of a chitosan-based gel (ChitoCare) for the treatment of non-healing diabetic foot ulcers (DFUs).

**Research design and methods** Forty-two patients with chronic DFUs were randomized to the ChitoCare or placebo gel for a 10-week treatment period and 4-week follow-up. The primary study end point was the rate of complete wound closure at week 10, presented as relative rate.

**Results** Thirty patients completed the 10-week treatment and 28 completed the 4-week follow-up. The ChitoCare arm achieved 16.7% complete wound closure at week 10 vs 4.2% in the placebo arm (p=0.297), 92.0% vs 37.0% median relative reduction in wound surface area from baseline at week 10 (p=0.008), and 4.62-fold higher likelihood of achieving 75% wound closure at week 10 (p=0.012). Based on the results of the Bates-Jensen Wound Assessment Tool, the wound state at week 10 and the relative improvement from the baseline were significantly better (median 20 vs 24 points, p=0.018, and median 29.8% vs 3.6%, p=0.010, respectively).

**Conclusions** ChitoCare gel increased the rate of the DFU healing process. Several secondary end points significantly favored ChitoCare gel.

**Trial registration number** NCT04178525.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Diabetic foot ulcers (DFUs) are the leading cause of lower-limb amputations.
⇒ Some studies indicate that chitosan products may have potential wound healing properties, but the efficacy and safety in DFUs treatment is not well studied.

### WHAT THIS STUDY ADDS

⇒ Efficacy and safety of a chitosan-based gel in treatment of DFUs were evaluated.
⇒ The chitosan-based gel accelerated the healing process of DFUs and significantly improved wound state after 10 weeks and showed a safety profile comparable to placebo.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Chitosan-based gel proved to be a safe and effective option for the treatment of DFUs.

## INTRODUCTION

The estimated number of people with diabetes was 537 million in 2021, and it is expected to reach 783 million by 2045.[1] About 15%–25% of patients with diabetes develop foot ulcers which are the leading cause of lower-limb amputations worldwide.[2–6] Based on data from 11 countries,[7] the major amputation rate in patients with diabetes was 17 times higher than in the general population in 2013, and 85% of these amputations were preceded by a foot ulceration that subsequently worsened to infection or gangrene.[8] Therefore, it is important to detect and treat diabetic foot ulcers (DFUs) in their early stages.

The current standard of care (SOC) for DFUs involves four processes: pressure relief, debridement, infection management, and revascularization when indicated.[9] Under ideal conditions, the median time to healing without surgery is around 12 weeks. However, >30% of DFUs would not heal despite 20 weeks of SOC.[10–12] Such results indicate that SOC may not properly address all factors underlying wound persistence, highlighting the need for new therapies to enhance the healing of chronic DFUs and prevent ulcer recurrence. Several new adjunctive therapies (negative pressure wound therapy, hyperbaric oxygen therapy, bioengineered skin substitutes, topical platelet-rich plasma, and stem cell therapy[9]) are available and have already been evaluated by the International Working Group on the Diabetic Foot (IWGDF).[13] Some of these therapies paired with the best SOC have already been demonstrated to provide clinically relevant benefits, but they

may require highly skilled professionals for their application and/or may not be cost-effective.[9 14 15] However, multifunctional hydrogels, such as chitosan-based gels, could provide a simple, easy-to-apply, cost-effective, and efficient therapy for the exceptionally complex pathology of DFUs.

Chitosan is composed of β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units, and it is structurally similar to tissue components such as glycosaminoglycans. These components interact with various growth factors, receptors, and adhesion proteins, in addition to being the biologically important mucopolysaccharides in all mammals.[16] Chitosan promotes cell chemotaxis in the first stage of the inflammatory response, reduces the duration of the inflammation phase, and allows the growth, proliferation, and differentiation of cells with histoarchitectural tissue organization. These characteristics make chitosan ideal for use in skin and wound repair, as well as tissue regeneration techniques.[17 18] In addition, chitosan can trigger blood clotting and block nerve endings, reducing pain.[19] When applied as a gel, chitosan forms a protective film on drying, provides moisture control and offers a physical barrier against external skin irritants and contaminants. The physical interaction and the non-pharmacological mode of action of the chitosan film on the wound confirm its use as a medical device.[20] Furthermore, chitosan carries a positive charge and can interact with negatively charged groups at the surface of bacterial cells, which consequently alters cell permeability and potentially affects microbial growth or survival.[21–23]

Since 2008, several clinical studies using different chitosan treatments on chronic wounds have shown the efficacy of chitosan in preventing infection, maintaining a moist environment, protecting the wound, and minimizing scar formation.[24–30] Only a few studies have evaluated chitosan-based products for DFU treatment, however, with most including only a small number of cases. Therefore, the aim of this study was to evaluate the efficacy of a chitosan-based gel (ChitoCare medical, Primex ehf, Iceland) in comparison with a placebo gel for the treatment of DFUs that failed to heal with SOC.

## RESEARCH DESIGN AND METHODS

### Study design

The CHITOWOUND study (NCT04178525) was designed as a prospective, multinational, multicenter, double-blind, placebo-controlled, randomized, two-arm clinical trial with four study centers in Slovenia and one center in Croatia. All study centers had specialized diabetic foot units. Monitoring of the study and statistical analysis were performed by independent institutions.

### Subjects

The main inclusion criteria for participation in the study were as follows: patients aged 18 years or more with type 1 or 2 diabetes mellitus and a non-healing, uninfected DFU of Wagner grade <3 (without osteomyelitis) or a postamputation wound that was present for at least 4 weeks and measured 0.5–12 $cm^2$. All selected patients had received SOC (off-loading therapy with healing shoe or vacuum cushioned removable cast walkers and inert dressing). Patients who did not receive off-loading before inclusion underwent a 4-week run-in period, after which their ulcer had to have diminished in size by <40%, which indicated that off-loading alone did not account for significant wound reduction. Patients had to have a hemoglobin 1c ($HbA_{1c}$) value of ≤12% (108 mmol/mol) and adequate perfusion of the affected limb with palpable foot pulses, as assessed by the investigators. Detailed inclusion and exclusion criteria for study enrollment are presented in figure 1.

