

Transdermal delivery of sinapine thiocyanate by gelatin microspheres and hyaluronic acid microneedles for allergic asthma in guinea pigs

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ABSTRACT

Dissolving microneedles (MNs) are an efficient, safe, and generally painless method for transdermal distribution of poorly permeable medicines. Here, dissolving composite MNs were prepared from sinapine thiocyanate (ST)-loaded gelatin microspheres (GMS) and hyaluronic acid (HA). To immobilize ST in MNs, we used a two-step centrifuging and molding method. When ST-GMS/ST-HA MNs were placed on the skin, they showed extraordinary mechanical strength and dissolved slowly. In vitro, skin implantation ability was assessed with fluorescein isothiocyanate staining, which revealed progressive penetration from the puncture site into deeper tissues. The feasibility of transdermal delivery of ST-GMS/ST-HA MNs in allergic asthma guinea pigs was then determined through in vivo pharmacodynamic and pharmacokinetic tests. The results indicated that ST-GMS/ST-HA MNs, in comparison with the traditional subcutaneous application approach, achieved both high efficiency and continuous release of ST. Therefore, this device is promising for the delivery ST for allergic asthma therapy.

1. Introduction

Allergic asthma is a heterogeneous illness that is becoming more common worldwide. It is a chronic inflammatory illness of the airways in which allergen exposure induces dyspnea, airway hyperreactivity, wheezing, and coughing (James et al., 2019).

A transdermal drug delivery system (TDDS) acts on the surface of the skin to deliver drugs to the blood circulation (Sun et al., 2014). The therapy of applying traditional Chinese medicine to the skin is an ancient method to treat diseases externally (Gao et al., 2020). Drug stimulation occurs by absorption through the skin and is used to cure illnesses such as bronchial asthma, allergic rhinitis, repeated respiratory infections, and other diseases (Lin and Shen, 2015). Baijiezi powder is used in the prevention and treatment of asthma through transdermal application. Modern pharmacological studies have shown that sinapine thiocyanate (ST), the main component of white mustard seed (Alqahtani et al., 2018; Shoji et al., 2016; Wu et al., 2020), has strong anti-asthmatic, antitussive, and expectorant effects through relaxing smooth muscles and inhibiting capillary permeability (Wang et al., 2011; Liu et al., 2015; Wu et al., 2016). However, transdermal drug delivery has many limitations, primarily the natural skin barrier of the

human body, through which most drugs are slowly and poorly absorbed, thus making effective therapeutic drug concentrations difficult to achieve (Wu et al., 2020). Because of the limitations of transdermal drug delivery, researchers are increasingly focusing on new transdermal drug delivery systems, among which microneedle (MN) drug delivery systems have attracted great attention. Because of its high efficiency, ease of application, and noninvasiveness, MN technology has been widely used in TDDS. Furthermore, drugs can be delivered to the dermis directly through the stratum corneum of the skin (Panda et al., 2021; Lopez-Ramirez et al., 2021). Dissolvable MNs, because of their excellent drug loading capacity, safety, and biocompatibility, are a good carrier for promoting the transdermal penetration of drugs (Yenkoidiok-Douti et al., 2021; Suriyaamporn et al., 2021). After the MNs are placed and dissolved, the medicine loaded inside the dissolvable MNs is released into the dermis (Pukfukdee et al., 2020; Wu et al., 2021; Hao et al., 2020; Xu et al., 2019).

In the present study, we developed an ST-loaded dissolvable hyaluronic acid (HA) and gelatin microsphere (GMS) MNs for allergic asthma therapy. The Food and Drug Administration (FDA) has authorized the needle material HA and gelatin, and the MNs have been demonstrated to have good skin insertion ability (Becker et al., 2009;

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Tezel and Fredrickson, 2008). HA releases drugs quickly (Liu et al., 2010; Li et al., 2021), and GMS can release drugs slowly and prolong the administration of transdermal absorption, distribution, and action. In the acute attack of allergic asthma, HA-loaded ST dissolves quickly and can rapidly relax tracheal calming muscles, expand the trachea to increase airflow, improve small airway function, and reduce the number of asthma attacks in patients. With the extension of administration time, ST in GMS will also slowly dissolve, thereby prolonging the action time of the drug. It maintains the durability of drug delivery, better prevents and controls the next paroxysmal asthma attack, and achieves the quick-acting and long-acting treatment of allergic asthma. To investigate the effect of ST-GMS/ST-HA MNs on the treatment of allergic asthma, we constructed a guinea pig model of allergic asthma and collected samples from the lung using microdialysis. In addition, pharmacokinetic (PK) and pharmacodynamic (PD) data were evaluated in allergic asthma guinea pigs. The results that we obtained should enable the development of a therapeutic strategy using drug-loaded dissolvable MNs to manage allergic asthma (Scheme 1).

2. Materials and methods

2.1. Materials

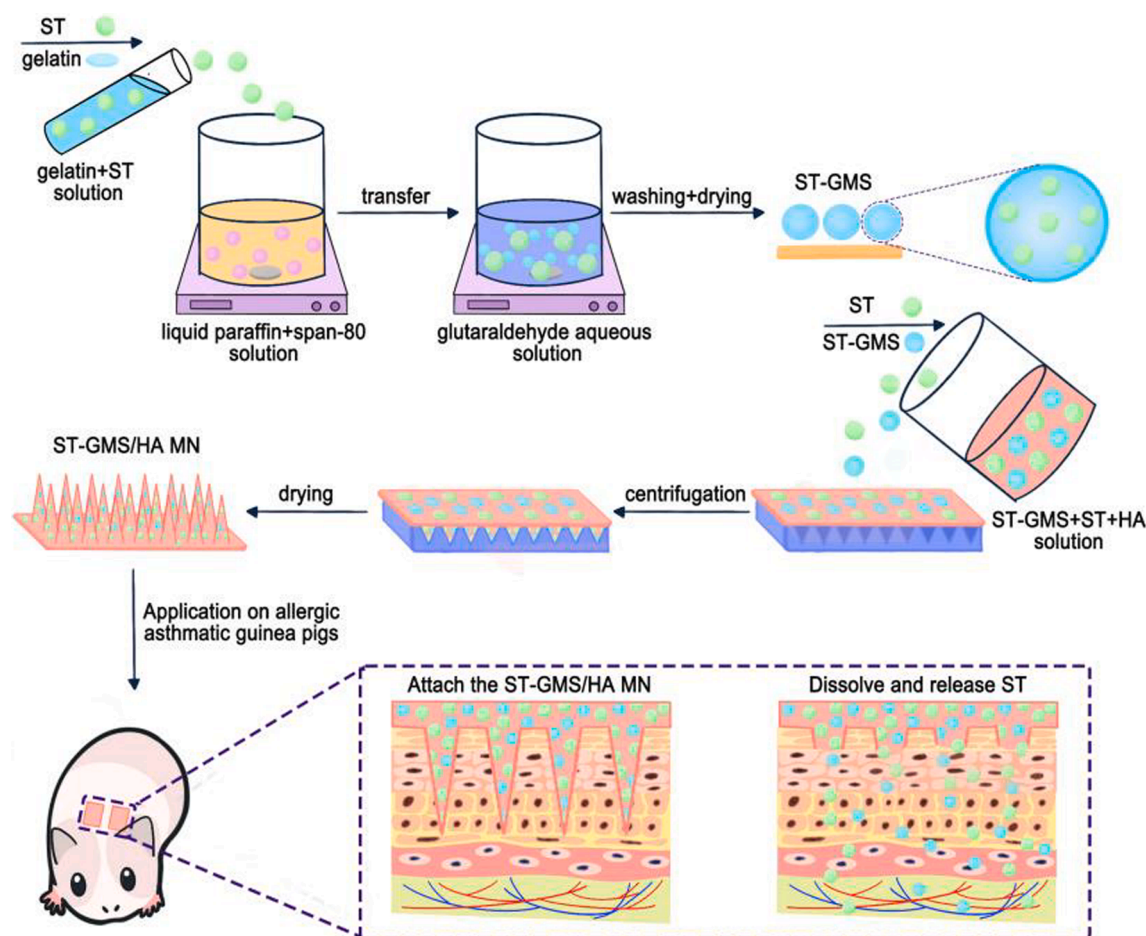
HA ($M_r = 1,000,000$, pure 96.3%, w/w) was from Huaxi Freda Biomedical Co., Ltd. (Jinan, Shandong, China), the ST reference substance (pure 99.39%, w/w, batch number 111702-200501) was from the National Institutes of Food and Drug Control, gelatin (batch number: 20190903) was from the Tianjin Guangfu Chemical Research Institute,

