Vaccine 37 (2019) 3810–3819

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Transcutaneous immunization of recombinant Staphylococcal enterotoxin B protein using a dissolving microneedle provides potent protection against lethal enterotoxin challenge

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ARTICLE INFO

Article history:
Received 22 October 2018
Received in revised form 16 May 2019
Accepted 19 May 2019
Available online 27 May 2019

Keywords:
Dissolving microneedle
Transcutaneous immunization
rSEB

ABSTRACT

Staphylococcal enterotoxin B (SEB) produced by the *Staphylococcus aureus* bacteriumis most commonly associated with food poisoning and is known to also cause toxic shock syndrome. Currently, no approved vaccine or specific drug is available to treat SEB intoxication. In this study, we fabricated dissolving microneedles (MNs) loaded with recombinant SEB (rSEB) protein, and evaluated its characteristics, including dissolution profile, protein particle size, insertion depth, antigen retention time in vivo, and skin irritation. Our results showed that rSEB protein-loaded dissolving MNs made of chondroitin sulfate (2%) and trehalose (0.8%) could easily penetrate into the mouse skin within 5 min. The rSEB particle size was unchanged before and after MN fabrication. The skin penetration depth of the MNs was 260 μm. Moreover, the MNs also significantly extended the antigen retention time in vivo. rSEB protein-loaded dissolving MNs also triggered slight erythema at the beginning of administration, but this erythema disappeared within a few hours. More importantly, we investigated the immunogenicity and protective efficacy of rSEB protein-loaded dissolving MNs. Challenge studies in mice revealed that mice in full-dose MN group had a high level of SEB specific antibody response thatprovided100% protection against a lethal SEB toxin challenge. However, there was only 60% protection observed in mice that were in the half-dose MN (dose sparing) group. We also determined the pathological alterations in the tissues of the immunized mice. Taken together, these dissolving MNs may present a promising transcutaneous immunization strategy for treating SEB intoxication.

1. Introduction

Staphylococcal enterotoxin B (SEB) is secreted by the gelanshi-negative bacterium *Staphylococcus aureus*, which produces several enterotoxins and superantigens. Currently, there are around twenty serotypes reported [1].SEB is one of the most serious exotoxins and enters the body through the digestive system and respiratory tract [2].When the toxin is ingested in food, it causes vomiting, abdominal pain, diarrhea and other symptoms. When the toxin enters the circulatory system, it causes vascular inflammation and severe toxic shock syndrome [3,4]. Currently, there is no effective vaccine or specific drug to treat the effect of SEB toxins.

Previously, SEB vaccine studies focused on the use of formalin inactivated toxoid vaccine, but they have not been approved for human use [5].In addition, other SEB mutants or subunit candidate vaccines are in preclinical stages of development [6]. There has also been a rationally designed recombinant SEB protein vaccine, termed STEBVax, containing triple mutations (L45R, Y89A, and Y94A) in the SEB molecule that has completed Phase 1 clinical trials in 2016. It was found to confer effective protection against SEB-induced diseases by inducing the generation of antibodies as well

Abbreviations: rSEB, recombinant staphylococcal enterotoxin B; BSA, bovine serum albumin.

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https://doi.org/10.1016/j.vaccine.2019.05.055
0264-410X/

as cellular immune responses and therefore is a promising SEB vaccine candidate [1,7].

Intramuscular injection is a traditional strategy of vaccine inoculation in humans. Direct needle injection provides a low-cost, rapid and direct way to deliver almost any type of molecule into the body. It allows for efficient delivery of protein to the target sites, but is associated with pain, poor compliance and requires the assistance of medical professionals [8]. Dissolving microneedles are micronscale structures that painlessly pierce the skin to administer vaccines in a minimally invasive and targeted manner [9]. They can result in antigen dose sparing, do not generate medical waste and have no risk of depositing harmful materials in the skin [10]. Due to the abundance of resident Langerhans and dermal dendritic cells in the skin, which are powerful antigen presenting cells, skin immunization has become an attractive direction for vaccine delivery [11,12]. In addition, MN delivery might not require a health care professional for vaccine administration, assuming that such a delivery would become available in the future.

In this study, a rSEB proteins was used as the model antigen to investigate the feasibility of transcutaneous immunization (TCI) using dissolving MNs and to determine whether MNs could induce potent immune responses that can provide effective protection against a lethal SEB challenge. After fabrication of rSEB protein-loaded dissolving MNs, we evaluated the characteristics of the prepared MNs, including dissolution profile, protein particle size, insertion depth, antigen retention time in vivo, and skin irritation. Finally, the protective efficacy of our dissolving MNs was evaluated with a wild type SEB toxin challenge and passive transfer studies.

2. Materials and methods

2.1. Fabrication of rSEB protein-loaded dissolving MNs

Recombinant SEB protein was prepared using the *E. coli* expression system as described previously [13]. Briefly, the plasmid was constructed according to the reported recombinant SEB vaccine (STEBVax) (GenBank no. AB479118.1). The plasmid was transformed into Escherichia coli BL-21 (DE3) competent cells and induced with 0.1 M IPTG (isopropyl- β-D-thiogalactopyranoside) for 12 h at 30 °C. The rSEB protein was purified by SP Sepharose 4FF (GE) ion exchange chromatography and washed with a buffer containing 50 mM CH3COONa and 300 mM NaCl (pH 5.5). After dialysis against 0.01 mol/L PBS (pH7.2), the concentration of rSEB was 4 mg/ml (BCA Protein Array kit, $R^2 = 0.998$). The purity of rSEB protein was analyzed by SDS-PAGE. The rSEB protein-loaded dissolving MNs were produced using a micro-mold casting method. The polydimethylsiloxane (PDMS) micro-molds used in this study included 14 × 14pyramidal needles (approximately 570 μm long and 180 μm wide at the base, with400 μm interspacing). The matrix solution contained chondroitin sulfate (2% (w/v)), trehalose (0.8%(w/v)) and rSEB protein. We incubated the mixture for 1 h at room temperature. Then, the mixture was spun into the PDMS mold by centrifuging at 3000gand applying a vacuum to completely fill the pinholes of the silicone rubber (PDMS) mold. Finally, it was dried in a 4 °C silica gel cartridge. Cy-7-labeled rSEB was incorporated into MNs using the same procedure.

2.2. In vivo dissolution of rSEB proteins from MNs

The rSEB protein-loaded MNs were inserted into the exposed mouse skin and left for different time intervals (0, 0.5, 1, 3 and 5 min) to dissolve the MNs. The MNs were then removed and the applied area was tape-stripped twice. The dissolved MNs and its needle lengths were examined with a digital microscope (Osaka, Japan).

2.3. rSEB protein size evaluation

After fabrication, the MNs were immersed and dissolved in 0.5 mL of PBS for evaluation of structural stability of the protein by dynamic light scattering (DLS). The particle size distribution of the rSEB proteins was measured by DLS using a Zetasizer Nano ZS particle analyzer (Malvern Instruments Ltd., UK). The native rSEB solution with a similar concentration was also analyzed as a control.

2.4. Skin insertion capability of rSEB protein-loaded dissolving MNs

To test its skin insertion capability, the Cy-7-labeled rSEB dissolving microneedles were applied to ex vivo porcine ear skin for 3 min and then removed. Porcine ear auricle back skin was purchased from Linxi Jingde Agricultural Products Sales Co., Ltd. Immediately, thereafter, the treated pig skin was placed on a glass slide and observed under a confocal laser scanning microscope (CLS, Olympus, Japan). Representative images were recorded in the xy-plane. The skin was scanned one layer at a time from the surface down into the deeper layers at intervals of 20 mm to determine the depth of skin puncture depth due to the microneedles. The 3D confocal reconstruction images were obtained using a XYZ-stack to visualize the penetration.

2.5. Distribution of rSEB after MNs insertion in vivo

Using in vivo imaging, we observed the retention time of Cy7-labeled rSEB protein in mice after insertion. Eight-week-old BALB/c female mice (18–20 g) were used for conducting the in vivo insertion test. A depilatory cream was used to remove the fur from the abdomen of the mice. The Cy-7-labeled rSEB dissolving microneedle was applied to the skin of the living mice for 5 min and then removed. An equal dose of the rSEB protein was intramuscularly injected into the hind leg muscle of each mouse. The mice were anesthetized with 1% pentobarbital sodium and observed under an In Vivo Imaging System (IVIS) (Kodak BioMax, Rochester, NY, USA). The mice were then placed in an incubator until they regained consciousness. This IVIS system was used to collect and analyze all fluorescence data. Follow-up inspections were also conducted at 4 h, 7 h, 2 days, 3 days, 4 days, 5 days, 6 days, and 7 days after insertion.

2.6. Skin irritation caused by MN application

The exposed dorsal skin of living mice was treated with rSEB protein containing MNs as described above. To evaluate skin irritation caused by MN application, the treated areas were photographed and scored blindly by an experienced dermatologist for signs of erythema or edema using the Draize scoring methods at both pre- and post-application time points (0, 1, 2, 4 and 24 h after MNs removal).

2.7. Draize test

The rSEB dissolving microneedle was applied to living mice skin for 5 min and then removed, and we observed the local skin immediately and after 1, 2, 4 and 24 h. Skin irritation was evaluated after MN immunization. Skin erythema and edema were graded based on a 0–4 scale as described previously [14]. Briefly, 0 denotes no erythema; 1, barely perceptible erythema; 2, well defined erythema; 3, moderate to severe erythema; and 4, severe erythema.

2.8. Immunization studies in mice

2.8.1. Immunization protocol

Groups of BALB/c mice aged 6–8 weeks were divided into four groups (n = 8per group): (1) the blank MNs group (negative control), in which unloaded MNs were administered to the dehaired dorsal skin using the applicator; (2) the MNs group (MN), in which 26 μg of rSEB protein loaded MNs were applied to the dorsal skin; 3) the MNs group (1/2 dose MN), in which 13 μg of rSEB protein loaded MNs were applied to the dorsal skin; and (3) the intramuscular (IM) injection group, with rSEB protein solution (26 μg/100 μL per dose). For MNs vaccinations, each mouse was anesthetized and dehaired before each immunization. For IM administration, the rSEB protein stock solution was diluted to the desired concentration using PBS. Mice received a prime immunization (week 0) followed by two booster immunizations at 3-week intervals (weeks 3 and 6), and blood samples were collected before immunization and 3 weeks after the last immunization. All mice experiments were approved by the Institutional Animal Care and Use Committee at the Beijing Institute of Microbiology and Epidemiology.

2.8.2. Antibody response measurement by ELISA

Total SEB-specific IgG and IgG subtype levels in the sera from immunized mice were measured by ELISA using rSEB protein (5 μg/ml) as the coating antigen. Briefly, each well of the 96-well plate was coated and incubated overnight at 4 °C. Plates were blocked with PBST containing 5% milk, incubated with diluted serum samples for 2 h at 37 °C, and then incubated with diluted HRP-conjugated goat anti-mouse IgG, IgG1 or IgG2a antibodies (Sigma) for 1 h at 37 °C.

2.8.3. Challenge test

Three weeks after the last immunization, groups of mice were challenged intraperitoneally (I.P) with 1 mg (∼2LD50) of wild type SEB and 4 h later with 50 μg of LPS, as previously described [15,16]. After the challenge, the mice were observed for 10 days to determine the status of vaccine protection.

2.8.4. Histological examination

Mice in each group (n = 3) were sacrificed at 3 days after the wild type SEB challenge. The intestines, lungs, kidneys, and spleen tissues of each mouse were separated, fixed in formalin, and embedded in paraffin. Then, these tissues were stained with hematoxylin-eosin and photographed under a microscope.

2.8.5. Passive immunization and challenge

The protective efficacy of rSEB protein dissolving MNs was further evaluated by a passive immunization/challenge experiment. Two groups of BALB/c mice were I.P inoculated with 100 μL of anti-SEB pooled serum/mouse. One day later, all mice were I.P injected with wild type SEB followed by LPS. The survival of the challenged mice was monitored and recorded for a period of 7 days.

2.9. Statistical analysis

Data are presented as mean value ± standard deviation. Antibody titers are expressed as the geometric mean values of each sample. Statistical data were analyzed by Student's t-test or one-way analysis of variance using SPSS version 18.0 (San Diego, CA, USA). Statistical differences were considered to be significant when $p < 0.05$.

3. Results

3.1. Fabrication of rSEB dissolving microneedles

The rSEB MN vaccine patch was produced using polyvinyl alcohol (PVA) as illustrated in Fig. 1, which shows an overview of the dissolving microneedles and the size of the MN patch containing 196 MNs (Fig. 1a and b). The whole patch has an effective encapsulation of 13 ± 1.4 μg/patch of rSEB. Fig. 1c displays the images of skin stained with trypan blue after the puncture experiments. The microneedle pinhole array is clearly shown in the image, revealing that the whole microneedle structure could penetrate the skin with each single needle.

Then, we analyzed the purity of recombinant SEB proteins with SDS-PAGE and found the purity of rSEB protein was more than 90%. Also, we found there is no obvious change in the intactness of the molecule before and after immunization with peptide mapping analysis (Fig. S1a and b). Subsequently, the distributions of the SEB protein in the MNs were observed under confocal microscope. The results showed that Cy7-labeled rSEB was distributed evenly in the upper region of the needles, whereas little or no red fluorescence was apparent near the shaft bases or base plate, suggesting that the majority of the encapsulated antigens were localized in the needle shafts (Fig. 1d). In addition, when viewed under a microscope, we found that the height of the rSEB MNs was 570 nm (Fig. 1e).

3.2. In vivo dissolution characteristics of MNs

The in vivo dissolution profile of the rSEB antigen from the dissolving MNs was evaluated in mice. After insertion into the skin for different durations, the dissolution process of the needles was monitored. As seen in Fig. 2, approximately one-third or half of the MN shafts were dissolved when left in the skin for 30 or 60 s, respectively. When the insertion time was extended to 5 min, almost all of the needles were dissolved. Meanwhile, the needle length shortened from 560 nm at 0 s to 150 nm at 3 min. Thus, these data showed that the rSEB MNs were fully dissolved into the mouse skin within 5 min.

3.3. rSEB size distribution

To examine the structural integrity of the rSEB during the drying process, we compared the rSEB before and after encapsulation by DLS (Fig. 3). Native rSEBs are in particulate form with a diameter of 568 nm (z average mean). After encapsulation into the matrix, the rSEBs exhibited a similar size distribution as before, suggesting that even when dried at room temperature, the rSEBs in the prepared MNs underwent negligible aggregation or degradation.

3.4. rSEB dissolving microneedle insertion

Previous studies showed that several factors could influence the depth of microchannels, including the geometry of MNs, pressure and the density of material [10]. The microchannels created by rSEB protein-loaded MNs could be indicated visually using the confocal laser scanning microscope. As shown in Fig. 4, we observed the 14 confocal images obtained by scanning the skin punctured with Cy-7-loaded rSEB at different depths beneath the skin surface. Scanning was conducted once from the initial fluorescence of the skin surface through the z-axis perpendicular to the xy-plane at an interval of 20 μm. The fluorescence intensity gradually weakened as the MN depth increases. Notably, quite weak fluorescence intensity was observed at a depth of 260 μm. All of these data indicate that the insertion of rSEB protein-loaded MNs into porcine ear

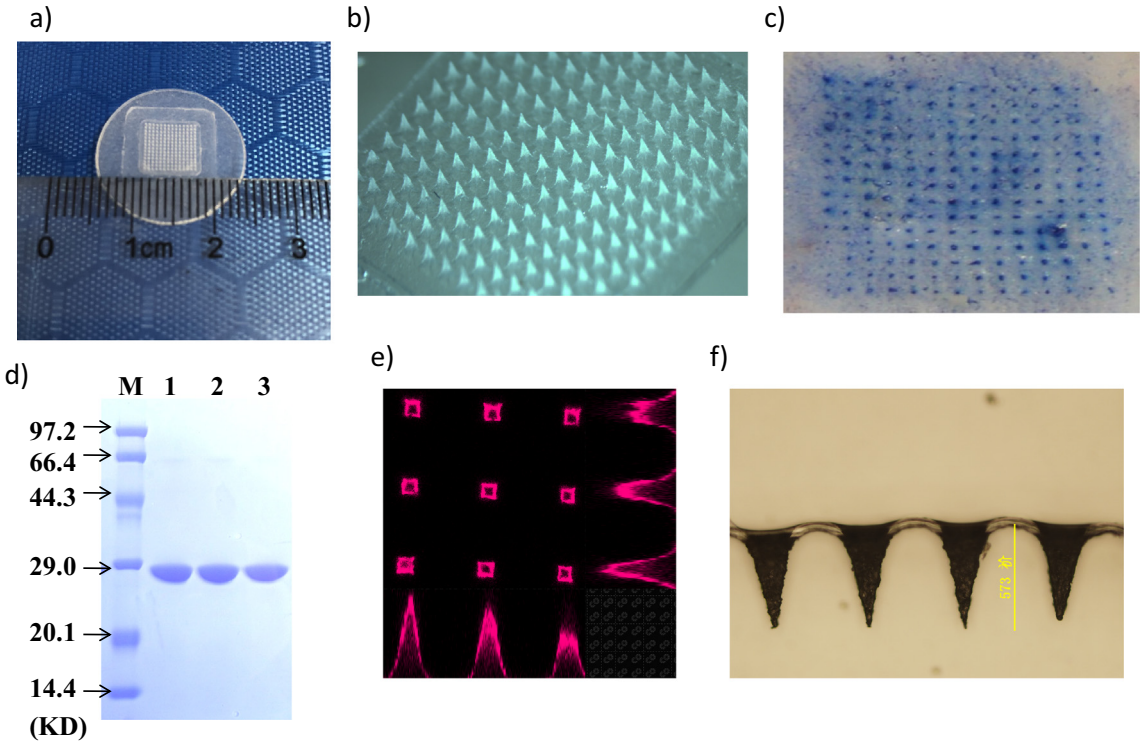

Fig. 1. Dissolving microneedles fabricated with recombinant staphylococcal enterotoxin B protein. (a) Side view of the dissolving microneedles and the relative size of the microneedles next to a ruler. (b) Digital images of arrays of a microneedle patch. (c) Sites of skin penetration from GRBS MNs with a force of 40 N. (d). SDS-PAGE of recombinant SEB protein. Lane 1–3: the purified rSEB protein before immunization. One sample was loaded and separated by electrophoresis gel for three times. M: marker. (e) Confocal microscope image of Cy-7-labeled rSEB upper needles. (f) The height of a single needle observed under a confocal microscope.

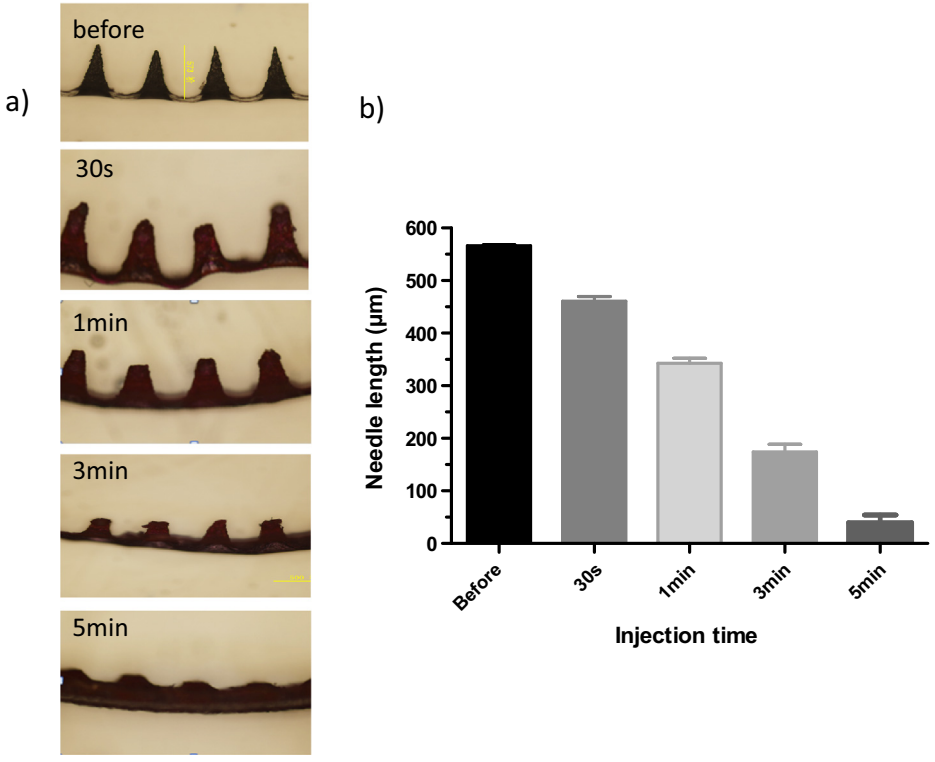

Fig. 2. rSEB released from microneedles. (a) MN dissolution before (0 s) and after insertion into skin for 30 s, 1 min, 3 min, and 5 min. (b) rSEB dissolving microneedles length before (0 s) and after insertion into skin for 10 s, 30 s, 1 min, 2 min, and 3 min.

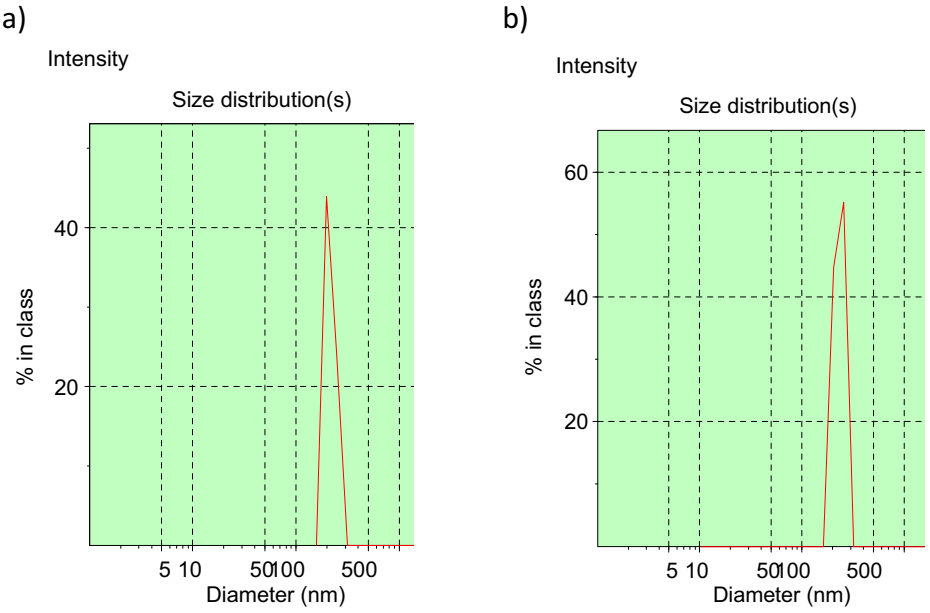

Fig. 3. Particle size distribution measured by DLS in native rSEB protein solution (a) and microneedles solution (b).

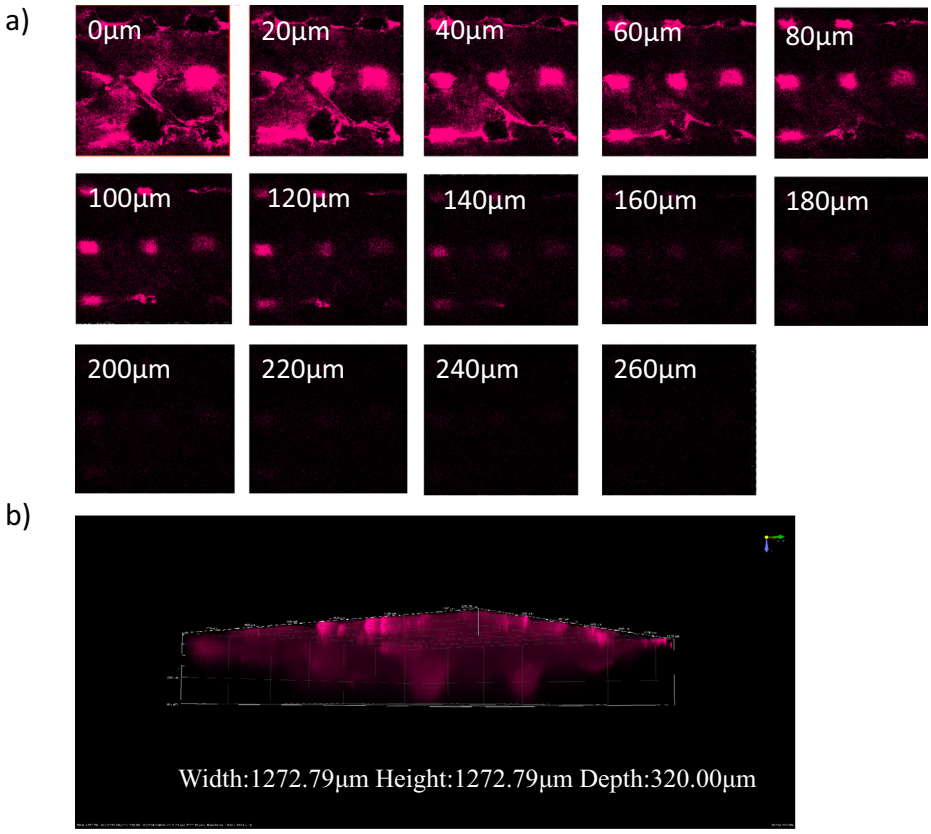

Fig. 4. Confocal image of rSEB dissolving the microneedle insertion. (a) Confocal microscope scanned rSEB microneedle through the z-axis. (b) 3D graphs of an overall view of micro conduits created by the rSEB microneedle.

skin was approximately 260 μm. The 3D reconstructed confocal image of penetration depth was illustrated in Fig. 4b.

3.5. Distribution of rSEB antigen after MN insertion

To characterize the cutaneous permeation of the released Cy-7-labeled rSEB, we inserted the dissolving MNs into BALB/c mouse skin for 5 min and then removed the patches from the skin. Meanwhile, the same concentration was IM injected as a control. All mice were visualized and photographed after 1hrs, 4hrs, 7hrs, 2 days, 3 days, 4 days, 5 days, 6 days and 7 days with IVIS. The skin sites that received Cy-7-labeled rSEB MNs exhibited a strong fluorescent signal, indicating that the rSEB antigen of MNs group could last up to6 days, whereas that of the IM group lasted only three

days, showing that the rSEB MNs significantly prolonged the antigen retention time in vivo (Fig. 5).

3.6. Skin irritation after MN insertion

An in vivo skin irritation study was performed using mice skin using the Draize scoring method. As seen in Fig. 6, the depilatory procedure before MNs insertion led to no significant edema or erythema on the mouse skin. After a 2 min application of dissolving MNs, the punctured skin showed slight or well-defined erythema (no edema) that rapidly lessened within the following 4 h. Thereafter, the affected area of the skin recovered to a similar to intact skin level within 24 h after MN application.

3.7. Immunization and antibody detection

Six-week-old female BALB/c mice (n = 8/group) were immunized with the rSEB proteins via MN or IM injection as described in the Materials and Methods. Mice were immunized with 13 μg or 26 μg of the rSEB protein three times at 3-week intervals. The sera collected 3 weeks after the last immunization was analyzed for antigen-specific antibody levels by indirect ELISA. As expected, SEB-specific antibodies were not detected in mouse sera from mice immunized with three doses of PBS. In contrast, MN, 1/2 dose MN and IM immunizations elicited detectable antibody responses after the first immunization, which were significantly increased after three booster immunizations (data not shown). Twenty-one days after the last immunization, the MN or 1/2 dose MN group had comparable serum IgG levels to that in the control group, and MN inoculation induced a higher titer of antigen-specific antibody responses than that after IM injection. The distribution of serum IgG subclasses induced by different immunization regimens were further determined as shown in Fig. 7B. The MN or 1/2 dose MN group showed higher IgG1 and IgG2a titers compared with the IM group. In all three immunization groups, IgG1 dominated the isotype profiles. The IgG1/IgG2a ratios for IM, MN or 1/2 dose MNs groups were 1.4, 2.6 and 1.9, respectively, indicating that MNs induced a more Th2-type immune response.

We tested the in vivo neutralization of SEB by the mice serum of three immunized with rSEB dissolving MNs in the LPS potentiation model of toxic shock in BALB/c mice. SEB (2 μg) was pre-incubated with each mice serum for 1 h at room temperature. Mice were then challenged with SEB alone or with SEB pretreated with the mice serum by intraperitoneal injection. After 4 h, mice were injected intra-peritoneally with 40 μg of LPS and monitored for 4 days for morbidity and mortality. As seen in Fig. 7e, whereas all mice treated with SEB and LPS alone were dead within 2–3 days, preincubation of SEB with the immunized mice serum resulted in 20% (IM), 50% (1/2 MN), and 80% (MN) protection from lethal challenge.

3.8. Protective effect of immunization with dissolving rSEB protein MNs

Next, the protective efficacy of dissolving rSEB protein MNs was evaluated in the experimental SEB toxin challenge model using BALB/c mice. Three weeks after the last immunization, the mice were challenged with 1 μg of wild type SEB (corresponding to 2LD50) by I.P injection and 50 μg LPS after 4 h [17]. As shown in Fig. 8, all mice vaccinated with MNs were completely protected from the toxin challenge. However, only 60% of 1/2 dose MNs vaccinated mice were protected. As expected, all mice in the control group died within 24 h after toxin challenge. Meanwhile, pathological alterations are shown; we found that the structure of the small intestine in the IM and PBS mice group was damaged, while it was intact in the MN group. Lungs in the IM and PBS groups were severely invaded compared with that in the 1/2 dose MN and MN group. Liver and kidney pathological results showed that the IM and PBS groups were seriously bleeding, while the results of the MN groups were effectively improved. Overall, the intestine, lung, kidney, and spleen histopathological changes in the MN or 1/2 dose MN mice were significantly alleviated in a dose dependent manner compared with the changes in the non-immunized mice.

To further examine the in vivo protective effect of MN immunization, naïve BALB/c mice were injected I.P. with the anti-SEB pooled sera from the immunized mice followed by I.P challenge at day 1 with 1 μg wild type SEB and 50 μg LPS after 4 h. Subsequently, the survival rate of the mice was observed for consecutive 7 days. As seen in Fig. 8, the mice inoculated with PBS gradually showed severe clinical symptoms, and all of them died at 24hr post-infection. In contrast, the mice administered anti-SEB pooled sera were free of severe clinical signs, and all of them survived over the entire 7-day study period. These results demonstrated that

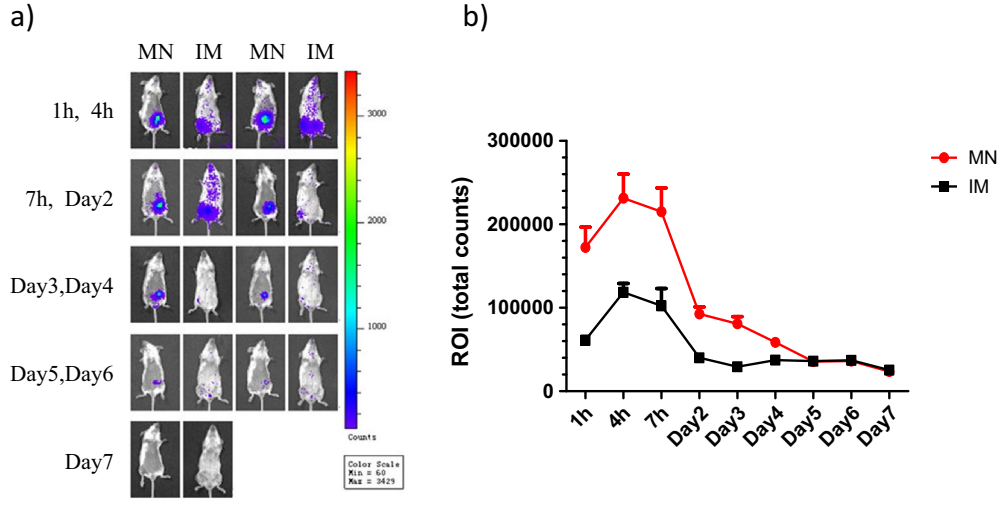

Fig. 5. Fluorescence intensity detected after MNs or IM of Cy-7-labeled rSEB protein at different time intervals. (a) In vivo fluorescence images of BALB/c mice at 1 h, 3 h, 7 h, 2 days, 3 days, 4 days, 5 days, 6 days, and 7 days after treatment with Cy-7-labeled rSEB microneedles (n = 5 for each group). (b) The amount of fluorescence was evaluated after 1 h, 4 h, 7 h, 2 days, 3 days, 4 days, 5 days, 6 days, and 7 days.

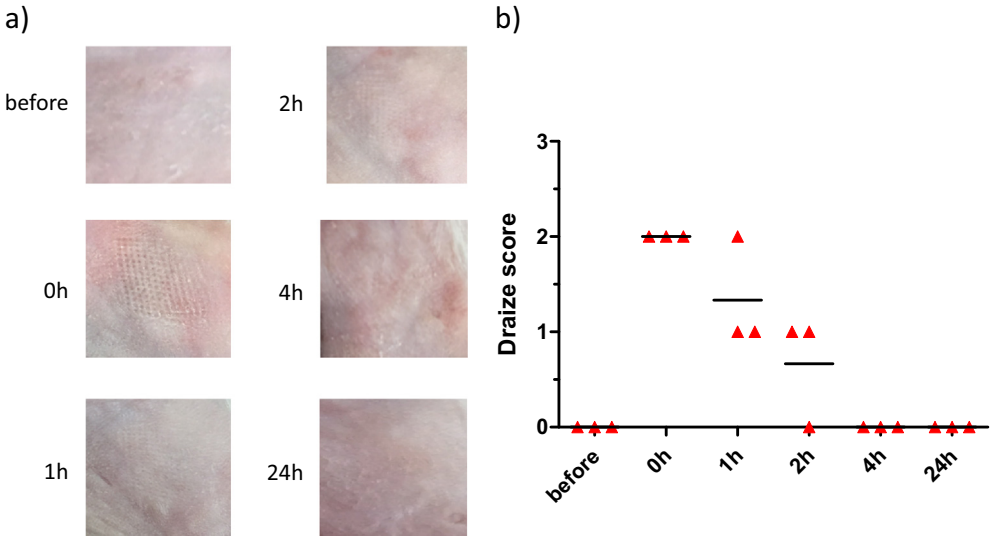

Fig. 6. Skin irritation after MNs insertion. (a) Depilatory procedure before MNs insertion caused no significant edema or erythema on the skin; (b) An in vivo skin irritation study was performed using mice skin by the Draize scoring method.

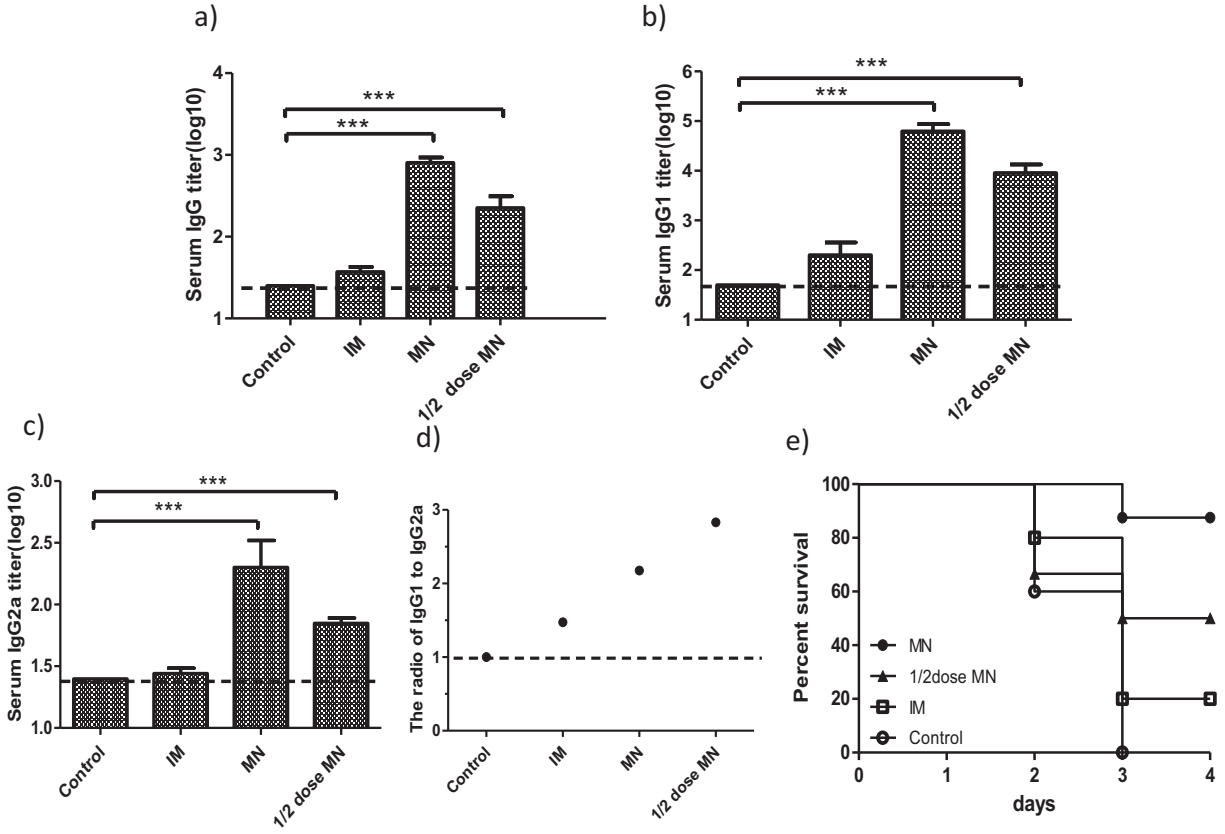

Fig. 7. Serum antibody responses after rSEB dissolving microneedles. (a) Serum IgG titers for ELISA after rSEB dissolving microneedles. The 6-to 8-week-old BALB/c mice (8 per group) were MN immunized with 13 μg or 26 μg rSEB, or IM immunized with 26 μg rSEB, or IM immunized PBS as a control group at three-week intervals, and serum samples at three weeks post last immunization were collected for ELISA.***$p < 0.001$. (b) Serum IgG1 subtypes titers for ELISA after rSEB dissolving microneedles. (c) Serum IgG2a subtypes titers for ELISA after rSEB dissolving microneedles. (d)The ratio of IgG1to Ig2a subtypes titers. (e) In vivo SEB neutralization by the serum of three immunized mice in mice model of toxic shock. BALB/c mice were challenged with 2 μg of SEB preincubated with the serum of three immunized mice and received 40 μg of LPS 4 h after SEB challenge. Analysis of data was performed using log-rank.

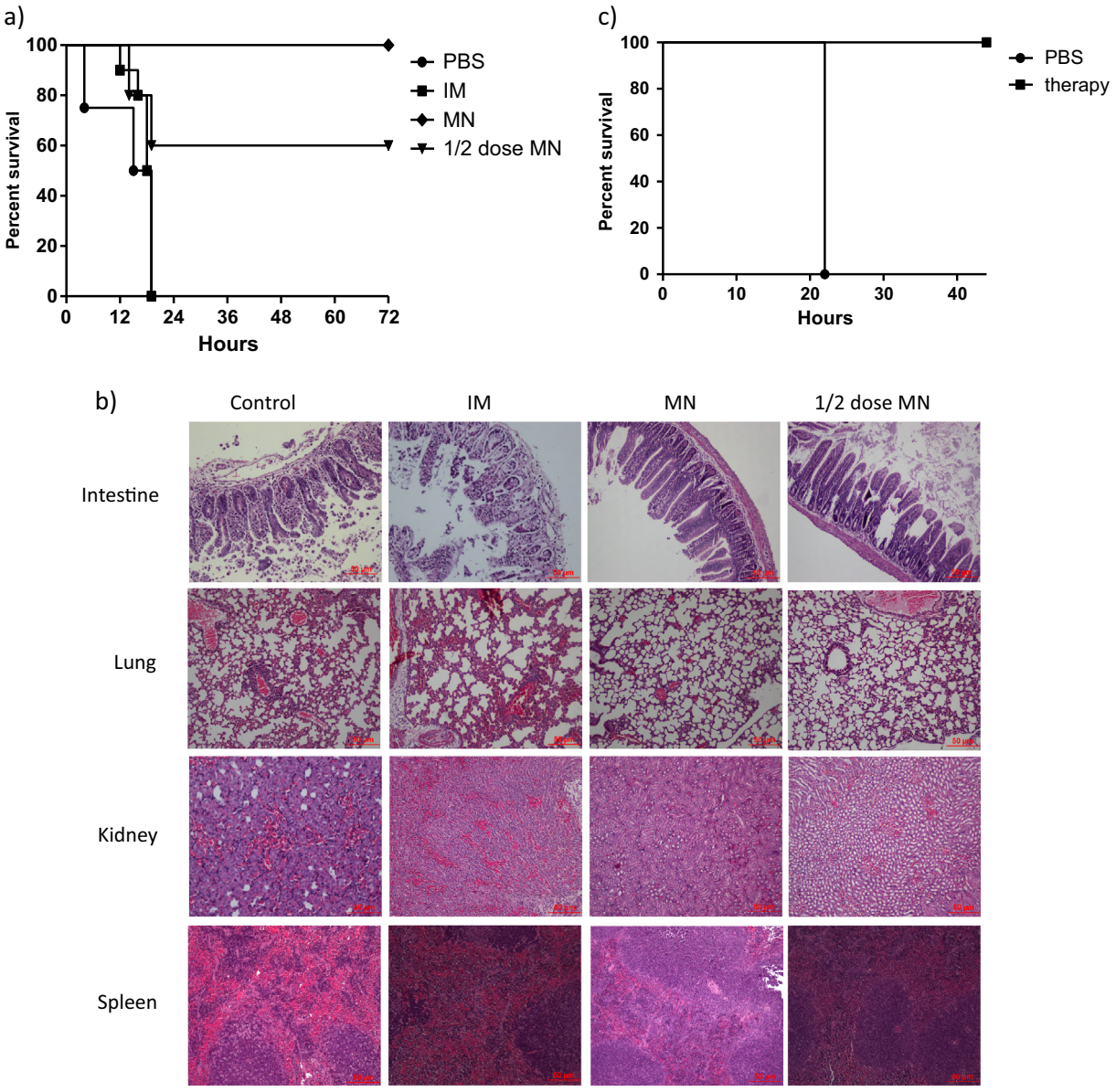

Fig. 8. Challenge test and pathology evaluation. (a) Protection of BALB/c mice vaccinated with rSEB proteins MNs. Mice (n = 8/group) were immunized IM with 26μgrSEB, or immunized MN with 13 μg or 26μgrSEB, followed by two booster immunizations with the same dose at 3-week intervals. Mice were challenged intraperitoneally with 1 μg wild type SEB (2LD50) and 50μgLPS after 4 h, three weeks after the final immunization. (b) Pathological findings from the intestines, lungs, kidneys, and spleens. Representative pathologic images for the tissues of mice are shown 3 days after wild type SEB toxin-challenge. (c) Immunized sera passively protected mice against a lethal SEB toxin challenge. Two groups of mice were IP injected with pooled antisera from mice treated with MNs (n = 8) and after 24 h, IP inoculated with wild type SEB and LPS after 4 h. The challenged mice were subsequently monitored daily for survival up to 7 days.

immunization with dissolving rSEB protein loaded MNs provides effective protection against a lethal SEB challenge.

4. Discussion

Microneedle technology as a practical and more convenient method has been used to deliver several active ingredients, including biological macromolecules, drugs and vaccines [14,18–20]. Previous studies have shown that dissolving MNs are currently used with polio vaccine immunization, diphtheria and tetanus Toxoid, vitamin K, Streptococcus suisbacterin and so on [21–23]. Another group reported that in situ nanomicelle-generating dissolving MNs could enhance cancer vaccination [24]. Strikingly, first in clinical study of vaccine-loaded MNs have been reported where MN were used to vaccinate humans against influenza [25]. Dissolving MNs, as an innovative vaccine immunization device, exhibit several advantages: (1) normally their application leads to no pain or bleeding, which makes it especially attractive for pediatric populations; (2) they do not require reconstitution and release the loaded antigen rapidly thereby simplifying usage, storage, transportation and disposal; (3) they offer the possibility of enhanced immunogenicity and dose sparing; and (4) they reduce the requirement for trained healthcare staff [26–28]. Of course, several challenges of dissolving MNs will require further investigations including antigen wastage, barriers to commercialization for industrialization, potential polymer accumulation in vivo [29]. With the prospect of self-vaccination, avoidance of sharps disposal, and dose sparing it is predicted that the full economic evaluation of

MNs will prove to be cost-effective. To this end, the purpose of this study was to evaluate the potential use of dissolving rSEB protein-loaded MNs as a TCI vaccine against SEB toxin.

In this paper, we first prepared the rSEB protein-loaded MNs using a micro-mold casting method and then evaluated its characteristics. As seen in Fig. 2, the prepared rSEB protein-loaded MNs almost completely dissolved and released their payload 5 min after in vivo insertion into the mouse skin. Due to their rapidly dissolving characteristics, we speculated that non trained individuals can finish the vaccination course by themselves within minutes, resulting in a huge improvement in vaccine delivery efficiency.

We further assessed the protective efficacy of rSEB dissolving MNs in mice and IM injections at the same dose. Meanwhile, to examine any dose-sparing effect, the 1/2 dose MNs group was deliberately set up at half the dose of the other injection groups. The kinetics of the SEB-specific antibody responses showed that these serum antibody levels were significantly elevated after two booster immunizations in all immunized mice (Fig. 7).

In mice, the T-cell proliferative response induced by SEB requires the presence of I-E-bearing antigen-presenting cells for optimal mitogenic activity [30]. Lethality occurred only when SEB was potentiated by LPS. Thus, SEB and LPS together was recognized as a widely accepted SEB lethal mice model [31]. Subsequently, mice challenge results showed that rSEB protein-loaded MNs could provide complete protection (100%), and a 1/2 dose MN patch afforded a significantly higher level of protection (60%) in immunized mice against a lethal SEB toxin. These findings further suggest that MN immunization provides comparable protective efficacy to that provided by conventional injection vaccination and that only a 1/2 dose (dose sparing) was needed.

Finally, BALB/c mice who received antisera therapy from each group only showed transient weak symptoms, and all mice survived; in addition, the treatment provided full protection against a subsequent lethal SEB challenge (Fig. 8). The reason for this outcome might be related to the high SEB specific antibody responses in the pooled antisera from the immunized mice. Moreover, there is a dose-sparing effect since antisera from mice immunized with 1/2 dose of the rSEB protein-loaded MNs provided100% protection against a lethal SEB challenge. However, whether rSEB protein-loaded MNs with a much lower 1/3, 1/5 or 1/10 dose are equally effective needs to be further explored.

To evaluate the potential for cold-chain storage, we also assessed the storage stability of rSEB protein-loaded MNs at different temperature (4, 25 or 37 °C). When the MNs were stored at 4 °C, the rSEB protein content showed no significant changes for up to 12 months of storage. When stored at 25 or 37 °C, the remaining rSEB protein activity slowed significantly during the first two weeks and then decreased slowly over the next four weeks (data not shown here). Consequently, we can conclude that rSEB proteins in dissolving MNs showed superior stability compared to those in an aqueous solution. The long-term stability of rSEB protein-loaded MNs also needs to be investigated in future studies. These findings demonstrate that these dissolvable MNs might present a potential and effective vaccination alternative against SEB toxin.

5. Conclusion

In the current study, we have developed dissolving microneedles (MNs) loaded with recombinant SEB (rSEB) proteins and evaluated their characteristics. rSEB protein dissolving MNs could easily penetrate into the mouse skin and significantly extend the antigen retention time in vivo. After MN fabrication, the rSEB particle size remained unchanged. The skin penetration depth was 260 μm. These MNs did trigger slight erythema that disappeared within several hours. Moreover, immunization with these MNs could elicit the production of antibody immune responses in mice. Mice challenge studies demonstrated that MN immunization conferred complete protection against a lethal SEB challenge, but only partial protection was observed in fractional (for example, 1/2) dose MNs. Therefore, our dissolving MNs have potential as a promising vaccine, including as an anti-SEB vaccine, using a transcutaneous immunization approach that needs further investigation in preclinical and clinical studies.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgement

This work was supported by the National Key Research and Development Plan of China (No. 2016YFC1000902).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.055.

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