### Randomization

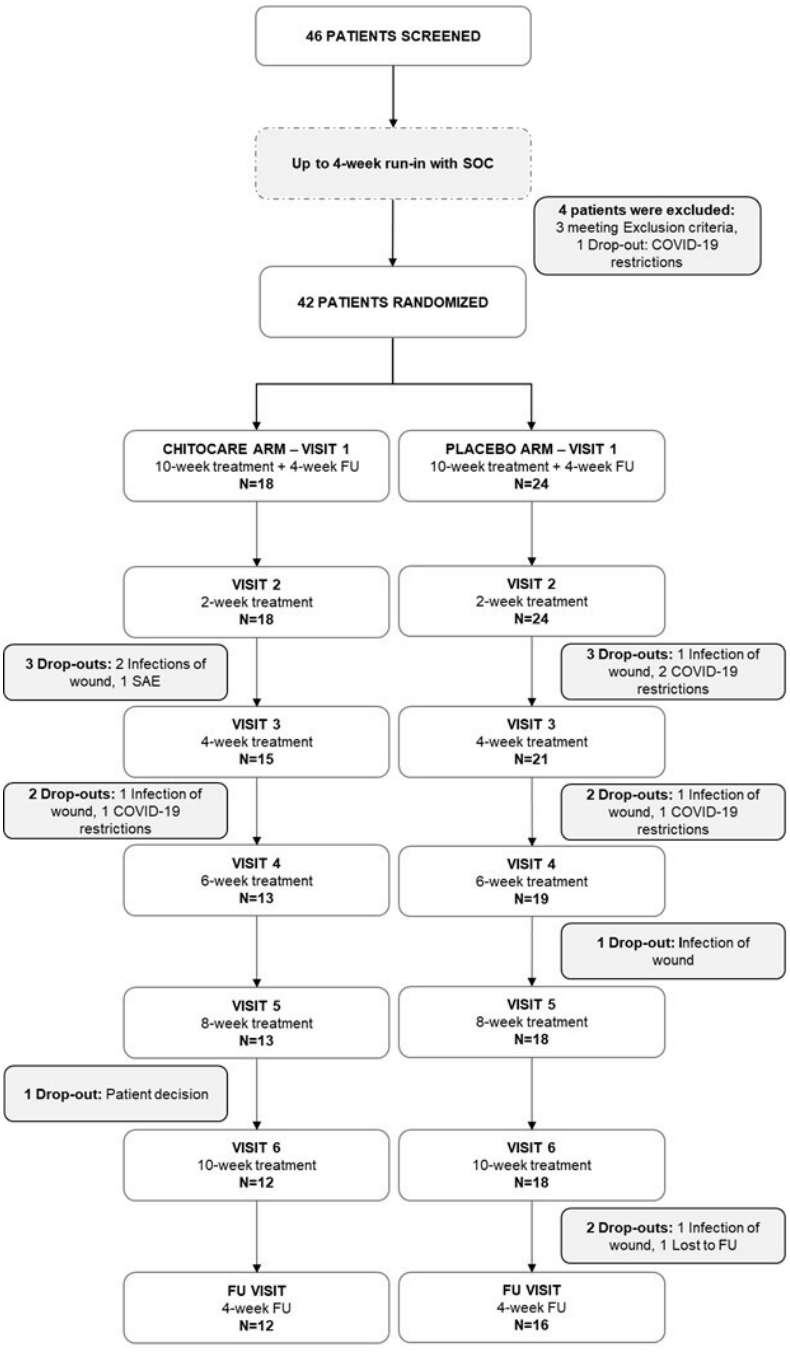
Eligible patients were randomized at the baseline visit (visit 1) to either the ChitoCare or the placebo arm. Simple randomization was performed at a 1:1 ratio at the study and study center level. Randomization was done via previously prepared sealed randomization envelopes containing unique three-digit randomization numbers, pre-allocated to one of the treatment arms. Crossover of patients from one treatment arm to another was not allowed at any time during the study.

### Interventions

All patients were recruited as outpatients at participating sites. The patients visited their study center eight times: screening visit, baseline visit with randomization and first product application, five visits until the end of treatment (weeks 2, 4, 6, 8, and 10), and the follow-up visit at week 14. All patients received SOC provided by nurses who specialized in DFU care and the application of a study product, either ChitoCare medical gel or placebo hydroxyethyl cellulose gel as described by Ravel *et al*.[31] The study products were supplied by the sponsor (Primex ehf) in identical 5 mL airless containers.

Study treatment with the ChitoCare or placebo gel started at the baseline visit (visit 1), up to 28 days post-screening. If multiple wounds were present, the ulcer with the largest area and at least 2 cm from other wounds was designated as the index ulcer. The study gel was directly applied to the wound as a thin layer, completely covering the wound and extending 1 cm from the edges. The gel then dried, forming a flexible film, and the wound was covered with an inert standard dressing. The frequency of application was done in accordance with the frequency of dressing change, which was at least two to three times a week. All secondary wounds were treated with SOC.

On each study visit, wound assessment and debridement were performed before digital photographs of the wound were taken. Off-loading therapy was provided for the duration of the study.




**Inclusion criteria:**
- Written informed consent signed
- Age ≥ 18 years
- Diagnosed with type 1 or type 2 diabetes mellitus
- $HbA_{1c}$ ≤ 12%
- Presence of foot ulcer that met the following criteria:
  a) Size of the wound 0.5 – 12 $cm^2$
  b) Wagner grade 1 or 2
  c) Wound was not infected
  d) Wound was present for at least 4 weeks
- With multiple wounds, the biggest wound fitting the criteria was selected and the distance from other wounds had to be at least 2 cm
- Post-amputation wound could be included if it fulfilled all other criteria
- Adequate perfusion (good, strong foot pulses). If foot pulses were weak, evaluation of perfusion pressures with Doppler ultrasound was performed.
- Able to understand and comply with the requirements of the trial

**Exclusion criteria:**
- Known history of alcohol or drug abuse
- Use of any antibiotic medication within the last 7 days prior to the first application of study product
- Ulcer grade Wagner 3 or higher ulcer
- Severe comorbidity (cancer, heart failure (NYHA class IV), severe anemia (Hb<100 g/L), neoplasia)
- Any significant condition that may preclude the participant from the study (e.g., severe depression or psychiatric illness)
- Severe ischemia of the affected limb requiring revascularization procedure
- Excessive lymphedema that could interfere with wound healing (if there was mild edema treated with compression therapy the patient could be included)
- Autoimmune connective tissue disease
- Previous participation in a study involving this study product
- Participation in another clinical trial involving a device or systematically administered investigational study drug or treatment within 30 days of the randomization visit
- Receiving or scheduled to receive a medication or treatment which, in the opinion of the investigator, was known to interfere with or affect the rate and quality of wound healing
- Medical condition likely to require systemic corticosteroids during the study period
- Allergic to shellfish, chitosan or one of product compounds
- Immobile patients
- Pregnant and lactating women
- Significant increase in wound healing during run-in period (reduction of wound area size ≥ 40%)
- BMI > 39 $kg/m^2$

**Figure 1** Diagram of study flow. BMI, body mass index; FU, follow-up; Hb, hemoglobin; $HbA_{1c}$, hemoglobin 1c; NYHA, New York Heart Association; SAE, severe adverse event; SOC, standard of care.

## Outcomes and end points

At each visit, the photograph of the index wound included a ruler next to the wound to enable assessment of complete wound closure, reduction of wound size area and time to heal. ImageJ software (NIH, V.1.51, 2017) was used to convert the measurements shown by the ruler to pixels for calculating the width, length and area size of the wound. This method enabled determining the wound size more accurately than the standard length by width approximation, especially because most of the wounds were not rectangular in shape. The wound state was assessed using the Bates-Jensen Wound Assessment Tool (BWAT)[32] and Wagner Ulcer Classification[33 34] at each visit.

Ulcer recurrence was tracked for the patients with a completely healed wound. Adverse events were evaluated for each patient at every visit to ensure patient safety during the study. Secondary infections, including infections of the index wound or any other diabetic ulcer, were recorded as adverse events.

The primary study end point was the rate of complete wound closure at week 10, presented as the relative rate

(RR). Secondary end points included (1) wound area reduction from baseline, (2) at least 50% and 75% wound closure, (3) HR (ChitoCare vs placebo) for time to 50%, 75%, and complete wound closure, (4) improvement of wound state assessed with BWAT score and Wagner Ulcer Classification, and (5) occurrence of adverse events. Study end points referring to 50% and 75% wound closure were introduced after study commenced due to low occurrence of complete wound closure at week 10.

## Statistical analysis

### Sample size and design rationale

The sample size calculation was based on the primary end point. Based on prior data,[35] 50% wound closure in the placebo arm at week 10 was expected. Assuming 85% wound closure rate for ChitoCare arm at week 10, 27 patients would be needed in each arm to enable rejection of the null hypothesis that wound closure for ChitoCare and placebo arms is equal. Type 1 error or alpha was set to 0.05 and power to 0.80. Assuming a 10% drop-out rate, a total of 60 patients would be needed (30 per study arm).

### Statistical methods

Numerical variables are presented as medians with an IQR and as means with an SD. Differences in study end points presented as categorical variables, such as number of patients achieving complete, at least 75% or 50% wound closure, number of patients with wound improvement (Wagner Ulcer Classification), incidence of adverse events, and secondary infection occurrence were analyzed using Fisher's exact test. The relative reduction of diabetic ulcer wound size and wound state improvements using BWAT score were evaluated using Mann-Whitney non-parametric U test. The time to complete, at least 75%, and at least 50% wound closure was analyzed by survival analysis using Kaplan-Meier method and log-rank test with HR calculation. Median times were reported if 50% probability was reached. Proportional hazards assumption was tested using $\chi^2$ test and by plotting Schoenfeld residuals versus time. The significance level was set at 0.05. Statistical analysis was performed in IBM SPSS Statistics for Windows (V.27.0, IBM, Armonk, New York, USA) and under R environment for statistical computing using RStudio (Boston, Massachusetts, USA).

## RESULTS

Between August 2018 and May 2020, 46 patients were screened for the study. Of these, three patients did not meet the inclusion criteria and were excluded from randomization and one patient dropped out owing to COVID-19 restrictions during the run-in period. Therefore, 42 patients were included in the intention-to-treat (ITT) population (figure 1), with 18 patients randomized in the ChitoCare arm and 24 in the placebo arm. During the treatment phase, 12 patients dropped out (6 in each arm) leading to 30 patients in total at week 10.

**Table 1** Intention-to-treat population characteristics (n=42)

| Characteristic | ChitoCare arm N=18 | Placebo arm N=24 | P value |
|---|---|---|---|
| Sex<br>N (%) female | 6 (33.3) | 3 (12.5) | 0.212* |
| Age, years<br>Mean (SD), median (min-max) | 59.2 (10.0), 58.7 (35.8–77.0) | 66.5 (9.3), 66.4 (45.9–84.9) | 0.016† |
| BMI, kg/m$^2$<br>Mean (SD), median (min-max) | 31.4 (4.1), 32.1 (22.5–39.7) | 30.7 (3.9), 30.6 (23.8–38.6) | 0.608† |
| Duration of diabetes at baseline, years<br>Mean (SD), median (min-max) | 17.5 (12.6), 14 (1–54) | 20.6 (11.1), 25 (1–37) | 0.273† |
| eGFR, mL/min/1.73 m$^2$<br>Mean (SD), median (min-max) | 80.9 (28.5), 90.4 (31.4–113)‡ | 70.4 (28.3), 76.6 (12.6–108) | 0.244† |
| HbA$_{1c}$<br>%: Mean (SD), median (min-max) mmol/mol: | 7.3 (1.1), 7.0 (5.6–9.7)<br>56 (12), 53 (38–83) | 7.3 (1.3), 6.9 (5.5–10.2)<br>56 (14), 52 (37–88) | 0.990† |
| Baseline wound area size, cm$^2$<br>Mean (SD), median (min-max) | 2.55 (1.87), 1.66 (0.50–5.80) | 2.01 (1.51), 1.33 (0.30–4.90) | 0.309† |
| Baseline Bates-Jensen score<br>Mean (SD), median (min-max) | 27.9 (4.2), 28 (19–36)‡ | 27.2 (3.1), 27 (21–34)‡ | 0.536† |
| Wound duration, months<br>Mean (SD), median (min-max) | 16 (28), 6.1 (1.3–109)‡ | 22 (28), 5.6 (1.0–97) | 0.459† |

*$\chi^2$ test.
†Mann-Whitney U test.
‡Data missing for one patient within study arm (n=17 or n=23).
BMI, body mass index; eGFR, estimated glomerular filtration rate using CKD-EPI 2009 equation; HbA$_{1c}$, hemoglobin A1c; max, maximum; min, minimum.

Table 2 Study outcomes at week 10 for ChitoCare and placebo arms

| | ChitoCare arm | Placebo arm | P value |
|---|---|---|---|
| Complete wound closure: patients with event/all (%) | 3/18 (16.7) | 1/24 (4.2) | 0.297* |
| 75% wound closure: patients with event/all (%) | 8/18 (44.4) | 3/24 (12.5) | 0.033* |
| 50% wound closure: patients with event/all (%) | 11/18 (61.1) | 7/24 (29.2) | 0.060* |
| Relative reduction in wound surface area, n total | 12 | 18 | 0.008† |
| Median ($Q_1$; $Q_3$), % | 92.0 (61.6; 98.7) | 37.0 (–22.9; 68.4)‡ | |
| Mean (SD), % | 76.1 (31.9) | 20.5 (68.2) | |
| Bates-Jensen Wound Assessment, n total | 10 | 17 | |
| Relative change from baseline score: median ($Q_1$; $Q_3$), % | –29.8 (–59.1; –15.8) | –3.6 (–15.4; 3.7) | 0.010† |
| Relative change from baseline score: mean (SD), % | –34.0 (22.6) | –10.3 (25.5) | |
| Score§ at week 10: median ($Q_1$; $Q_3$) | 20 (10; 22) | 24 (21; 28) | 0.018† |
| Adverse events; no. of patients with (%) | 6 (33.3) | 4 (16.7) | 0.281* |
| Infection of primary wound, n (%) | 2 (11.1) | 1 (4.2) | |
| Infection of secondary wound, n (%) | 1 (5.6) | 2 (8.3) | |
| Acute bronchitis, n (%) | 1 (5.6) | 0 (0) | |
| Injury (twisted ankle, humerus fracture due to fall), n (%) | 2 (11.1) | 0 (0) | |
| Hospitalization for pre-existing condition¶, n (%) | 0 (0) | 1 (4.2) | |

Total n is smaller for some outcomes due to drop-outs and some missing data at week 10.
*Fisher's exact probability test.
†Mann-Whitney U test.
‡A negative value implies an increase in wound surface area.
§A lower score implies a better wound state.
¶Benign prostate hyperplasia.

Characteristics of the ITT population are presented in table 1. Patients in the ChitoCare arm were statistically younger compared with the placebo arm (mean difference of 7.3 years). No other major differences were observed between the two arms (table 1). The frequency of applications of the study product (ChitoCare or placebo gel) differed between study sites, with some patients applying the gel six to seven times per week and others two to four times per week, all in accordance with the study protocol. Randomization was performed per study site; therefore, there were no statistical differences between ChitoCare and the placebo arms regarding the number of applications per week, with an overall mean 4.4 applications per week for ChitoCare arm (n=12) and 4.8 applications per week for placebo arm (n=17) (Mann-Whitney U test, p=0.375).

## Primary outcome

In the ChitoCare arm, three patients (16.7%) achieved complete wound closure compared with one patient (4.2%) in the placebo arm at week 10 (RR 4.00, 95% CI 0.453 to 35.4, p=0.297; table 2). Detailed results for weeks 2–8 are presented in online supplemental table S1. Additionally, the ChitoCare arm showed a 4.44-fold higher likelihood to completely heal over 10 weeks compared with the placebo arm (95% CI for HR 0.461 to 42.7), but the difference was not statistically significant (p=0.158). The Kaplan-Meier curves presented in figure 2A clearly show the difference in the rate of complete wound closure between ChitoCare and placebo arms. Median times were not reached in either of the study arms. The proportional hazards assumption was not violated (p=0.560).

## Secondary outcomes

### Rate of 75% and 50% wound closure

Eight patients (44.4%) achieved 75% wound closure in the ChitoCare arm compared with three patients (12.5%) in the placebo arm at week 10, and the difference was statistically significant (RR 3.56, 95% CI 1.09 to 11.6, p=0.033). A similar trend was observed for 50% wound closure (61.1% vs 29.2% patients, RR 2.10, 95% CI 1.02 to 4.32, p=0.060). Detailed results for weeks 2 to 8 are presented in online supplemental table S1. Additionally, the ChitoCare arm showed a statistically significant 4.62-fold higher likelihood to achieve 75% wound closure over 10 weeks compared with the placebo arm (95% CI for HR 1.22 to 17.2, p=0.012). The median time for 75% wound closure in the ChitoCare arm was 8 weeks (figure 2B). A statistically significant finding was also observed for 50% wound closure, with an HR of 3.46 (95% CI 1.31 to 9.10, p=0.008). The median time for 50% wound closure in the ChitoCare arm was 6 weeks (figure 2C). The proportional hazards assumption was not violated in either of the outcomes.

### Reduction in wound surface area

The median relative reduction in wound surface area from baseline was 92.0% for the ChitoCare arm compared

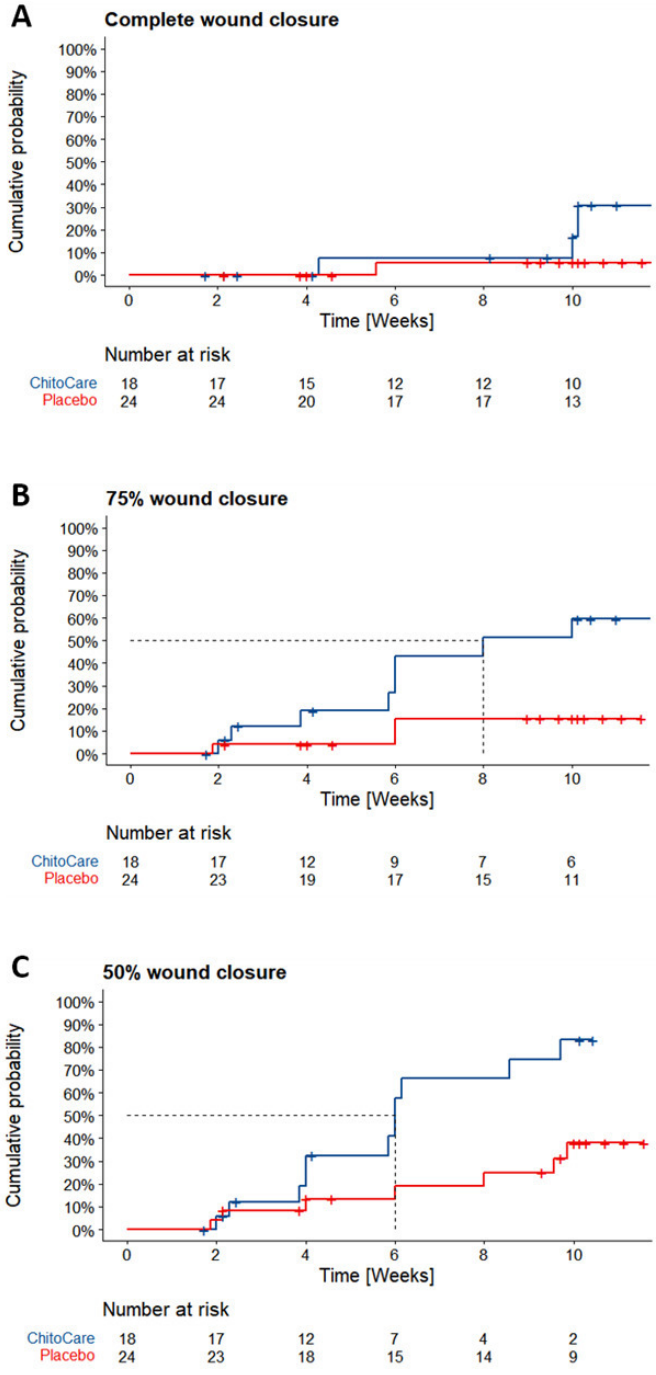



**Figure 2** Kaplan-Meier curves showing probability of complete, 75%, and 50% wound closure for ChitoCare (blue line) and placebo (red line) arms. The dashed line in each graph indicates the median time to wound closure.

with 37.0% for the placebo arm (p=0.008) at week 10 (table 2). Results for weeks 2–8 are presented in online supplemental table S2.

### Improvement in wound state

A significantly better wound state was reported in the ChitoCare arm (median 20 points) compared with the placebo arm (median 24 points) at week 10 based on BWAT (p=0.018, table 2). The relative improvement from the baseline wound state was also significantly higher in the ChitoCare arm (median 29.8%) compared with the placebo arm (median 3.6%, p=0.010). Figure 3 compares the observed improvement of each BWAT domain from baseline to week 10 in both arms and displays the main effects of ChitoCare gel. Seven domains (size, undermining, necrotic tissue type, necrotic tissue amount, skin color surrounding wound, peripheral tissue edema, and peripheral tissue induration) had low scores at baseline, leaving little room for improvement during the study. Six domains (depth, edges, exudate type, exudate amount, granulation tissue, and epithelialization) had higher baseline scores, enabling observation of noticeable improvement during the study. The share of patients with improvement in these six domains at week 10 was greater in the ChitoCare arm (50%–80%, n=10) compared with the placebo arm (15%–45%, n=17), with the greatest improvement in the epithelialization domain. However, the sample size was too small to permit adequate power for statistical analysis.

The results of the Wagner Ulcer Classification (the number of subjects with wound improvement) showed no statistical difference between ChitoCare and placebo arms (data not shown). This method of wound state assessment is less sensitive to change compared with BWAT, because there is only one domain with six levels of wound assessment (grades 0–5).

### Ulcer recurrence

In the 4 weeks of follow-up, no ulcer recurrence was reported in any patient with a completely healed wound in either arm of the study.

### Safety

A total of 12 adverse events occurred during the treatment and follow-up phase in 12 patients, 7 (38.9%) patients in the ChitoCare arm and 5 (20.8%) patients in the placebo arm (p=1.000, table 2). All reported adverse events were determined to be non-related to the treatments. Hospitalization of patients owing to wound infection was not considered a serious adverse event per this study protocol because it was an expected progression of the primary disease. However, severe adverse event was reported in one patient in the ChitoCare arm (hospitalization due to a humerus fracture) and in one patient in the placebo arm (hospitalization due to pre-existing benign prostate hyperplasia).

## DISCUSSION

Although several studies have evaluated chitosan in the promotion of chronic wound healing,[24–30] only a few have evaluated chitosan in DFUs and only one of these studies[25] was assessed in the 2019 IWGDF guidelines for DFU treatment. The goal of the CHITOWOUND study was to explore the effect of chitosan as an easy-to-apply gel on healing of DFUs.

### Outcomes related to wound healing

The primary end point of the study was to compare complete wound healing after a 10-week treatment period

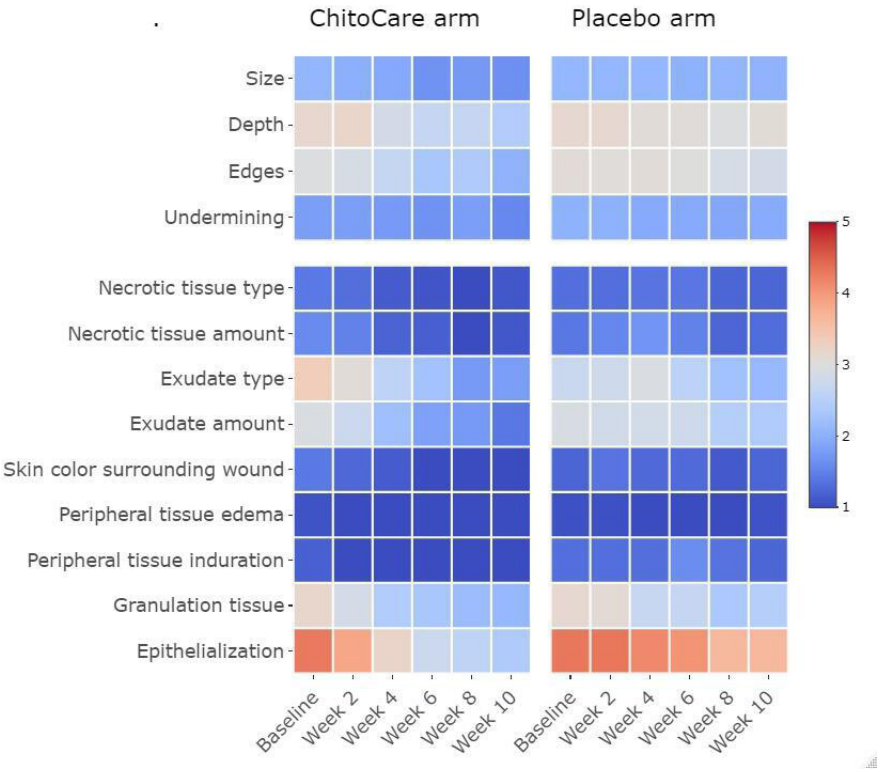



**Figure 3** Heatmaps of mean scores in each domain of the Bates-Jensen Wound Assessment Tool for the ChitoCare and placebo arms from baseline to week 10. Lower scores (blue color) indicate better wound state. The upper heat map includes the four domains with a score scale of 0–5 and the lower heat map includes the other nine domains with a score scale of 1–5. Improvement in wound state is seen in the domains that have a higher mean baseline score. In these domains, the improvement (shift to blue shades by week 10) is more pronounced in the ChitoCare arm than in the placebo arm.

between patients receiving ChitoCare treatment and those receiving a placebo treatment. Although the effect size favoring the ChitoCare study arm was high, no statistically significant difference for this outcome was found between the arms, likely owing to the small number of healed wounds at week 10. The low healing rate could be due to the fact that only chronic wounds, lasting at least 4 weeks, were included in the study. It is noted that the mean duration of wound prior to inclusion in the study was >1 year in both study arms (table 1). Additionally, a 4-week run-in period was performed prior to the start of the intervention, during which wounds that had already healed significantly (≥40%) were excluded from the study. In comparison with the study by Totsuka Sutto *et al*,[25] in which a threefold higher effect was observed for the chitosan arm after 75 days of treatment, the effect of ChitoCare gel was fourfold higher compared with placebo after 70 days. A comparison of our placebo arm (4.2% patients healed at week 10) with patients receiving other standard treatments[10–12 36 37] indicates the importance of a longer treatment time for enhanced healing.

The time to complete wound closure analysis also favored ChitoCare gel. The likelihood of complete wound closure at any time point within the treatment period was more than fourfold higher for the patients in the ChitoCare gel arm compared with the placebo arm, although the outcome did not reach statistical significance. However, statistical analysis revealed that significantly more patients in the ChitoCare arm achieved at least 75% wound closure at week 10. The statistically significant difference for rate of at least 50% wound closure, which favored the ChitoCare arm at weeks 6 and 8 but not at week 10, suggests that wound healing in the placebo arm had slightly improved at the end of treatment period. Altogether, these results imply a favorable effect of ChitoCare gel on wound healing, although longer treatment would be needed for complete wound healing.

Additionally, it was observed that wounds treated with ChitoCare gel healed faster than those treated with placebo gel. This observation is supported by the outcomes of several secondary end points in the study. First, the relative reduction of wound size area at weeks 6, 8, and 10 was significantly in favor of the ChitoCare arm, with a mean relative reduction estimated at 60.3%, 70.3%, and 76.1%, respectively, compared with 24.8%, 19.1%, and 20.5% in the placebo arm. Interestingly, during the 10-week treatment, the mean relative reduction of wound size area in the ChitoCare arm of our study was higher than in the chitosan arm of the study by Totsuka Sutto *et al*.[25] However, the methods of wound size area calculation were different. Totsuka Sutto *et al*[25] used multiplication of the largest by the smallest wound diameter to calculate the wound size area, while we calculated the area based on the number of pixels from wound photographs.

The enhanced ChitoCare gel efficacy observed in our study compared with the chitosan arm in the study by

Totsuka Sutto *et al*[25] could be due to differences in gel composition. The higher chitosan concentration and pH[25] could have caused the gel to be less mucoadhesive and biologically active. In addition, the organic acid used in the gel may influence chitosan properties. Previously, acetic acid was found to be less desirable than lactic acid, which allowed for a more flexible and bioadhesive chitosan film that had better skin suitability.[38 39]

The improvements in 75% and 50% wound closure coincide with the significant improvement in wound state (BWAT) observed at all time points favoring the ChitoCare arm. Specifically, the BWAT domains of epithelialization, exudate type and amount, wound depth, and edges improved faster in the ChitoCare arm than in the placebo arm. This coincides with earlier publications indicating that chitosan can help regulate an appropriate inflammatory microenvironment conducive for healing,[40] promote tissue granulation,[41] and enable faster wound contraction,[18] while providing a matrix for tissue growth and stimulating cell proliferation.[19]

## Study strengths and limitations

The CHITOWOUND study aimed to achieve objective results; therefore, the primary outcome was set as complete wound closure rather than wound size reduction or a similar outcome. Still, a calculation based on ImageJ analysis of wound photographs was used to determine the wound size, instead of the classic length by width calculation. For determining other wound characteristics, the study used BWAT, a very detailed tool. The run-in period with off-loading allowed for an equal baseline between participants and excluded 'fast-healers'. Prohibition of the use of antibiotics or other medication that could affect the healing process also allowed for more objective comparisons; however, the strict inclusion/exclusion criteria resulted in drop-outs.

Although the participants in the ChitoCare arm were statistically younger, other more important characteristics such us BMI, $HbA_{1c}$, eGFR, and duration of wound did not differ. In fact, the mean duration of a wound before inclusion was >1 year in both arms, which could account for the few completely healed wounds at week 10. Nevertheless, other important outcomes, such as wound duration, size, and depth clearly showed positive effects due to chitosan and could be a predictor of complete wound healing. This prediction is consistent with previous findings[42] that the per cent change in foot ulcer area after 4 weeks of observation is a robust predictor of healing at 12 weeks.

The study was performed during the COVID-19 pandemic, which hindered recruitment, caused drop-outs and protocol deviations, and consequently led to an early termination in order to safeguard patient safety and data integrity. The original sample size calculation was 60 patients (30 per arm) but ultimately, 46 patients were recruited and 42 patients were included in the ITT analysis. However, the number of patients included in the ITT analysis per arm (18 in ChitoCare and 24 in placebo) was comparable to that of Totsuka Sutto *et al*[25] (4 arms, 17 patients per arm).

Despite its limitations, our study also had numerous strengths. This study represents one of the few randomized controlled studies of DFU treatment with chitosan, and to the best of our knowledge, it is the largest one to date. The high number of analyzed outcomes offers an important contribution to the broader understanding of the effects of chitosan in DFU treatment.

## Conclusions

DFUs are often associated with adverse outcomes including protracted healing, failure to heal, infection, sepsis, amputation, a high risk of recurrence in those that do heal, and death. The management of DFUs remains a challenge.[15] The cornerstones of DFU management are off-loading, wound debridement, restoration of foot perfusion, control of infection, and choice of appropriate dressing. A wide variety of adjunctive treatments are available on the market, but appropriate good-quality evidence of their efficacy and effectiveness is often lacking.

Our study addressed the effect of ChitoCare gel on DFU healing. Although the primary end point of complete wound closure at week 10 was not met, the ChitoCare gel obviously increased the rate of DFU healing, demonstrating a fourfold effect compared with placebo. This conclusion is supported by several secondary end points that significantly favor ChitoCare gel treatment. We speculate that a slightly longer treatment time could contribute to complete healing of complex DFUs. ChitoCare gel, paired with SOC, proved to be safe to use as a topical treatment for DFUs. One of its advantages is its easy application, which enables home use and thus eliminates the need for frequent clinic visits. This could considerably reduce DFU treatment costs.

The study contributed new data on chitosan application and DFU healing. However, additional studies with unified SOC, larger sample sizes, and longer treatment and follow-up periods are required to obtain definitive evidence regarding the positive effect of chitosan on DFU healing and optimal treatment.

**Acknowledgements** The authors would like to thank all study patients, and study personnel who actively participated in the study. The authors would also like to thank Barbara Bates-Jensen for providing and approving the use of Bates-Jensen Wound Assessment Tool.

**Contributors** All authors contributed to the writing and review of the manuscript. MS was involved in protocol writing, study execution, and data management. MNP and VUR were principal investigators in the study. JJ, NČL, and IL performed the statistical analysis of the study. MS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Funding** This study was supported by two grants (R 18 044-18 and R 19 044-18 for the research project 'ChitoCare as a solution for chronic wounds') received by Primex ehf from the AVS Research Fund of The Ministry of Fisheries in Iceland.

**Competing interests** This study was sponsored by Primex ehf (Siglufjörður, Iceland). The sponsor covered all costs for the study including all institutional fees, CRO services, statistical services, and provided study product and supplies. HLL

is an employee of Primex ehf, however, was not involved in the execution of the study, data management, or statistical analysis.

**Patient consent for publication** Not applicable.

**Ethics approval** The study protocol and implementation were approved by the national ethics committees (approval number 0120-658/2017/18 and 530-07/19-01/36 for Slovenia and Croatia, respectively), and competent authorities (approval number 340-14/2018-9 and UP/I-530-09/19-06/49 for Slovenia and Croatia, respectively) of both countries, as well as institutional ethics committees where applicable. The study was performed in accordance with the Declaration of Helsinki, applicable recommendation from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guidelines for good clinical practice, and the International Organization for Standardization ISO 14155. Before any study procedures were performed, the participants provided written informed consent.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available on reasonable request. The data of this study are property of Primex ehf, and therefore not publicly available. Data are however available from the authors on reasonable request and with permission from Primex ehf.



**ORCID iDs**
Matevž Slivnik http://orcid.org/0000-0002-6339-6060
Igor Locatelli http://orcid.org/0000-0002-0052-8986

## REFERENCES

1 International Diabetes Federation. *IDF Diabetes Atlas*. 10th edn. Brussles, Belgium, 2021. Available: https://www.diabetesatlas.org
2 Rodríguez-Rodríguez N, Martínez-Jiménez I, García-Ojalvo A, *et al*. Wound Chronicity, impaired immunity and infection in diabetic patients. *MEDICC Rev* 2022;24:44–58.
3 Pickwell KM, Siersma VD, Kars M, *et al*. Diabetic foot disease: impact of ulcer location on ulcer healing: ulcer location influences ulcer healing. *Diabetes Metab Res Rev* 2013;29:377–83.
4 Morbach S, Furchert H, Gröblinghoff U, *et al*. Long-term prognosis of diabetic foot patients and their limbs: amputation and death over the course of a decade. *Diabetes Care* 2012;35:2021–7.
5 Yazdanpanah L, Shahbazian H, Nazari I, *et al*. Incidence and risk factors of diabetic foot ulcer: a population-based diabetic foot cohort (ADFC Study)—Two-year follow-up study. *Int J Endocrinol* 2018;2018:7631659.
6 Senneville É, Lipsky BA, Abbas ZG, *et al*. Diagnosis of infection in the foot in diabetes: a systematic review. *Diabetes Metab Res Rev* 2020;36 Suppl 1:e3281.
7 Carinci F, Uccioli L, Massi Benedetti M, *et al*. An in-depth assessment of diabetes-related lower extremity amputation rates 2000–2013 delivered by twenty-one countries for the data collection 2015 of the Organization for Economic Cooperation and Development (OECD). *Acta Diabetol* 2020;57:347–57.
8 Zhang P, Lu J, Jing Y, *et al*. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. *Ann Med* 2017;49:106–16.
9 Aldana PC, Khachemoune A. Diabetic foot ulcers: appraising standard of care and reviewing new trends in management. *Am J Clin Dermatol* 2020;21:255–64.
10 Margolis DJ, Allen-Taylor L, Hoffstad O, *et al*. Healing diabetic neuropathic foot ulcers: are we getting better? *Diabet Med* 2005;22:172–6.
11 Margolis DJ, Allen-Taylor L, Hoffstad O, *et al*. Diabetic neuropathic foot ulcers: predicting which ones will not heal. *Am J Med* 2003;115:627–31.
12 Ince P, Game FL, Jeffcoate WJ. Rate of healing of neuropathic ulcers of the foot in diabetes and its relationship to ulcer duration and ulcer area. *Diabetes Care* 2007;30:660–3.
13 Rayman G, Vas P, Dhatariya K, *et al*. Guidelines on use of interventions to enhance healing of chronic foot ulcers in diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev* 2020;36 Suppl 1:e3283.
14 Game FL, Apelqvist J, Attinger C, *et al*. Effectiveness of interventions to enhance healing of chronic ulcers of the foot in diabetes: a systematic review: effectiveness of interventions to enhance healing - A systematic review. *Diabetes Metab Res Rev* 2016;32:154–68.
15 Vas P, Rayman G, Dhatariya K, *et al*. Effectiveness of interventions to enhance healing of chronic foot ulcers in diabetes: a systematic review. *Diabetes Metab Res Rev* 2020;36 Suppl 1:e3284.
16 Domard A. A perspective on 30 years research on Chitin and Chitosan. *Carbohydrate Polymers* 2011;84:696–703.
17 Zuluaga F SD. Evaluation of biocompatibility of Chitosan films from the Mycelium of Aspergillus Niger in connective tissue of Rattus Norvegicus. *J Mol Genet Med* 2015;09.
18 Liu H, Wang C, Li C, *et al*. A functional Chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing. *RSC Adv* 2018;8:7533–49.
19 Paul W, Sharma CP. Chitosan and Alginate wound dressings: a short review. *Trends Biomater Artif Organs* 2004;18.
20 Racchi M, Govoni S, Lucchelli A, *et al*. Insights into the definition of terms in European medical device regulation. *Expert Rev Med Devices* 2016;13:907–17.
21 Rabea EI, Badawy ME-T, Stevens CV, *et al*. Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules* 2003;4:1457–65.
22 Helander IM, Nurmiaho-Lassila EL, Ahvenainen R, *et al*. Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria. *Int J Food Microbiol* 2001;71:235–44.
23 Chung Y-C, Chen C-Y. Antibacterial characteristics and activity of acid-soluble Chitosan. *Bioresour Technol* 2008;99:2806–14.
24 Mo X, Cen J, Gibson E, *et al*. An open multicenter comparative randomized clinical study on Chitosan. *Wound Repair Regen* 2015;23:518–24.
25 Totsuka Sutto SE, Rodríguez Roldan YI, Cardona Muñoz EG, *et al*. Efficacy and safety of the combination of isosorbide Dinitrate spray and Chitosan GEL for the treatment of diabetic foot ulcers: a double-blind, randomized, clinical trial. *Diab Vasc Dis Res* 2018;15:348–51.
26 Kordestani S, Shahrezaee M, Tahmasebi MN, *et al*. A randomised controlled trial on the effectiveness of an advanced wound dressing used in Iran. *J Wound Care* 2008;17:323–7.
27 Hampton S. A new spray-on Chitosan FH02TMdressing for venous leg ulcers: an evaluation. *Journal of General Practice Nursing* 2018;32:1–4.
28 Sharpe A, Tickle J, Hampton S, *et al*. A multicentre evaluation of a new Chitosan FH02TMspray-on dressing in patients with chronic wounds in the UK. *Journal of Community Nursing* 2018;32:30–8.
29 Campani V, Pagnozzi E, Mataro I, *et al*. Chitosan GEL to treat pressure ulcers: a clinical pilot study. *Pharmaceutics* 2018;10:15.
30 Sandoval M, Albornoz C, Muñoz S, *et al*. Addition of Chitosan may improve the treatment efficacy of triple bandage and compression in the treatment of venous leg ulcers. *J Drugs Dermatol* 2011;10:75–9.
31 Ravel J, Gajer P, Fu L, *et al*. Twice-daily application of HIV microbicides alters the vaginal microbiota. *mBio* 2012;3:e00370-12.
32 Bates-Jensen BM, McCreath HE, Harputlu D, *et al*. Reliability of the Bates-Jensen wound assessment tool for pressure injury assessment: the pressure ulcer detection study. *Wound Repair Regeneration* 2019;27:386–95.
33 Meggit B. Surgical management of the diabetic foot. *Br J Hosp Med* 1976;2:227–332.
34 Wagner FW. The dysvascular foot: a system for diagnosis and treatment. *Foot & Ankle* 1981;2:64–122.
35 Roth-Albin I, Mai SHC, Ahmed Z, *et al*. Outcomes following advanced wound care for diabetic foot ulcers: a Canadian study. *Can J Diabetes* 2017;41:26–32.
36 Smith-Strøm H, Iversen MM, Igland J, *et al*. Severity and duration of diabetic foot ulcer (DFU) before seeking care as predictors of healing time: A retrospective cohort study. *PLoS ONE* 2017;12:e0177176.
37 Margolis DJ, Kantor J, Berlin JA. Healing of diabetic neuropathic foot ulcers receiving standard treatment. A meta-analysis. *Diabetes Care* 1999;22:692–5.
38 Velasquillo C, Silva-Bermudez P, Vázquez N, *et al*. In vitro and in vivo assessment of lactic acid-modified Chitosan scaffolds for potential

treatment of full-thickness burns: in vitro and in vivo assesment of La-modified CTS scaffolds. *J Biomed Mater Res* 2017;105:2875–91.
39 Peh K, Khan T, Ch'ng H. Mechanical, bioadhesive strength and biological evaluations of Chitosan films for wound dressing. *J Pharm Pharm Sci* 2000;3:303–11.
40 Jayakumar R, Prabaharan M, Sudheesh Kumar PT, *et al*. Biomaterials based on Chitin and Chitosan in wound dressing applications. *Biotechnol Adv* 2011;29:322–37.
41 Dai T, Tanaka M, Huang Y-Y, *et al*. Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. *Expert Rev Anti Infect Ther* 2011;9:857–79.
42 Sheehan P, Jones P, Caselli A, *et al*. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust Predictor of complete healing in a 12-week prospective trial. *Diabetes Care* 2003;26:1879–82.