ribavirin reference substance (pure 99.27%, w/w, batch number 140629-201703) was from the China Institute of Food and Drugs, Merck KGaA provided high-performance liquid chromatography (HPLC) grade acetonitrile and methanol (Darmstadt, Germany), and the tulobuterol patch (Imported medicine standard H20110366) was from the Nitto Denko Corporation (Osaka, Japan). Male specific-pathogen-free (SPF) guinea pigs were purchased from Heilongjiang University of Chinese Medicine's Safety Evaluation Center (Heilongjiang, China, license number: SCXK (Hei) 2020-001, initial weight 370 ± 20 g) and placed in the animal experimentation center. Biyuntian Biotechnology Co., Ltd. (Shanghai) provided histamine, IL-10, and INF-kits for guinea pigs. Sigma-Aldrich (St. Louis, MO, USA) provided the ovalbumin (OVA).

2.2. Preparation and characterization of microneedles

2.2.1. Sinapine thiocyanate-loaded gelatin microsphere preparation

The emulsification-linkage approach was used to make ST-GMS. A certain amount of ST was added to the gelatin solution (15%, w/v) and then mixed well. Then, the solution was dropped into 50 °C liquid paraffin containing span-80 (2%, v/v). After emulsifying for 15 min, the system was transferred immediately into a 4 °C ice water bath and stirred for 20 min. Then, a small amount of glutaraldehyde aqueous solution was added to the system to cross-link the gelatin droplets for 50 min. Isopropyl alcohol was also added to the solution to dehydrate the gelatin droplets. The resulting solution was suction filtered and washed three times and left to dry to obtain ST-GMS. The amount of ST was determined by HPLC. The encapsulation efficiency and drug loading were calculated according to the following formulas (1) and (2):



Scheme 1. ST-GMS/HA MNs production procedure and application on the skin of guinea pig allergic asthma model for the transdermal distribution of ST-GMS/HA MNs.

$$EE\% = W_{drug}/W_0 \times 100\% \quad (1)$$

$$DL\% = W_{drug}/W_m \times 100\% \quad (2)$$

where W_{drug} is the amount of ST in the microspheres, W_0 is the amount of initial feeding ST, and W_m is the amount of microspheres.

2.2.2. Fabrication of ST-GMS/ST-HA MNs

Blueacre Technology, Ltd.'s identical silicone molds were used to create all MNs. Each needle had a spherical base of 200 μm by 200 μm that tapered to a height of 600 μm . The hands were positioned in a 15 $\times$ 15 array with a tip-to-tip distance of 500 μm . To make an MN with ST-GMS, we added ST-GMS to 10% HA ($M_r = 1,000,000$) solution containing ST at a ratio of 4:3 and mixed it well to prepare a uniform and stable HA solution. Then, the uniform solution was poured into the MN mold. The system was centrifuged for 5 min at 2862 g and wholly absorbed into the microhole of the MN mold. The autolytic MNs of ST were obtained by complete drying under vacuum conditions at 25 $^{\circ}\text{C}$ for 6 h.

2.2.3. Characterization of ST-GMS/ST-HA MNs

The morphology of as-prepared ST-GMS/ST-HA MNs was characterized with a German Zeiss stereomicroscope and scanning electron microscope (SEM; Apreo C; Thermo Scientific Company, Waltham, MA, USA).

2.2.3.1. In vitro skin implantation test. The ability to implant transcutaneous medicine delivery systems into the skin is crucial. We used trypan blue staining methods to verify whether the ST-GMS/ST-HA MNs could enter the skin. (Luo et al., 2019; Lee et al., 2017). In brief, The ST-GMS/ST-HA MNs were infiltrated into the skin of the guinea pigs for 5 min, the insertion was subsequently subjected to trypan blue dye for another 5 min, and the stain was washed away with normal saline. According to the approach described above, fluorescein isothiocyanate (FITC) was loaded into ST-GMS/ST-HA MNs, which were used to test the skin insertion ability in divided guinea pig skins. The sites of MN insertion could be visually perceived after the FITC MNs were placed into the skin under microscopic imaging. Skin samples were sliced to a thickness of 50 μm after being frozen in liquid nitrogen to obtain micrographs of skin implantation. The tissue slices were then examined with a confocal laser scanning microscope (Axio Observer A1, Carl Zeiss, Germany).

2.2.3.2. Mechanical strength of ST-GMS/ST-HA MNs. ST-GMS/ST-HA MNs were tested for mechanical strength against both static and dynamic forces. The MNs were subjected to the first test of resistance, which consisted of placing various weights against them. In brief, ST-GMS/ST-HA MNs were placed with the needle tips facing down on the underside of the aluminum thin sheet; subsequently, 50, 100, 200, and 500 g weights were gently placed on top of each patch and maintained in place for 1 min (Liu et al., 2018). The MNs' mechanical resistance to dynamic forces was determined by pushing the needle against a stainless steel plate on a mechanical properties tester by using a stress-strain gauge (LD Lishi Digital Technology Equipment Co., Ltd., Shanghai, China). Between the MN tip and the stainless-steel plate, the gauge was initially fixed at 2.00 mm, with a cell loading capacity of 10.00 N. The upper stainless-steel plate traveling toward the MNs was configured to move at 0.1 mm s^{-1} speed (Liu et al., 2018).

2.2.3.3. Dissolution of ST-GMS/ST-HA MNs in the skin of guinea pigs. The dissolution of MNs by bodily fluid was investigated on the backs of guinea pigs. The receiving site for MNs was cleansed with 75% alcohol before the ST-GMS/ST-HA MNs were pressed onto the skin with the experimenter's thumb. After 10, 30, and 60 min, the MNs were removed from the skin and imaged using a digital microscope. After removal of

the MNs at 10, 30, and 60 min, a site was chosen to examine the live skin regeneration with a digital camera.

2.3. Determination of in vitro release rate

The in vitro release of ST in GMS, HA, and ST-GMS/ST-HA MNs in the release medium was determined by dialysis. Specifically, 1 mL GMS solution (32 mg GMS), 45 mg 10% HA solution (ST content 0.5 mg), 18.3 mg ST-GMS/ST-HA MN (ST content 0.5 mg), and free ST solution (ST content 0.50 mg/mL) were added to a pre-treated dialysis bag (molecular weight cut off 6,000–8,000 Da) and immersed in 500 mL of release medium with a stirring rate of 50 rpm at 37 $^{\circ}\text{C}$. The release medium was 1% SDS and 0.02% NaN_3 in PBS solution (pH 7.4). Subsequently, 2 mL of release medium was collected at 0.5, 1, 2, 4, 8, 12, 24, and 36 h, and equal quantities of fresh release medium were added to the drug release medium. The ST content of the sample was determined, and the cumulative release at each time point was computed with the formula below. The cumulative in vitro release of the microspheres was calculated with formula (3) below, and a release curve was then created.

$$E_r = \frac{V_e \sum_{i=1}^{n-1} C_i + V_0 C_n}{M_{drug}} \times 100\% \quad (3)$$

where E_r denotes the cumulative release of ST, V_e denotes the release medium's displacement volume, C_i denotes the drug concentration released during the i -th replacement sample, and V_0 denotes the release medium's volume. In the n th option, C_n indicates the release medium. The ST content in the microspheres was used to calculate the drug concentration, M_{drug} .

2.4. Cytotoxicity of blank GMS and HA

NIH 3T3 fibroblasts were seeded into 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h. Blank GMS and HA were diluted into the culture medium to different concentrations, and the original 96-well plate was placed in an incubator at 37 $^{\circ}\text{C}$ and 5% CO_2 for 48 h. Cells containing blank medium were used as a negative control. Finally, the medium in each well was discarded, and 100 μL of CCK-8 medium containing 10% was added and incubated for 2 h. The absorbance was measured at 450 nm with an enzyme marker, and cell viability was calculated according to the following formula.

$$\text{Cell viability (\%)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100\%$$

2.5. In vivo studies using ovalbumin (OVA)-induced allergic asthma guinea pigs

2.5.1. Establishment of a guinea pig allergic asthma model and drug application

All animal experiments were conducted according to the guidelines of Heilongjiang University of Chinese Medicine's Experimental Animal Administrative Committee. A total of 42 healthy male guinea pigs 350–500 g were divided into seven groups. They were maintained in an animal house and given unlimited food and water. On days 1 and 5, 36 guinea pigs were sensitized by injection of 100 mg OVA (purity 98%) plus 1 mg aluminum hydroxide as an adjuvant in 1 mL saline intraperitoneally. The OVA-sensitized guinea pigs were tested on day 15 by inhalation of a 1% OVA aerosol for 1 h. On day 29, 36 challenged guinea pigs were divided into six groups at random: an OVA sensitization model group, ST-GMS/ST-HA MN transdermal administration high-dose group, ST-GMS/ST-HA MN transdermal administration low-dose group, and tulobuterol patch transdermal administration group. Six guinea pigs in the normal control group were sensitized and challenged with aerosolized 0.9% saline. (Evans et al., 2012; Li et al., 2020).

The patching area was on the left and right sides of the guinea pigs'

backs. The ST gel patch administration in the transdermal application groups was calculated with an ST content of 3.2 mg in each piece and a 2 g mass for each piece. The preparation process of the gel is shown in the SI. ST-GMS/ST-HA MNs were loaded with different doses of ST in each piece (0.8 mg and 3.2 mg). For the positive drug tulobuterol patch group, a tulobuterol content of 0.5 mg in each piece was calculated.

2.5.2. Pharmacodynamic (PD) and pharmacokinetic (PK) analyses

In this study, bolus pumps (model is CMA 402) and probes (model is CMA 20 Elite, 20 kD molecular weight cutoff) were used for microdialysis fluid collection in experiments. The effects of temperature, perfusate concentration, and perfusate velocity on the in vitro recovery of the probe were investigated with the incremental method, and the effects of probe stability on the in vitro relative recovery (RR) and relative loss rate (RL) of ST in dialysate and the in vivo RL rate were investigated. The established microdialysis method was stable and reliable, and consequently could be utilized for local pharmacokinetic and pharmacodynamic analyses of ST-GMS/ST-HA MNs in guinea pig lungs. The specific experimental steps and results are shown in the SI.

Guinea pigs were extubated, connected to a small animal ventilator, and ventilated with a frequency of 75–80 breaths/min and a tidal volume of 3.5 mL. The skin on the right side of each guinea pig's chest was cut, the right chest wall was positioned between the 5th and 6th ribs, an oblique incision was made along the rib gap, the 5th rib was severed with a hemostat clamp, the right lung was fully exposed, a small incision was made in the dirty pleura, a microdialysis probe was implanted obliquely along the incision, the incision was sutured to fix the probe to the chest wall, and the chest cavity was closed layer by layer.

30% absolute ethanol-physiological saline was used as the perfusion fluid, at a perfusion rate of 2 $\mu\text{L}/\text{min}$. After equilibrating for 1 h, about 60 μL of blank dialysate was collected as an empty control group for PKs. After administration, a 60 μL volume of receiving solution was collected once every hour for 12 h. An internal standard ribavirin solution (100 μL , 1 $\mu\text{g}/\text{mL}$) was added to 100 μL of the microdialysis sample and spun for 30 s. After filtering with a 0.22 μm microporous membrane, 4 μL of supernatant was taken for HPLC-MS analysis.

The concentration–time profiles were used to visually assess PK characteristics such as peak plasma drug concentration (C_{max}) and time to peak plasma drug concentration (T_{max}). Other PK parameters included the area under the time–concentration curve and the area under the time–concentration curve $AUC_{0-\infty}$, AUC_{0-t} , average residence time (MRT_{0-t}), ($MRT_{0-\infty}$), and half-life of the drug ($t_{1/2}$), were obtained using the WinNonlin Examples Guide version 5.2.1 (Huang et al., 2019).

2.6. Statistical analysis

Data were analyzed using SPSS (Version 26.0) software. A two-way repeated-measures analysis of variance was used for intergroup comparison. PD data comparison between groups was analyzed using a two-factor multi-level LSD method, with $p < 0.05$ and $p < 0.01$ representing significant and highly significant differences, respectively.

3. Results and discussion

3.1. Preparation and characterization of ST-GMS/ST-HA MNs

Drug-loaded GMSs were prepared by an emulsification method as delivery vehicles for ST. The morphology of microspheres is shown in Fig. 1a. GMSs were spherical and had smooth surfaces. As shown in Fig. 1b, the size of GMS approximately followed a Gaussian distribution. The mean length of the GMS was 4 μm , the average drug loading of the microspheres was 1.54%, and the encapsulation rate was 80.36%.

3.2. Release characteristics of drug-loaded GMS, HA, and ST-GMS/ST-HA MNs

In release media, the release characteristics of drug-loaded GMS, HA, and ST-GMS/ST-HA MNs were determined. The results shown in Fig. 1c indicated that the cumulative release of free ST solution was 92.98% at 4 h; the ST in HA solution was 80.8% at 4 h; the ST in GMS was about 39.5% at 4 h, 56.8% at 8 h, and 80.4% at 24 h; and the ST in ST-GMS/ST-HA MNs was 60.31% at 4 h, 73.05% at 8 h, 83.48% at 12 h, and 88.67%

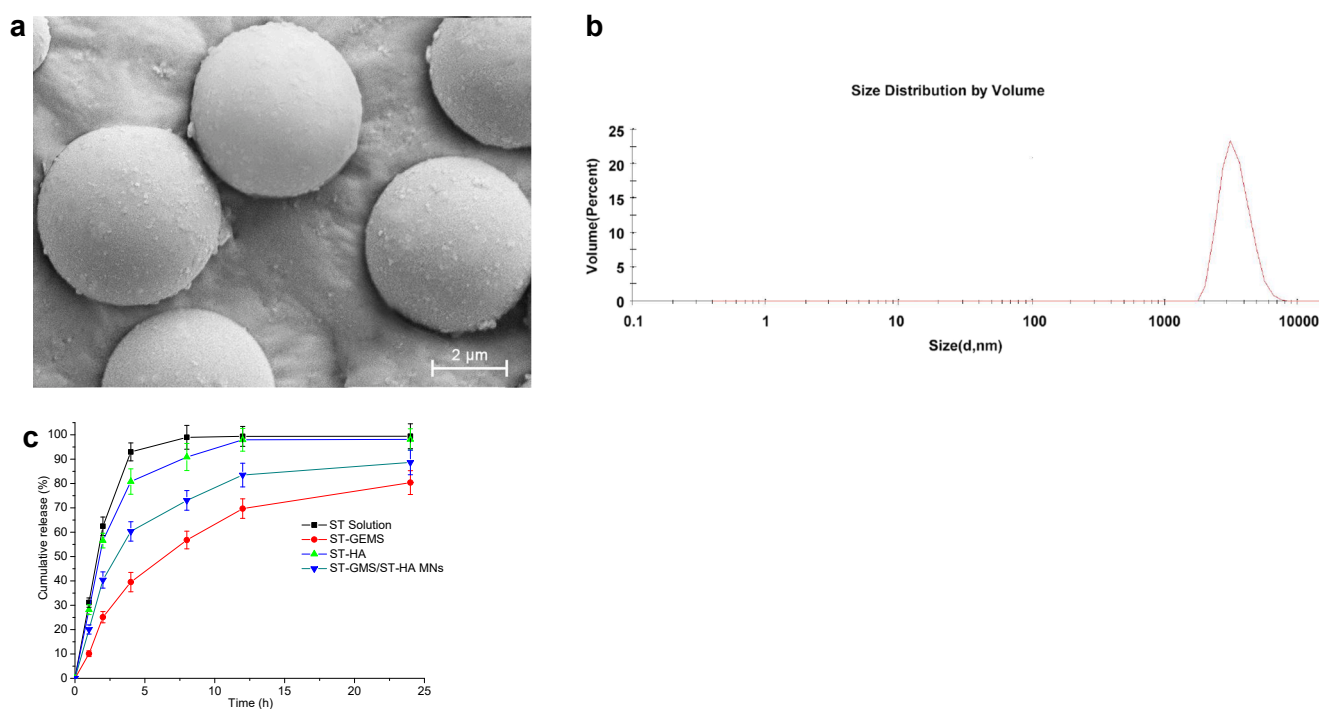


Fig. 1. SEM image of gelatin microspheres (GMS), scale bar = 2 μm . (a), Size, distribution of GMS (b), cumulative release of ST-GMS, HA, ST-GMS/ST-HA MNs and free ST solution in the release medium (means $\pm$ SD, $n = 3$) (c).

at 24 h. These results indicated the rapid-release effect of HA solution, the better slow-release effect of ST-GMS, and at the beginning, the effects of rapid release and then slow release in ST-GMS/ST-HA MNs.

3.3. Morphology of ST-GMS/ST-HA MNs

A template approach was used to create biodegradable composite MNs in this study. Fig. 2a shows the optical photographs of ST-GMS/ST-HA MNs, indicating that the PDMS mold successfully replicated ST-GMS/ST-HA MNs. Under SEM, the completed MN arrays were examined. As shown in Fig. 2b and Fig. 2c, square cone-shaped needles with sharp ends and translucent edges were fashioned from the biodegradable composite. Each patch contained a 15×15 array of MNs. The ST-GMS MNs were approximately 600 and 260 μm tall and wide at the base, respectively (Fig. 2d and f). The distance between the tips of the two microneedles was 502.6 μm (Fig. 2e).

3.4. Mechanical properties of ST-GMS/ST-HA MNs

MNs are intended to be used as a channel for transdermal medication administration. Therefore, they must be made of biocompatible materials and must not penetrate the skin (Luo et al., 2019; Gholami et al., 2019). The initial resistance test consisted of applying different weights against the MN array to analyze the mechanical behaviors of ST-GMS/ST-HA MNs. As shown in Fig. 3A, when the 50 or 100 g weight was placed on the MN array, the morphology of the MNs was scarcely altered from their initial form (Fig. 3A-b, c). When the weight was increased to 150 g, the tip of MNs displayed little bending (Fig. 3A-d). When the weight was increased to 250 g, the majority of MNs remained upright (Fig. 3A-e). All MN tips displayed varying degrees of bending after the 500 g weight was placed on the MN array for 10 min (Fig. 3A-f). However, no broken MNs were found, thus indicating that the developed biodegradable composite MNs had high mechanical strength and toughness.

The patches were pressed against a stainless-steel plate, and a stress-strain gauge was used to assess the mechanical strength of the resulting ST-GMS/ST-HA MNs (Fig. 3B). The force was practically linear to a level of 0.5 N/needle, with displacement of approximately 1.0 mm,

as shown in Fig. 3B, thus demonstrating no severe buckling throughout the test. However, even under high stress, the MN did not break but crumbled, thereby demonstrating high mechanical strength and toughness. The toughening of polymers by GMS explained this outcome. The microspheres, as insoluble stiff particles, may considerably increase the hybrid system's toughness and strength (Heng et al., 2015; Zhang et al., 2017). This is a significant feature for practical applications because it ensures that no needles remain in the patient's dermis, even under abuse scenarios (Lhernould et al., 2015). According to the previous studies (Yu et al., 2017), an MN with a tip diameter of 20 μm is inserted into the skin, and a maximum force of ~ 0.15 N is required. As a result, the mechanical strength of the ST-GMS/ST-HA MNs generated in this work was sufficient to pierce the skin.

3.5. Dissolution of ST-GMS/ST-HA MNs

The biodegradable composite MNs of different materials can exhibit different dissolution rates, and the loaded drug also has different release rates (Yu et al., 2017; Lee et al., 2020; Dabholkar et al., 2021; Adithya et al., 2020). ST-GMS/ST-HA MNs were affixed to the backs of guinea pigs to replicate the swelling and dissolution of MNs in skin fluid, as depicted in Fig. 4A, to investigate the dissolution capabilities of MNs in live skin. In live skin, morphological alterations in ST-GMS/ST-HA MNs were discovered. The sharp MNs in the first stage are shown in Fig. 4A-a. However, the tips of MNs became blunt after penetrating the skin (Fig. 4A-b, c), owing to HA's ability to substantially dissolve in watery environments; after 60 min, the MNs' morphology had nearly vanished. These findings indicated that the MNs entered the skin and came into contact with the skin's interstitial fluid, but the morphology of the ST-GMS/ST-HA MNs remained essentially unchanged.

The animal model's skin spot recovery rate was used to assess the insertion's skin compatibility after treatment with ST-GMS/ST-HA MNs. The skin's apparent puncture marks could be seen after removing the MNs (Fig. 4B-a), and the effects progressively diminished over time, as illustrated in Fig. 4B. These microholes became weak after removal of the patches for 10 min (Fig. 4B-b), and the puncture marks were scarcely evident after 30 min (Fig. 4B-c). After 60 min, the patches on the skin had entirely healed (Fig. 4B-d). The findings demonstrated that MN

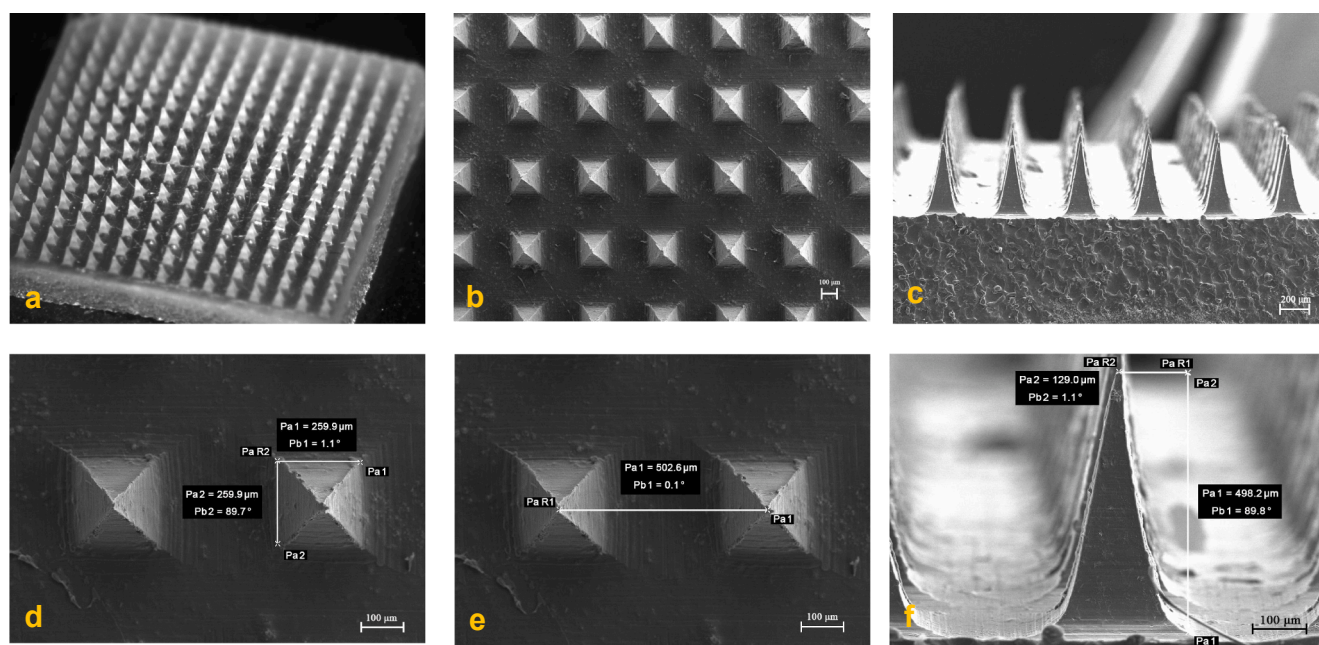


Fig. 2. Optical photographs of ST-GMS/ST-HA MNs (a), SEM images of ST-GMS/ST-HA MNs from the front. Scale bar = 100 μm (b), SEM images of ST-GMS/ST-HA MNs from the side. Scale bar = 100 μm (c), The microneedle base is square with a side length of 259.9 μm (d); The distance between the tips of the two microneedles was 502.6 μm (e), the height of the microneedle was 498.2 μm (f).

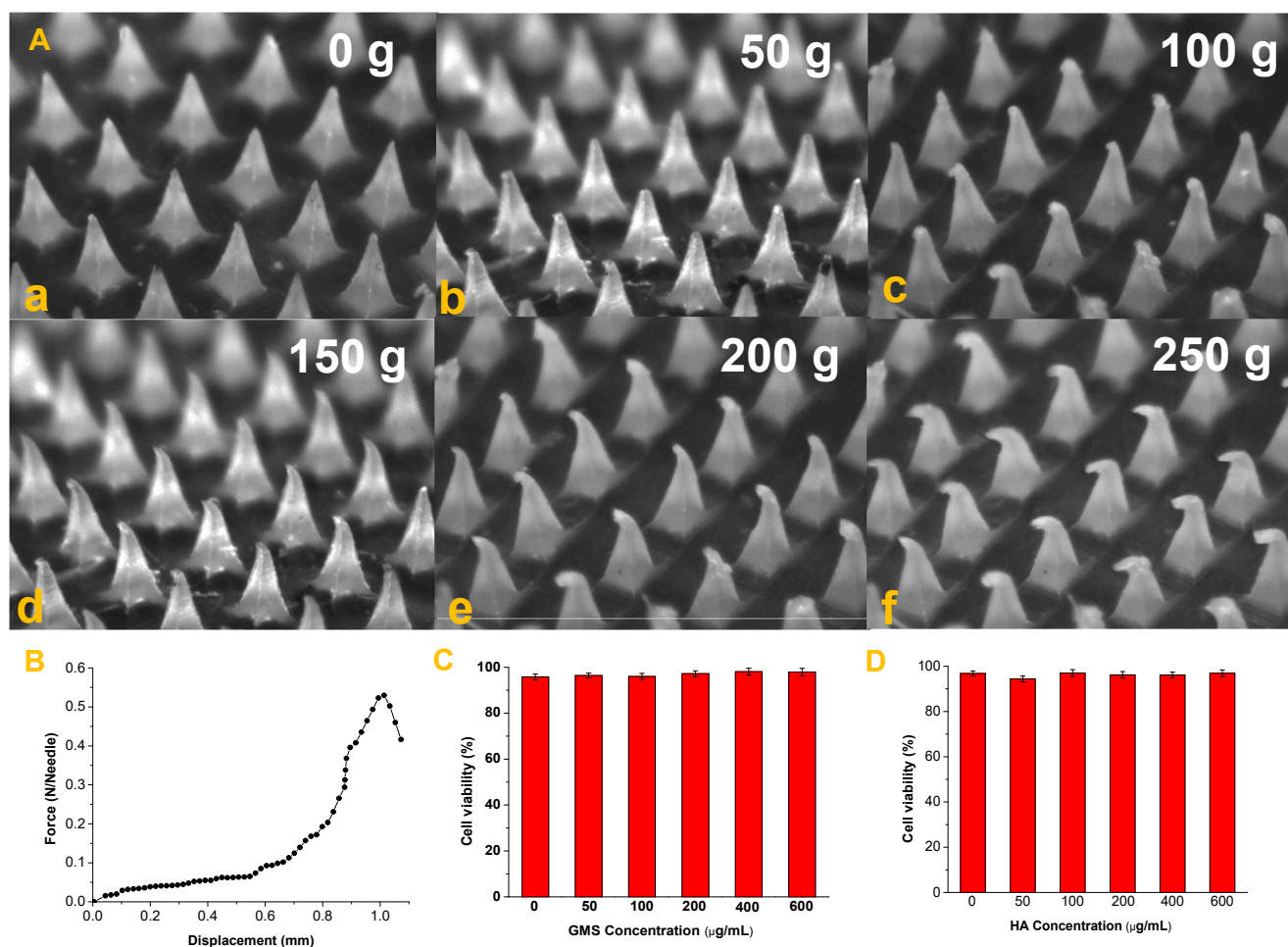


Fig. 3. MN shape with varied weight resistance (A); compression test of MNs (B); GMS and HA against NIH3T3 cells (C, D).

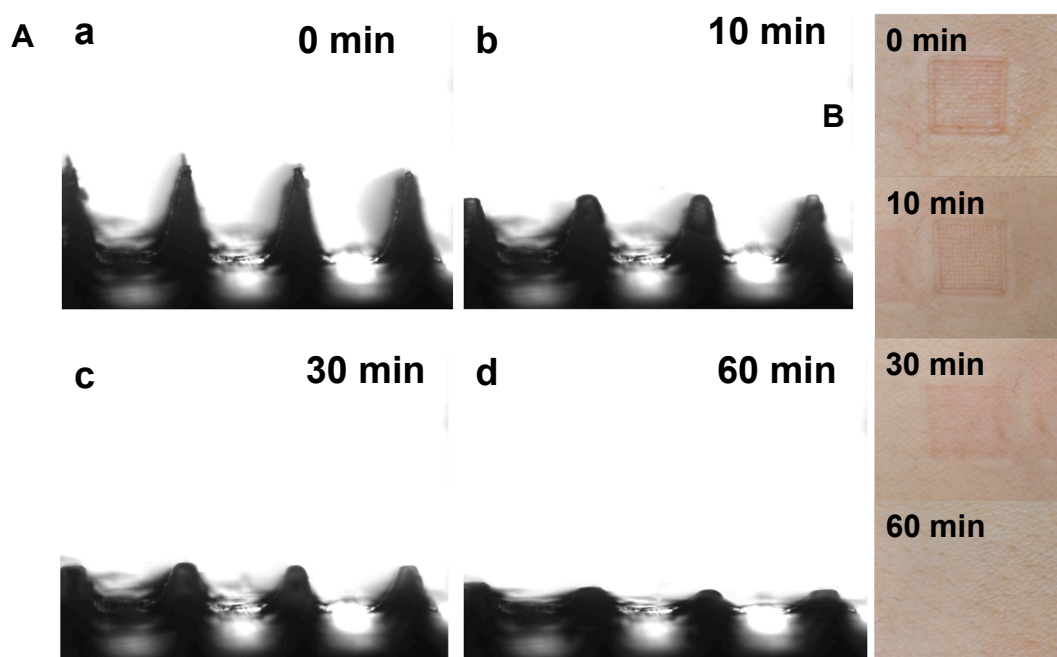


Fig. 4. Dissolution of ST-GMS/ST-HA MNs on the skin of guinea pigs' backs before and after insertion for 0, 10, 30, and 60 min. (A: a-0 min, b-10 min, c-30 min, and d-30 min; B: a-0 min, b-10 min, c-30 min, and d-30 min); After the MNs have been detached, a spot appears on the human skin (B).

therapy resulted in faster recovery of epidermal barrier function. Furthermore, no substantial inflammation was found in the area after MN administration, thus demonstrating the benefit of repeated transdermal medication delivery. With concentrations ranging from 100 to 600 $\mu\text{g}\cdot\text{mL}^{-1}$, NIH3T3 cells maintained viability of $>90\%$. As shown in Fig. 3C and D. These findings revealed that the GMS and HA composite had minimal toxicity and excellent biocompatibility.

3.6. Skin penetration in vivo

Using the whole thickness of guinea pig skin, we examined the ability of the generated biodegradable composite MNs to penetrate the skin and assessed drug diffusion. The MNs were first loaded with FITC (Fig. S1 A). The biodegradable composite MN was then physically placed into the skin, which was then frozen for two reasons: to lyse the cells and to simplify slicing. A sharp mark was imprinted on the skin, according to a histological cross-section (Fig. S1 B). After the MNs were introduced into the skin, the stratum corneum was ruptured. The MNs' tips penetrated past the granular layer and into the dermis layer, and finally were embedded in the skin. To further evaluate the diffusion property of the drugs from the MNs, we used fluorescence microscopy. After a 10-minute incubation period in the skin, the green fluorescence around the MN became visible, indicating the rapid permeation from the MNs in vivo (Figure S1 C). To visualize the penetration of ST-GMS/ST-HA MNs into the skin of guinea pigs at different depths, as shown in Fig. 5, we further examined the skin tissues with CLSM. Images were taken from the stratum corneum in the x-y plane with a 50 μm step size on the z-axis until the visual field was dark. We used trypan blue to stain the skin that had been treated (Fig. S1 D). MNs penetrated the epidermis of the guinea pigs with 100% efficacy, according to our observations.

3.7. In vivo treatment of allergic asthma

To investigate the effect of anti-allergic asthma drugs in vivo, we applied the ST-GMS/ST-HA MNs to a guinea pig model of asthma. Traditional research on lung drug PK is generally performed by surgical removal of the whole lung and homogenization or bronchoalveolar lavage to obtain the epithelial cell lining fluid (Bruning et al., 2011). The common disadvantage of this method is that it cannot perform continuous sampling and cannot distinguish the difference between intracellular fluid and extracellular fluid drug concentration. The data obtained may not accurately reveal the drug PK. The tissue homogenization method requires many samples, and differences between individuals also lead to experiments. The error is relatively large. Microdialysis technology can continuously monitor the concentration of free drugs in the interstitial fluid around the lung and has sound spatial localization and time resolution (Xie, 2013). The chromatograms of the blank saline solution, control solution, and mass spectrum microdialysis solution of samples are shown in Fig. S2(a)–2(d). No impurity peak was observed at the peak site, according to the MS analysis of ST and the internal standard ribavirin, and the peak shapes of ST and ribavirin were good. Fig. 6 shows the IL-10, TNF- α , HIS levels, and ST levels in the lung microdialysis fluid of guinea pigs with asthma in the groups after the application.

The effects of ST-GMS/ST-HA MNs on the levels of TNF- α , HIS, and IL-10 in the lung microdialysis fluid of guinea pigs is shown in Fig. 6 a–6c. The model group showed a very significant difference ($p < 0.01$) with respect to the blank group (as shown in the figure). At 1 h after administration, the levels of TNF- α and HIS in the high dose MNs transdermal administration group decreased rapidly, and the level of IL-10 increased significantly compared with the model group ($p < 0.01$),

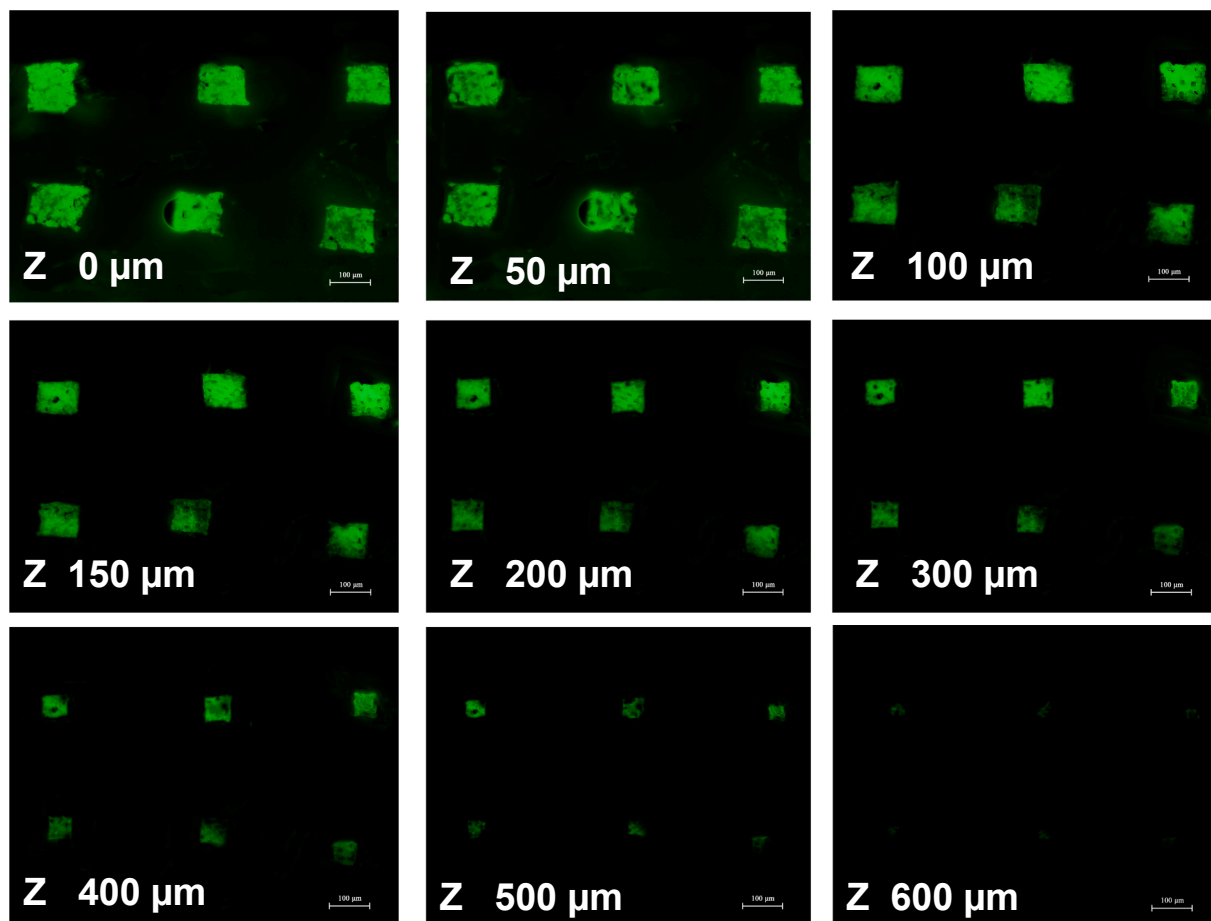


Fig. 5. Skin penetration experiments on ex vivo guinea pig skin using confocal laser scanning microscopy (CLSM).

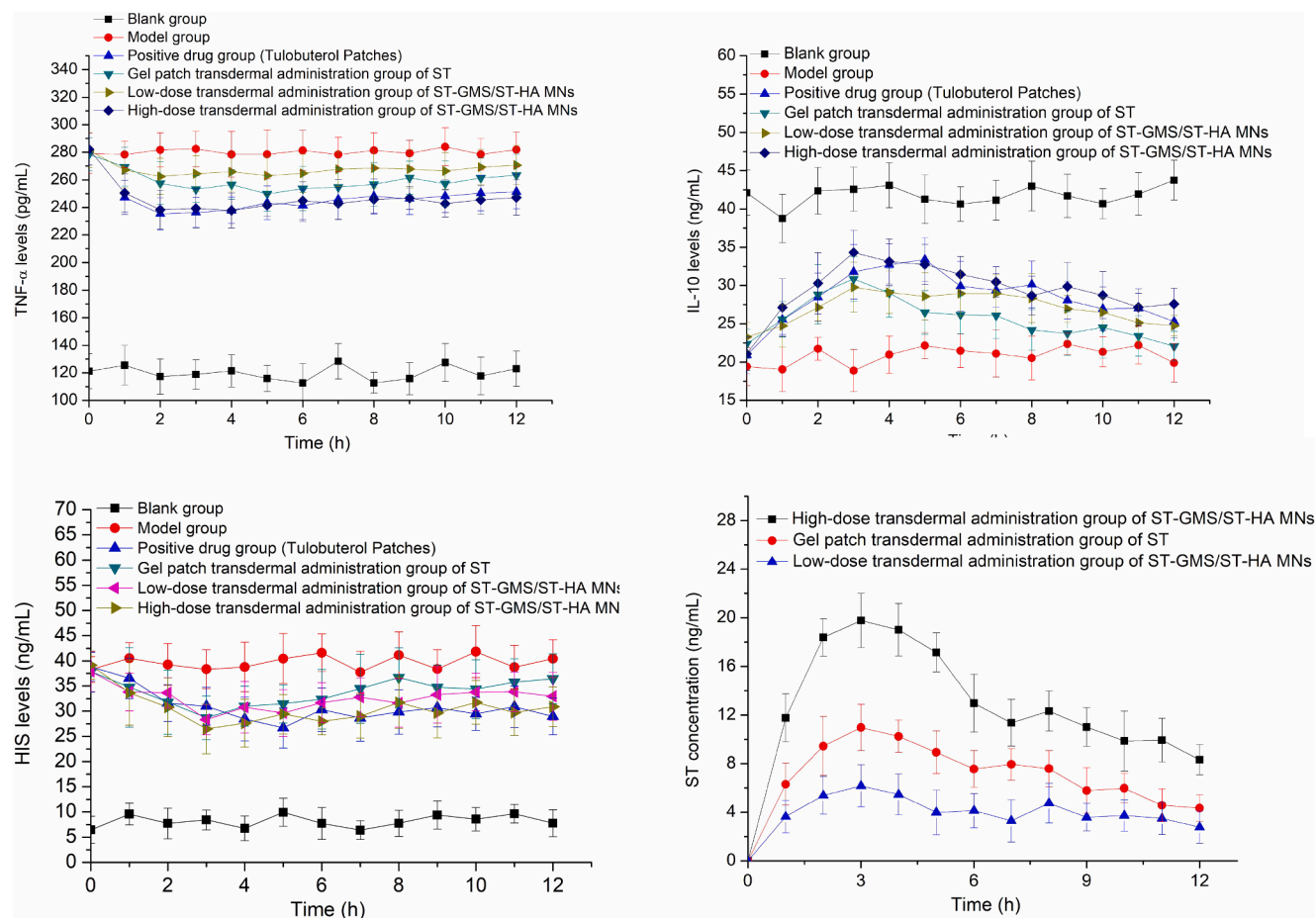


Fig. 6. The effect of ST-GMS/ST-HA MNs on the levels of TNF- α , HIS, and IL-10 in the lung microdialysis fluid of guinea pigs (a–c). The lung microdialysis fluid ST concentration–time curves after the application of ST-GMS/ST-HA MNs on the skin of allergic asthma guinea pigs (d). Data are means $\pm$ s (n = 6, for each group).

indicating that ST was absorbed through the skin of guinea pigs and distributed to the lungs, reducing the level of TNF- α . As the concentration of ST increased in MNs, TNF- α and HIS levels rebounded, and IL-10 levels decreased slowly, indicating that ST in the high-dose MNs transdermal administration group was absorbed smoothly and eliminated in the lung tissue of guinea pigs achieving the effect of continuous administration. There were significant differences in each index ($p < 0.05$) compared with the gel patch transdermal administration group, indicating that the high dose MNs transdermal administration group had a better therapeutic effect. After administration, the levels of TNF- α and HIS in the low dose MNs transdermal administration group also decreased slightly, the IL-10 level increased slightly, which was significantly different from the model group ($p < 0.05$).

The lung microdialysis fluid ST concentration–time curves after the application of ST-GMS/ST-HA MNs on the skin of allergic asthma guinea pigs are shown in Fig. 6 d. The PK results of each administration group of

ST-GMS/ST-HA MNs are shown in Table 1. According to the PK results, ST in the high dose MNs transdermal administration group reached its peak at 3 ± 0.236 h after administration, the highest drug concentration was 19.77 ± 1.32 ng·mL $^{-1}$, and then gradually decreased, the area under the drug-time curve $AUC_{(0-t)}$ was 155.272 ± 12.112 h·ng·mL $^{-1}$. ST in the gel patch transdermal administration group reached a peak at 3 ± 0.302 h after administration. The highest drug concentration was 10.97 ± 1.89 ng·mL $^{-1}$ and then decreased. The area under the drug-time curve $AUC_{(0-t)}$ was 86.605 ± 8.125 h·ng·mL $^{-1}$. ST in the low dose MNs transdermal administration group reached its peak at 3 ± 0.219 h after administration. The area under the drug-time curve $AUC_{(0-t)}$ was 48.088 ± 4.255 h·ng·mL $^{-1}$. The $t_{1/2}$ of the ST-GMS/ST-HA MNs high dose transdermal administration group was 1.8 times that of the ST gel patch transdermal administration group ($p < 0.05$), which was the same as that of the ST low dose transdermal administration group (3.2 times, $p < 0.05$). The $AUC_{(0-t)}$ indicated that the ST-GMS/ST-HA MNs high dose

Table 1

Comparison of pharmacokinetic parameters between ST-GMS/ST-HA MNs and ST gel patch (means $\pm$ s, n = 6).

parameter	High-dose transdermal administration group	Gel patch transdermal administration group	Low-dose transdermal administration group
C_{max} (ug/L)	19.77 ± 1.32	10.97 ± 1.89	6.17 ± 1.73
T_{max} (h)	3 ± 0.236	3 ± 0.302	3 ± 0.219
$t_{1/2}$ (h)	7.141 ± 0.228	4.959 ± 0.034	5.14 ± 1.117
$AUC_{(0-t)}$ (ug·L $^{-1}$ ·h)	155.272 ± 12.112	86.605 ± 8.125	48.088 ± 4.255
$AUC_{(0-\infty)}$ (ug·L $^{-1}$ ·h)	240.819 ± 18.092	118.091 ± 11.477	68.825 ± 7.215
$MRT_{(0-t)}$ (h)	5.668 ± 0.057	5.689 ± 0.055	5.908 ± 0.039
$MRT_{(0-\infty)}$ (h)	11.595 ± 0.294	9.21 ± 0.008	9.924 ± 1.494
$VRT_{(0-t)}$ (h ^{2})	9.643 ± 0.166	9.299 ± 0.396	10.123 ± 0.04
$VRT_{(0-\infty)}$ (h ^{2})	107.405 ± 6.212	55.705 ± 1.233	61.94 ± 6.358

transdermal administration group was more significant than the ST gel patch administration group ($p < 0.05$). No significant difference in $MRT_{(0-t)}$ was observed between the groups. The reason may be that HPLC-MS was a sensitive detection method. The drug can be detected when the concentration is lower than the therapeutic concentration, but there is no therapeutic effect.

PK results indicated that ST in ST-GMS/ST-HA MNs slowly entered the lung tissue of guinea pigs, and was readily absorbed and eliminated gradually. The effective concentration was maintained for a long duration, and the treatment effect in allergic asthma was improved.

4. Conclusions

In conclusion, the ST-GMS/ST-HA MNs consisting of HA and GMS were fabricated successfully, exhibiting mechanical strength and excellent swelling properties. In vivo, allergic asthma guinea pigs were used as models for the transdermal distribution of ST utilizing the as-prepared MNs. The pharmacological action of encapsulated ST in MNs was maintained, revealing a sustained suppression of asthma in allergic asthma guinea pigs, compared with the same dose in transdermal delivery with an ST gel patch. Our results indicated that the as-prepared composite MNs may be used to treat allergic asthma through transdermal administration.

CRedit authorship contribution statement

Yufei Feng: Writing-original draft. **Shuyuan Chang:** Writing – review & editing. **Zhongxu Jing:** Data curation. **Haibo Jiang:** Formal analysis. **Yuwei Liu:** Methodology. **Guozhao Qin:** Conceptualization, Software.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpharm.2022.121899>.

References

- Adithya, S.P., Sidharthan, D.S., Abhinandan, R., Balagandharan, K., Selvamurugan, N., 2020. Nanosheets-incorporated bio-composites containing natural and synthetic polymers/ceramics for bone tissue engineering. *Int. J. Biol. Macromol.* 164, 1960–1972. <https://doi.org/10.1016/j.ijbiomac.2020.08.053>.
- Alqahtani, S.A., Alsultan, A.S., Alqattan, H.M., Eldemerdash, A., Albacker, T.B., 2018. Population Pharmacokinetic Model for Vancomycin Used in Open Heart Surgery: Model-Based Evaluation of Standard Dosing Regimens. *Antimicrob. Agents Chemother.* 62, e00088–e118. <https://doi.org/10.1128/aac.00088-18>.
- Becker, L.C., Bergfeld, W.F., Belsito, D.V., Klaassen, C.D., Marks, J.G., Shank, R.C., Slaga, T.J., Snyder, P.W., 2009. Final report of the safety assessment of hyaluronic acid, potassium hyaluronate, and sodium hyaluronate. *Int. J. Toxicol.* 28, 5–67. <https://doi.org/10.1177/1091581809337738>.
- Bruning, J.B., Murillo, A.C., Chacon, O., Barletta, R.G., Sacchetti, J.C., 2011. Structure of the Mycobacterium tuberculosis D-alanine: D-alanine ligase, a target of the

- antituberculosis drug D-cycloserine. *Antimicrob. Agents Chemother.* 55, 291–301. <https://doi.org/10.1128/AAC.00558-10>.
- Dabholkar, N., Gorantla, S., Waghule, T., Rapalli, V.K., Kothuru, A., Goel, S., Singhvi, G., 2021. Biodegradable microneedles fabricated with carbohydrates and proteins: Revolutionary approach for transdermal drug delivery. *Int. J. Biol. Macromol.* 170, 602–621. <https://doi.org/10.1016/j.ijbiomac.2020.12.177>.
- Evans, R.L., Nials, A.T., Knowles, R.G., Kidd, E.J., Ford, W.R., Broadley, K.J., 2012. A comparison of antiasthma drugs between acute and chronic ovalbumin-challenged guinea-pig models of asthma. *Pulm. Pharmacol. Ther.* 25 (6), 453–464. <https://doi.org/10.1016/j.pupt.2012.08.004>.
- Gao, T., Bai, M., Xu, E.P., Zuo, T., Miao, M.S., 2020. Current situation and analysis of acupuncture medication of Chinese medicine. *China J. Tradit. Chin. Med. Pharm.* 35, 5691–5694.
- Gholami, S., Mohebi, M.M., Hajizadeh-Saffar, E., Ghanian, M.H., Zarkesh, I., Baharvand, H., 2019. Fabrication of microporous inorganic microneedles by centrifugal casting method for transdermal extraction and delivery. *Int. J. Pharm.* 558, 299–310. <https://doi.org/10.1016/j.ijpharm.2018.12.089>.
- Hao, Y., Chen, Y.W., He, X.L., Yang, F., Han, R.X., Yang, C.L., Li, W., Qian, Z.Y., 2020. Near-infrared responsive 5-fluorouracil and indocyanine green loaded MPEG-PLGA nanoparticle integrated with dissolvable microneedle for skin cancer therapy. *Bioact. Mater.* 5, 542–552. <https://doi.org/10.1016/j.bioactmat.2020.04.002>.
- Heng, Z.G., Chen, Y., Zou, H.W., Liang, M., 2015. Simultaneously enhanced tensile strength and fracture toughness of epoxy resins by poly(ethylene oxide)-block-carboxyl terminated butadiene-acrylonitrile rubber diol copolymer. *RSC Adv.* 5, 42362–42368. <https://doi.org/10.1039/C5RA05124J>.
- Huang, C., Wang, Z., Liu, L., Liu, X., Dong, J.I., Xu, Q., Zhang, B., Miao, L., 2019. Predicting the dopamine D₂ receptor occupancy of ropinirole in rats using positron emission tomography and pharmacokinetic-pharmacodynamic modeling. *Xenobiotica* 49 (2), 143–151. <https://doi.org/10.1080/00498254.2018.1428383>.
- James, B., