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A prospective multicenter phase III clinical trial evaluating the efficacy and safety of silk elastin sponge in patients with skin defects

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Silk elastin sponge, a novel recombinant protein used for wound healing, has been shown to be effective in promoting macrophage migration, epithelial growth, granulation, and angiogenesis in both preclinical (in vitro and in vivo) and clinical studies. This study aimed to evaluate the efficacy and safety of silk elastin sponges in the treatment of chronic and acute wounds. A prospective multicenter, single-arm, uncontrolled clinical trial included 20 patients with chronic wounds and five with acute wounds, applying the sponge after debridement. The primary endpoints were the percentage of patients with chronic wounds and well-prepared wound beds after 14 days of treatment. The safety of the procedure was also assessed. The results showed that 90.0% of chronic wound patients had well-prepared wound beds by day 14, and 24 out of 25 patients completed the treatment, with one case discontinued due to local infection. This study concluded that silk elastin sponges may be an effective new option for wounds that are unresponsive to existing treatments.

***Trial registration*: jRCT2052210072. Registered on 11 July 2023 in the Japan Registry of Clinical Trials (http://jrct.niph.go.jp).**

Keywords Wound healing, Recombinant protein, Silk-elastin sponge, Chronic wound, Acute wound, Clinical trial

The skin acts as a barrier to the external environment and plays various important roles, such as regulating important physiological processes in the body, sensing stimuli, synthesizing vitamin D, and monitoring the immune system[1]. When the skin is damaged by burns or trauma, and a wound is formed, the healing process consists of four phases: coagulation, inflammation, proliferation, and remodeling. During the coagulation phase, platelets aggregate to form a thrombus. During the inflammatory phase, the vascular permeability of the wound increases, and monocytes and neutrophils move into the wound to remove necrotic tissue and foreign substances. During the proliferative phase, collagen, fibroblasts, vascular endothelial cells, and keratinocytes proliferate and granulation, angiogenesis, and epithelial growth occur. During the remodeling phase, collagenase degrades excess collagen and the capillaries degenerate, resulting in a mature scar[2].

Wounds can be broadly classified into two categories: acute and chronic. Acute wounds arise from disruptions in skin continuity, such as trauma or surgical incisions, or from progressive tissue damage caused by burns and other factors. These wounds exhibit normal wound healing processes, including granulation, angiogenesis, functional connective tissue contraction, reepithelialization, and remodeling. The wound healing mechanism in acute wounds functions properly, and the healing process involves the removal of inhibitory factors, such as necrotic tissue and foreign bodies. In contrast, when the wound healing process is inhibited by microvascular dysfunction or susceptibility to infection caused by macrophage dysfunction due to immunodeficiency, the wound becomes a refractory chronic ulcer that has a significant impact on patient prognosis[3]. Chronic wounds, such as bedsores, ischemic ulcers, and diabetic ulcers, are wounds in which the normal wound healing process is impaired and a disorder is present. As chronic wounds fail to heal, treating the underlying disease is crucial before providing local treatment.

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The inflammatory phase, a critical component of chronic wounds, is distinct from acute wounds[4]. It is essential to eliminate or control factors that hinder normal wound healing processes and promptly shift the wound from the inflammatory phase to the proliferative phase. This constitutes the primary clinical distinction between chronic and acute wounds, and serves as the point of intervention in chronic wound care. Wound management is important for promoting wound healing processes, such as the removal of necrotic tissue, control of edema, suppression of bacterial colony formation on the wound surface, control of exudate, formation of a wound bed with angiogenesis, and maintenance of an appropriately moist environment on the wound bed[5]. Existing treatments, such as negative pressure wound therapy (NPWT), are effective for wound bed preparation[6–8]; however, some reports suggest that they increase costs[9] and have limited efficacy in treating difficult-to-heal ulcers[10]. Therefore, developing a highly desirable treatment that can effectively promote the formation of granulation tissue in difficult-to-heal wounds, similar to or better than NPWT, while minimizing the risk of bacterial infection[11], is of great importance.

Silk elastin (SE) is a recombinant protein developed by Protein Polymer Technologies, Ltd. This protein was produced by introducing an amino acid sequence encoding a plasmid encoding silk fibroin and elastin into the *Escherichia coli* expression system[12]. One of its subtypes, SE-P47K-WAS (Fig. 1), is a polymer containing 12 repeats consisting of four elastin-like motifs, a V-K substituted elastin-like motif, three additional elastin-like motifs, and four silk fibroin-like motif sequences with the unique feature of temperature-mediated self-gelling. It is water-soluble at room temperature and undergoes an irreversible sol-to-gel transition at 37 °C at a concentration of 4 wt% or higher[13–15]. Their practical application on wounds has been reported in animal studies[16–19]. It has also been previously reported that SE promotes macrophage migration[20,21], neoepithelial growth, and angiogenesis[19]. Furthermore, a phase I/II clinical trial was conducted with the primary objective of confirming the safety and feasibility of the SE sponge in patients with chronic ulcers; four out of six patients completed the study without inflammation or infection, highlighting its safety[22].

This multicenter clinical trial was conducted to evaluate the efficacy and clinical usefulness of an SE sponge (SE-P47K-WAS) for the treatment of skin ulcers.

Materials and methods

Study setting

This was a prospective, multicenter, single-arm, uncontrolled clinical trial (registration no. jRCT2052210072, registered on 11/07/2023). All individuals participating in this study adhered to the ethical principles outlined in the Declaration of Helsinki and complied with relevant laws, regulations, and international standards, including ISO 14155:2020, which pertain to clinical trials of medical devices. The target number of cases was 20 chronic wounds and five acute wounds. The different types of wounds, pre-study treatment, interventions, and assessments are summarized in the study design flowchart (Fig. 2) and the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) (Fig. 3). Written informed consent was obtained from all patients. The participants were treated with SE-P47K-WAS for 14 d and observed for another 14 d to evaluate the course of wound healing and adverse events. Previously reported inclusion and exclusion were utilized[23]. A summary of these criteria is provided in Fig. 4.

This study was conducted at nine hospitals in Japan: Kyoto University Hospital (Kyoto); Tokyo Medical University Hospital (Tokyo); Juntendo University Hospital (Tokyo); Kansai Medical University Hirakata Hospital (Osaka); Kobe University Hospital (Kobe); Hakodate Central General Hospital (Hakodate); Yao Tokushukai General Hospital (Osaka); Oita Oka Hospital (Oita); and Tokyo-Nishi Tokushukai Hospital (Tokyo). Institutional Review Board (IRB) approval was obtained from all investigational sites for the design and modification of the study.

Study schedule

For chronic wounds, only patients with a wound area reduction of less than 50% after 28 days of standard treatment prior to entry were considered eligible.

Fig. 1. Appearance of SE-P47K-WAS.

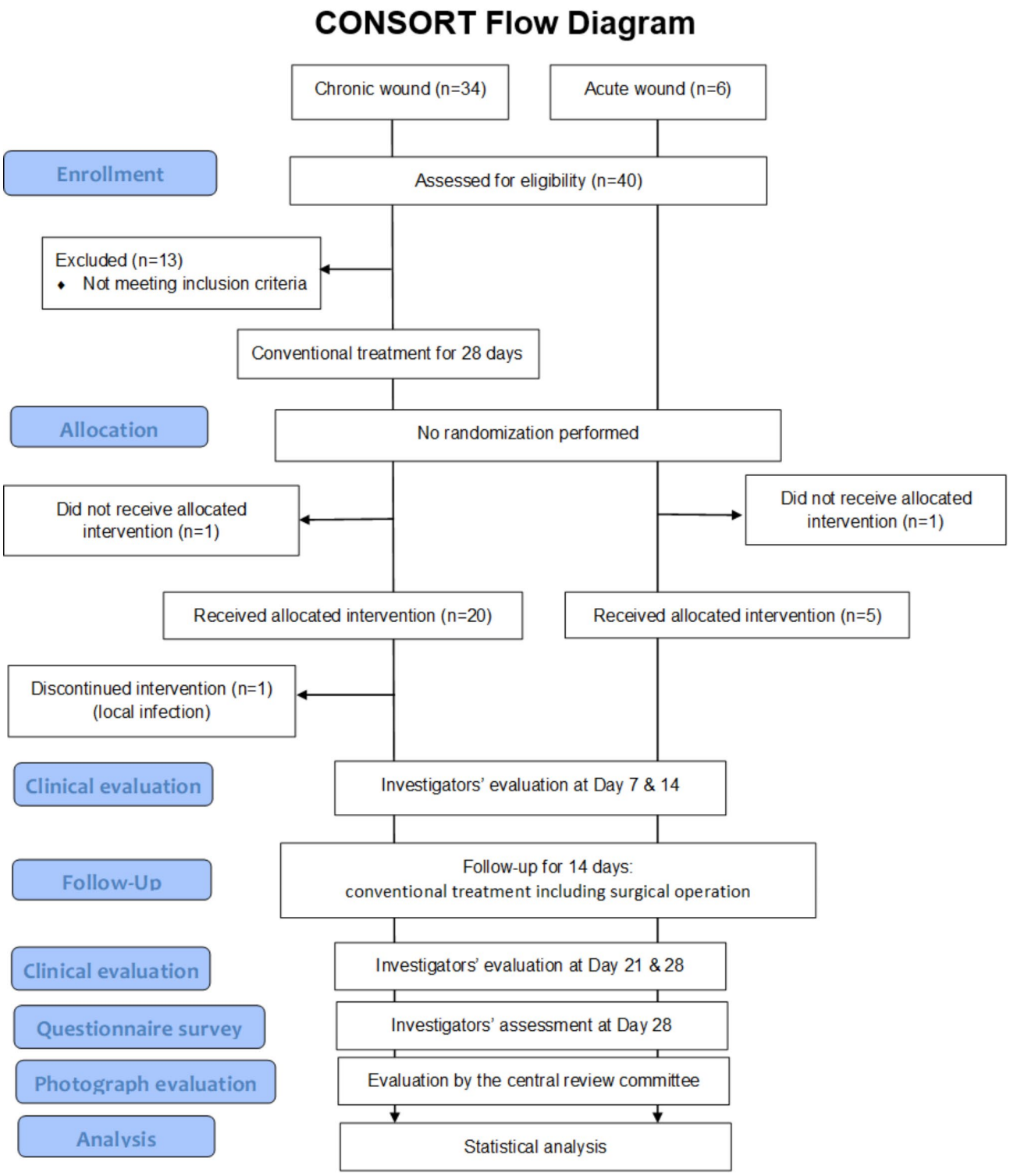

Fig. 2. Study flowchart.

After taking pictures of the wound at the beginning of treatment, the target wound was debrided under appropriate anesthesia to remove non-viable tissue. After hemostasis and washing with saline solution, a sheet of SE-P47K-WAS trimmed to fit the wound's shape was applied to the wound and covered with a wound dressing, which was selected from one of the following four options: polyurethane film dressing (3 M Tegaderm® Transparent Film Dressing; 3 M, Maplewood, Minnesota, USA), silicone-faced wound dressing (SI-Aid®; ALCARE, Tokyo, Japan), low-adherent absorbent dressing (Melolin®; Smith & Nephew, Hull, UK), or polyurethane form dressing (Hydrosite® thin type; Smith & Nephew). The wound was secured using appropriate external fixation.

	Study period									
	Enrollment	Conventional treatment	Before debridement	After debridement	Application of study device	Reapplication	Removal	Follow-up		Discontinuation of trial
TIMEPOINT:										
Chronic wound		>28 days	Day -1/0		Day 0	Day 7 ± 2	Day 14 ± 2	Day 21 ± 3	Day 28 ± 3	
Acute wound		None								
ENROLLMENT:										
Eligibility screen	O									
Informed consent	O									
Confirmation of eligibility		O								
INTERVENTIONS:										
Application of study device					O	O				
ASSESSMENTS:										
Wound size measurements	O	O		O		O	O	O	O	O
Assessment of granulation				O		O	O	O	O	O
Photography of wound	O	O	O	O	O	O	O	O	O	O
Assessment of wound bed						O	O	O	O	O
Questionnaire survey									O	O
Adverse events			←	—	—	—	—	—	—	→
Inflamation or infection related symptoms	O	O			O	O	O	O	O	O
Laboratory tests	O		O				O		O	O
Vital signs	O		O		O	O	O		O	O
Wound bacterial culture tests			O		O	O	O			
Drugs and Devices prohibited for use		←	—	—	—	—	—	—	—	→

Fig. 3. SPIRIT figure. Schedule of enrollment, interventions, and assessments.

If, after debridement, it was anticipated that the conditions would not be appropriate for SE-P47K-WAS hydrogel formation owing to bleeding or an excessive amount of exudate, the wound was temporarily covered with a wound dressing or ointment without applying SE-P47K-WAS. In such instances, SE-P47K-WAS was applied on the day after surgery. Antibiotics were administered intravenously on the day of surgery. After seven days, the wound dressing was removed, and the wound was assessed and photographed. Subsequently, the wound was washed with saline solution, and a new SE-P47K-WAS was applied unless there was already sufficient granulation and did not require additional application.

SE-P47K-WAS was maintained on the wound surface for 14 days (± 2 days) after the initial application, with or without additional application. After this period, conventional treatment was administered following the removal of SE-P47K-WAS, and the wound was observed until day 28. No further application of SE-P47K-WAS was permitted during the observation period.

Drugs and devices prohibited for use in combination with the study device

From enrollment to the end of the 28-day observation period, the application of NPWT, basic fibroblast growth factor solution, and artificial dermal substitutes (e.g. Pelnac®) on the target wound was strictly prohibited. The utilization of prostaglandin preparations, prostaglandin ointment, dibutyryl cyclic AMP ointment, and tretinoin tocopheril ointment is limited to long-term chronic use only. The use of the topically administered drugs or therapies mentioned above at sites other than the target wound was not restricted. This was performed to prevent any difficulties in evaluating the clinical efficacy of SE-P47K-WAS using these agents, which promote granulation formation, epithelial growth, and angiogenic properties. Antimicrobials were prohibited except for administration on the day of the surgery.

Primary and secondary endpoints

The primary endpoint in the chronic wound group was the percentage of patients who attained the status of a "well-prepared wound" (WPW) 14 days after the initial application of SE-P47K-WAS. WPW was defined as a wound that was ready for closure using simple surgical procedures, such as skin grafts, skin flaps, simple sutures, or through secondary healing. In other words, wounds had to meet the following three criteria: "healthy granulation tissue" covering at least 80% of the wound, less than 5% of necrotic tissue, and no obvious signs of infection. The secondary endpoints were as follows: (1) the percentage of patients who attained a WPW at days 7, 21 and 28 for chronic wounds, and at days 7, 14, 21 and 28 for acute wounds; (2) the wound area ratio

Inclusion Criteria for Chronic Wounds:

1. Baseline wound area ≥ 50% of original size after 28 days of conventional treatment.
2. Wound areas after debridement between 2 and less than 25 cm2.
3. Wound dressings adequately covered study device-applied wounds.
4. Perilesional skin was intact if another wound was located nearby.
5. No infection observed in wounds after debridement.
6. Bone exposure area ≤ 10% if the wound involved bones.
7. Perfusion pressure ≥ 30 mmHg in lower-extremity ulcers.
8. Underlying factors affecting healing (e.g., diabetes, venous insufficiency, bedsores).

Inclusion Criteria for Acute wounds:

1. Wounds requiring wound bed preparation with no systemic healing impact.
2. Wound areas after debridement between 2 and less than 100cm2.
3. Wound dressings adequately covered study device-applied wounds.
4. Perilesional skin was intact if another wound was located nearby.
5. No infection observed in wounds after debridement.
6. Bone exposure area ≤ 10% if the wound involved bones.

Exclusion Criteria for Chronic and Acute Wounds

1. Age <20 years at the time of trial consent.
2. Pregnant, nursing, or unwilling to use contraception.
3. History of allergy to silk, urethane, and other reagents(e.g., local anesthetics and disinfectants) using in the trial.
4. Uncontrolled diabetes (HbA1c was greater than 10% in the latest laboratory findings within 28 days before enrollment).
5. Hypoalbuminemia (less than 2g/dl).
6. Malignant disease requiring systemic treatment
7. Continuous systemic administration of steroids (a dose exceeding an equivalent prednisolone dose of 10 mg/day).
8. Wound on weight-bearing site.
9. Recent participation in another trial (≤ 3 months).
10. Previous participation in the trial.
11. Unable to provide written consent.
12. Deemed as inappropriate by investigators.

Discontinuation Criteria:

1. Decline in overall health owing to disease or complications.
2. Sudden worsening of a wound or failure to meet the requirements for the device before application.
3. The wound areas after debridement were ≥ 25 cm2 and ≥ 100 cm2 for chronic and acute wounds, respectively.
4. The severe allergic reactions to the device used in this study could not be controlled.
5. Local infections were observed after the device application.
6. Serious adverse events were caused by or linked to the device, or where a link could not be ruled out.
7. Requests from patients to discontinue the trial.
8. Complete discontinuation of the trial.
9. The investigators decided that continuing the trial was inappropriate.

Fig. 4. Study inclusion, exclusion, and discontinuation criteria.

(wound area at observation divided by wound area after debridement) at 7, 14, 21, and 28 days after the initial application of SE-P47K-WAS for chronic wounds; (3) the healthy granulation area ratio (healthy granulation area divided by wound area) at 7, 14, 21, and 28 days after the initial application of SE-P47K-WAS for chronic wounds; (4) the healthy granulation assessment score (Supplementary Table S1) frequency at after debridement, 7, 14, 21, and 28 days after the initial application of SE-P47K-WAS for chronic wounds; (5) the frequency of applying SE-P47K-WAS to each patient; (6) the accessibility of SE-P47K-WAS assessed through a questionnaire survey (Supplementary Table S2) conducted by the investigators at the end of the observation period. Secondary endpoints (2), (3), (4) were evaluated only for chronic wounds.

Safety endpoints

The safety endpoints were as follows: (1) the incidence of adverse events, where adverse events were defined as medically unfavorable events that could not be attributed to the study intervention; (2) study equipment failure; (3) the incidence of local symptoms related to inflammation or infection, including redness, heat, swelling, pain

exacerbation, increased exudate, exudate cloudiness, and malodor at each time point; and (4) abnormal results from blood tests, wound bacterial cultures, and vital signs observed at each time point.

Evaluation of wound bed, wound area, and healthy granulation area

Digital photographs of the wounds were captured using a calibrator (Casmatch; Bear Medical, Tokyo, Japan). Their color and size were calibrated according to the manufacturer's instructions, and the size of the area was measured using a raster graphics editor (Adobe Photoshop; Adobe, San Jose, California, USA).

The achievement of the WPW was evaluated by the investigators at each institute. To this end, the wound area was measured using photographs taken by three independent plastic surgeons without any involvement in the treatment process. The post-debridement wound area was used as the baseline to determine the percentage of the wound area on days 7, 14, 21, and 28 after application. Moreover, the formation of granulation tissue was evaluated using two methods: (1) Healthy Granulation Assessment Score: The investigators at each institute assessed the score (ranging from 0 to 6, Supplementary table S1) on days 0 (after debridement), 7, 14, 21, and 28; (2) Healthy Granulation Area Ratio: The percentage of healthy granulation area was assessed by three independent plastic surgeons and calculated by dividing the healthy granulation area by the wound area at each time point.

Statistical analysis

Analysis set

The study population analyzed for efficacy was the full analysis set (FAS). For the primary endpoint, a reference analysis of the per protocol set (PPS) was also performed. The safety analysis population comprises the safety analysis set (SAS).

FAS was defined as the population of subjects enrolled in the study to whom the device was applied at least once and for whom the necessary efficacy data were available. The PPS was defined as the population of participants in the FAS with no significant violations of the study protocol. A significant violation of the protocol is defined in Supplementary Table S3. SAS was defined as the number of subjects enrolled in the study to whom the device was applied at least once. Descriptive statistical analysis was conducted to determine summary statistics and to express 95% confidence intervals for each survey item.

Monitoring and audit

Regular monitoring by a Clinical Research Associate (CRA) was conducted to ensure that the study was conducted in accordance with the study protocol, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

Auditing

Independent audits were conducted by a third-party accepting agency, and these audits confirmed compliance with regulatory requirements, study protocols, and ethical guidelines.

Results

Patient demographics and summary

A summary of the populations considered in this analysis is provided in Table 1, while ineligible cases are listed in Supplementary Table S4 and Fig. S1-5.

Of the 27 eligible patients, 21 had chronic wounds and six had acute wounds. Two patients were excluded from the study because one had a wound area exceeding 25 cm^2 before debridement and the other was taking prohibited concomitant medication. In total, the investigational device was applied to 25 subjects (20 with chronic wounds and 5 with acute wounds). One patient with a chronic wound developed a local infection five days after the initial application, leading to termination of the study. All 25 patients who received the investigational device were included in both the FAS and SAS, while two of the 27 enrolled patients who did not receive the application of SE-P47K-WAS were excluded from these analyses. The demographic data of all the patients are presented in Table 2. SE-P47K-WAS was applied once in one case and twice in 19 cases for chronic wounds, and once in one case and twice in four cases for acute wounds.

Population subject to analysis		Overall	Chronic wounds	Acute wounds
Registrations		**27**	**21**	**6**
FAS	Included	25	20	5
	Excluded	2	1	1
PPS	Included	24	19	5
	Excluded	1	1	0
SAS	Included	25	20	5
	Excluded	2	1	1

Table 1. The study participants included in the analysis.

Cases				
Variable		**Overall**	**Chronic wound**	**Acute wound**
Sex	Male	11(44.0)	8(40.0)	3(60.0)
	Female	14(56.0)	12(60.0)	2(40.0)
Age (y)		65.5 ± 14.5	66.4 ± 14.5	62.0 ± 15.6
Hight (cm)		160.3 ± 10.8	159.9 ± 11.6	162.0 ± 6.9
Weight (kg)		61.6 ± 13.0	62.1 ± 13.7	59.4 ± 10.7
Number of wounds	One	13(52.0)	12(60.0)	1(20.0)
	Two	9(36.0)	6(30.0)	3(60.0)
	Three or more	3(12.0)	2(10.0)	1(20.0)
Cause of the chronic wound	Diabetes		3(15.0)	
	Venous insufficiency		8(40.0)	
	Pressure sore		5(25.0)	
	Others		5(25.0)	
Cause of the acute wound	Complicated wound			2(40.0)
	Burn			3(60.0)
Location of the wound	Lower extremity	9(36.0)	6(30.0)	3(60.0)
	Foot/ankle	10(40.0)	10(50.0)	0(0.0)
	Hip/gluteal	2(8.0)	1(5.0)	1(20.0)
	Thigh/knee	1(4.0)	0(0.0)	1(20.0)
	Sacral region	3(12.0)	3(15.0)	0(0.0)
Wound area after debridement (cm^2)			7.5 ± 5.8	28.6 ± 20.7
Wound dressing	3 M Tegaderm®	1(4.0)	1(5.0)	0(0.0)
	SI-Aid®	15(60.0)	13(65.0)	2(40.0)
	Melolin®	9(36.0)	6(30.0)	3(60.0)
	Hydrosite®	0(0.0)	0(0.0)	0(0.0)

Table 2. Study cohort demographic characteristics. N (%) was calculated for categorical variables. Continuous variables are presented as mean ± standard deviation (SD).

Chronic wound			
	Well-prepared wound bed		
Analysis (number)	**Number**	**Percentage**	**95% CI**
FAS (*N* = 20)	18	90	68.3, 98.8
PPS (*N* = 19)	17	89.5	66.9, 98.7

Table 3. Results of the primary endpoint.

Evaluation of efficacy

Primary endpoint

Table 3 shows the results of the wound bed evaluation on day 14 after the application of SE-P47K-WAS for chronic wounds in FAS and PPS. The percentage of patients who attained a WPW 14 days after the initial application of SE-P47K-WAS (95% confidence interval (CI)) was 90.0% (68.3, 98.8) for FAS and was 89.5% (66.9, 98.7) for PPS.

Secondary endpoints

In terms of the evaluation of WPW, for chronic wounds, the proportion (95%CI) who were judged to achieve WPW on days 7, 21, and 28 after the application was 60.0% (36.1–80.9), 85.0% (62.1–96.8), and 85.0% (62.1–96.8), respectively (Table 4). For acute wounds, the proportion (95%CI) of subjects who achieved WPW on days 7, 14, 21, and 28 after the application of the investigational device was 60.0% (14.7–94.7), 100% (47.8–100.0), 100% (47.8–100.0), and 100% (47.8–100.0), respectively (Table 5).

The wound area ratios (95% CI) on days 7, 14, 21, and 28 after application were 79.92% (66.5–93.3), 61.76% (42.3–81.2), 46.37% (27.9–64.9), and 38.84% (22.9–54.8), respectively (Table 6). Wound area measurement was performed in 17 cases, excluding two cases in which area measurement from photographs was deemed impossible due to pocket formation and one case that dropped out of the study due to wound infection. The wound area ratio was calculated as the reference value. Supplementary table S5 shows the results at each time point for 19 patients, including two cases in which evaluation through photographs was not possible owing to the formation of pockets. The healthy granulation rates (95% CI) at days 7, 14, 21, and 28 after application were 74.10% (60.44–87.77), 83.66% (71.25–96.06), 83.65% (70.31–96.99), and 82.89% (68.37–97.41), respectively

Chronic wound			
	Well prepared wound bed		
Time point	Number	Percentage	95% CI
Day 7	12	60	68.3, 98.8
Day 21	17	85	62.1, 96.8
Day 28	17	85	62.1, 96.8

Table 4. Results of the secondary endpoint (wound bed for chronic wounds, FAS).

Acute wound			
	Well prepared wound bed		
TIme point	Number	Percentage	95% CI
Day 7	3	60	14.7, 94.7
Day 14	5	100	47.8, 100
Day 21	5	100	47.8, 100
Day 28	5	100	47.8, 100

Table 5. Results of the secondary endpoint (wound bed for acute wounds, FAS).

Chronic wound						
	Wound area (%)					
Time point	Number	Mean (SD)	95% CI	Minimum	Median	Maximum
Day 7	17	79.92 (26.6)	66.52, 93.31	3.6	80.24	116.1
Day 14	17	61.76 (37.77)	42.34, 81.18	0.8	73.7	145.4
Day 21	17	46.37 (35.99)	27.86, 64.87	0	34.59	128.7
Day 28	17	38.84 (30.99)	22.90, 54.77	0	43.92	99.6

Table 6. Results of the secondary endpoint (wound area, FAS).

chronic wound						
	Healthy granulation area (%)					
Time point	Number	Mean (SD)	95% CI	Minimum	Median	Maximum
Day 7	17	74.1 (26.58)	60.44, 87.77	4.4	82.73	95.7
Day 14	17	83.66 (24.13)	71.25, 96.06	0	91.57	99.8
Day 21	17	83.65 (25.95)	70.31, 96.99	0	92.33	100
Day 28	18	82.89 (29.2)	68.37, 97.41	0	91.6	100

Table 7. Results of the secondary endpoint (healthy granulation area, FAS).

(Table 7). The healthy granulation assessment scores are shown in Supplementary Table S6. The healthy granulation area, which was measured from photographs, was performed on 17 cases at days 7, 14, and 21, excluding two cases that were impossible due to pocket formation and one case that dropped out of the study due to wound infection. One case that had been excluded due to pocket formation was evaluated on day 28 and included in the results; on the other hand, the healthy granulation assessment score was evaluated in all 20 cases at day 0. However, after day 7, results were obtained in 19 cases, except for one case in which the trial was terminated because of infection.

In a questionnaire survey on the accessibility of SE-P47K-WAS, 85% of the investigators stated that they had no problems with its operability of SE-P47K-WAS, indicating its potential for widespread use of the material (Supplementary Tables S7 and S8).

Case presentation

Case #1: acute wound

A 71-year-old male with a deep dermal burn on the lumbar back due to the ignition of clothing was enrolled in a clinical trial as an acute wound case (Fig. 5). SE-P47K-WAS was applied to the wound surface without any complications after debridement on post-burn day 9 and promptly gelated and was retained on the wound surface. After application, a dressing change was not necessary for seven days, and the size of the wound exhibited

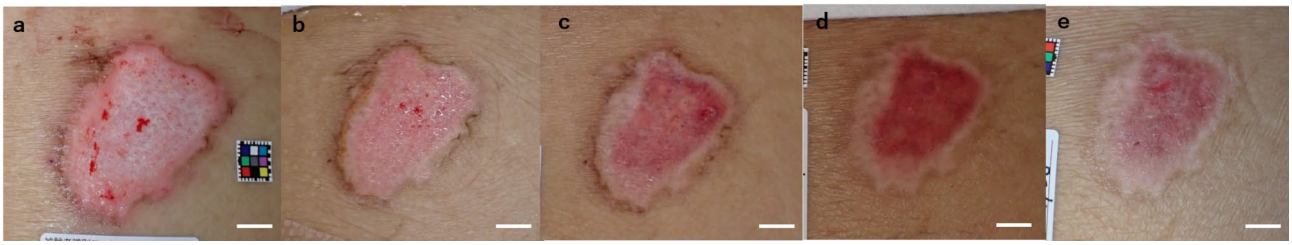

Fig. 5. Case #1. (**a**) Day 0, post-debridement wound. (**b**) Day 7, the wound area decreased. The healthy granulation area was observed. (**c**) Day 14, the wound had epithelialized. (**d**) Day 21. (**e**) Day 28. Scale bar (white line) = 1 cm.

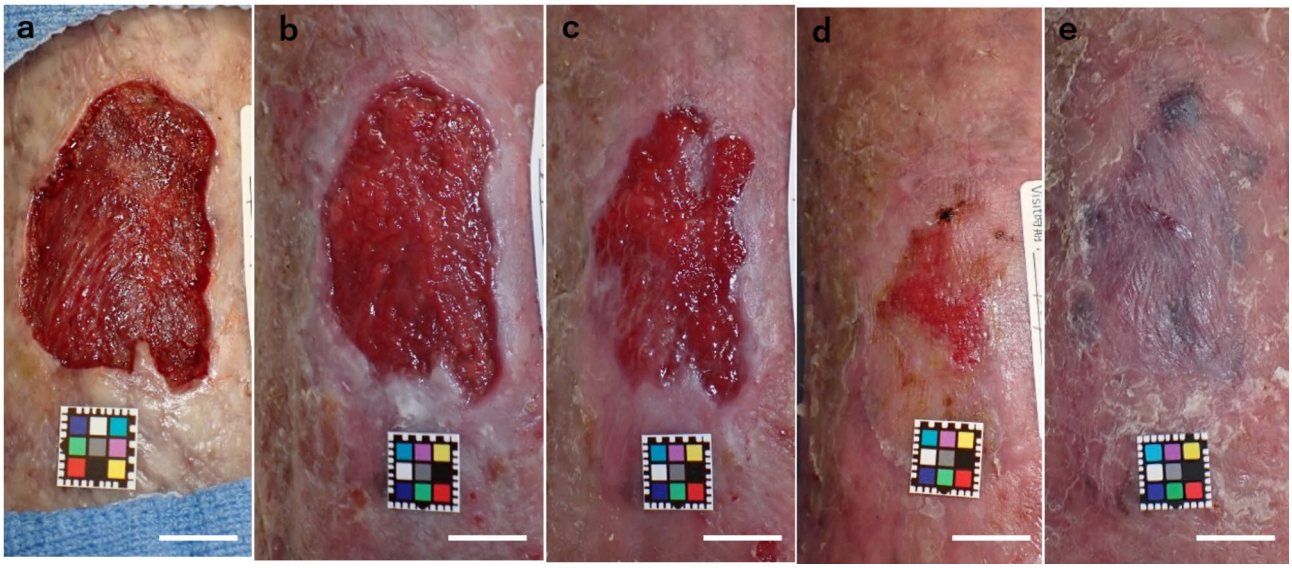

Fig. 6. Case #11. (**a**) Day 0, post-debridement wound. (**b**) Day 7, the healthy granulation was observed. (**c**) Day 14, the wound was well prepared with > 90% healthy granulation tissue, less than 5% necrotic tissue, and no apparent signs of infection. (**d**) Day 21, the wound area decreased. (**e**) Day 28, the wound had epithelialized. Scale bar (white line) = 1 cm.

a notable decrease. The wound was completely epithelialized on day 14. No excess exudate or signs of infection were observed during the observation period.

Case #11: chronic wound

A 57-year-old male patient with a history of venous stasis dermatitis for 20 years presented with an ulcerated left lower leg that did not heal in 15 months (Fig. 6). The patient was enrolled in the clinical trial. The wound surface and ulcer base were refreshed through the wound margin. Subsequently, SE was applied. The SE-covered wound showed no signs of infection and progressively decreased in size over the following 14 days. At this point, the wound was evaluated as well-prepared, with > 90% healthy granulation tissue and less than 5% necrotic tissue. The wound area ratios on days 7, 14, 21, and 28 were 116.1%, 83.9%, 14.9%, and 0%, respectively, and the healthy granulation assessment scores on days 0, 7, 14, 21, and 28 were 4, 4, 4, 4, and 5, respectively. The healthy granulation areas (%) on days 7, 14, and 21 were 7.54 cm^2 (91.9%), 5.92 cm^2 (99.8%), and 0.99 cm^2 (93.7%), respectively. On day 28, the wound was completely epithelialized and the healthy granulation area was not measurable.

Case #13: chronic wound

A 43-year-old male patient with a diabetic foot ulcer was enrolled in a clinical trial.

after a blister on his right big toe ulcerated and failed to heal for two months (Fig. 7). The wound surface was refreshed by removing the entire wound margin and ulcer base, to which SE was applied. The SE-covered wound area gradually decreased, without infectious complications. Fourteen days later, the SE-covered wound area was further reduced owing to epithelialization from the wound margin and wound contraction. At this point, the wound exhibited more than 90% healthy granulation, less than 5% necrotic tissue, and was found to have achieved the status of a well-prepared wound. The wound area ratios on days 7, 14, 21, and 28 were 77.7%, 19.5%, 26.5%, 43.9%, respectively, and the healthy granulation assessment scores on days 0, 7, 14, 21, and 28 were

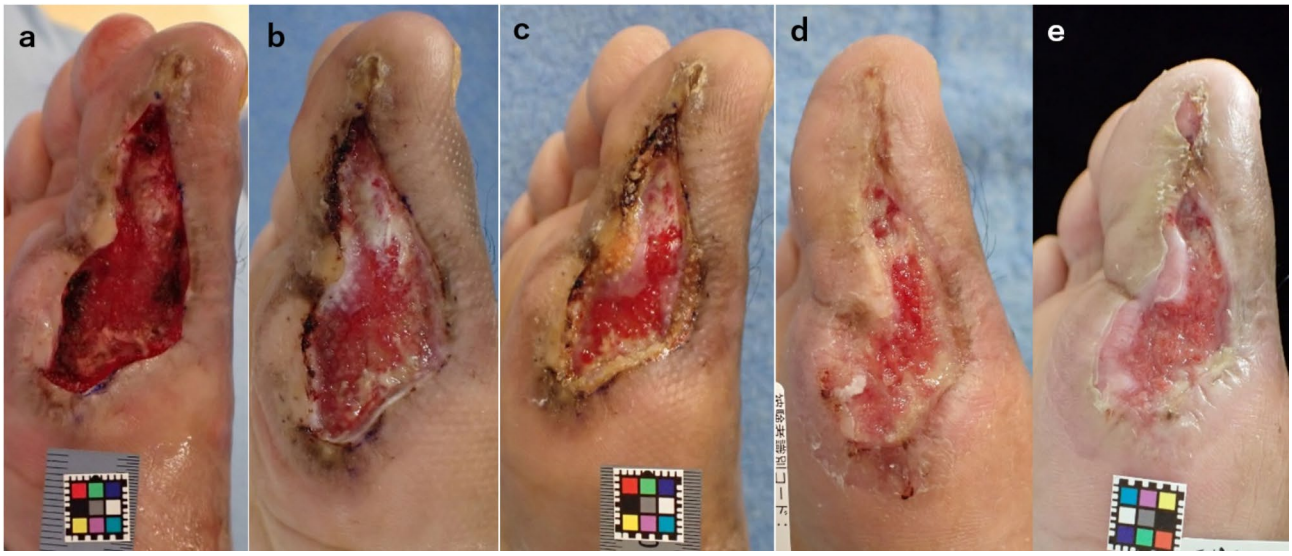

Fig. 7. Case #13. (**a**) Day 0, post-debridement wound. (**b**) Day 7, the wound area decreased and the healthy granulation area was observed. (**c**) Day 14, the wound was well prepared with > 90% healthy granulation tissue, less than 5% necrotic tissue, and no apparent sign of infections. (**d**) Day21 and (**e**) Day 28, the wound was well prepared. Scale bar (white line) = 1 cm.

3, 3, 4, 3, and 3. The healthy granulation area at days 7, 14, 21, and 28 were 3.46 cm^2 (80.7%), 0.99 cm^2 (92.6%), 1.21 cm^2 (83.1%), and 1.97 cm^2 (81.4%).

Safety

No deaths or notable adverse events were reported. Eleven patients (44.0%) experienced adverse events (Supplementary Table S9). Three adverse events (12.0%) were related to the application site of the investigational device, and a causal relationship with the study device could not be excluded. One patient (4.0%) was excluded from the study because of local *Pseudomonas* infection. Signs of infection appeared five days after application, necessitating debridement and termination of further clinical studies. The local infection was treated nonsurgically, and the patient recovered. In addition to the instances above, bacterial culture tests did not reveal any additional cases of severe bacterial infections. The SE-P47K-WAS device functioned without malfunction, and no abnormalities in the blood tests or vital signs were reported.

Discussion

This study aimed to demonstrate the safety, efficacy, and clinical application of a genetically modified SE sponge (SE-P47K-WAS). Patients with chronic wounds were included if they had not experienced a ≥ 50% reduction in wound area after 28 days of standard treatment before participating in the trial. The primary efficacy endpoint was the proportion of patients with chronic wounds who achieved WPW 14 days after SE-P47K-WAS application. WPW was defined as meeting the following three criteria: (1) healthy granulation tissue accounting for at least 80% of the wound area, (2) nonviable tissue accounting for < 5% of the wound area, and (3) the wound was free of infection. Consequently, 90% of the patients achieved WPW, exceeding the target achievement rate (66.7%) calculated based on the results of a preceding safety study[22]. Furthermore, in comparable Renasys and PICO studies, the percentage of patients achieving WPW at 14 days after treatment was 33.9%[24]. The responses to the SE-P47K-WAS convenience questionnaire, administered to investigators at the end of the observation period, indicated that the device was comparable to existing NPWT devices in terms of usability. These results suggest that SE-P47K-WAS is an effective treatment for intractable wounds that do not respond to existing treatments. The observation that 100% of the acute wounds achieved WPW after 14 days also suggests that SE effectively treats acute wounds. Based on these findings, SE likely contributes to the formation of WPW, reduction in the wound area, and formation of healthy granulation tissue in acute and chronic wounds.

In a previous study, the application of the SE-P47K-WAS sponge to full-thickness skin defects in a murine model promoted granulation tissue formation, epithelialization, and angiogenesis, compared to conventional collagen sponges (Pelnac®)[18,19]. In addition, the application of an SE sponge increases the migration of macrophages[20] and promotes their polarization into anti-inflammatory macrophages (M2)[21], accelerating wound healing. In this clinical study, well-prepared wounds, reduced wound areas, and healthy granulation areas were observed, suggesting that the SE sponge can increase macrophage migration, as suggested in animal studies[19].

The initial investigation showed that the application of SE-P47K-WAS did not cause severe adverse effects on the wounds. The current clinical study demonstrated similar results, with the exception of a case of *Pseudomonas* infection. These findings suggest that SE-P47K-WAS can be safely administered in the clinical setting.

Although SE-P47K-WAS has a sponge-like shape, its behavior after application to the wound surface differs from that of a conventional artificial dermis, which has a similar shape. Although the artificial dermis made of collagen sponge functions as a useful scaffold for skin defects[25,26] after the resection of malignant tumors and trauma/burn wounds by allowing blood vessels and cells to penetrate its pores[27], SE-P47K-WAS is dissolved by exudate and converted to hydrogel at body temperature. This transformation enables it to adhere effectively to the wound surface, promoting macrophage migration and enhancing wound healing. This process promotes rapid epithelialization and angiogenesis. In addition, no cell or blood vessel invasion was observed in the gelatinized material[19], which differed from that in the artificial dermis. SE-P47K-WAS may exhibit greater efficacy than artificial dermis in wounds, necessitating rapid epithelialization or angiogenesis. However, this hypothesis was not substantiated in the present study but was proposed as an alternative approach. Further investigation is required to evaluate the comparative efficacy of SE in human wounds.

This study has several limitations. The primary limitation was the small sample size of 25 patients in the phase III clinical trial without a control group. The sample size was determined using a power analysis based on a previous safety study[22] that calculated a minimum of 17 patients with chronic wounds. Accounting for potential dropouts, 20 patients had chronic wounds, and five had acute wounds. A descriptive analysis referencing previous NPWT clinical trials was conducted[24], and the investigators were surveyed to compare the convenience of different NPWT devices to compensate for the lack of a control group. However, these measures cannot eliminate the potential bias in this uncontrolled study, necessitating further research with larger sample sizes and comparison groups to demonstrate the efficacy and safety of SE-P47K-WAS. Additionally, although no allergy-related adverse events were observed, patients should be cautioned about the potential risk of anaphylaxis or other adverse reactions to the raw material, particularly given the limitations of the sample size. Furthermore, the short follow-up period of 28 days may have inadequately addressed long-term complications and scarring outcomes. Further studies with extended follow-up periods are required to address these issues. The efficacy of the treatment for infection control was also insufficiently verified in this study. Kawai et al. reported that an aqueous silk elastin solution inhibited bacterial growth in a mouse model of bedsores. However, the findings of this study did not show such antibacterial effects. Consequently, the use of this product for infected wounds should be approached with caution.

In conclusion, SE-P47K-WAS is a versatile gel for treating skin wounds. Its superior adhesive properties render it an efficacious solution for complex wounds, promoting macrophage migration to the wound surface and facilitating efficient wound healing. Moreover, this product does not require daily bandage changes and has demonstrated an efficacy comparable to that of conventional wound-healing devices for acute and chronic wounds. Its adhesive properties are believed to provide an advantage in managing complex acute wounds that are challenging to close. For chronic wounds, it may help improve the wound environment when other treatments prove ineffective. These findings indicate that SE-P47K-WAS is a safe and efficient option for wound healing. In summary, this study evaluated the potential efficacy and safety of SE sponges in wound healing, highlighting their promise as an alternative treatment for wounds unresponsive to conventional therapies.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

NM and SK designed and conceived of the study. ES, MS, YK, KN, and NM collected data.ES, MS, SK, and SS analyzed and interpreted the results and wrote and revised the manuscript. SK and SS supported the statistical analysis. All authors reviewed and approved the manuscript.

Declarations

Competing interests

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Additional information

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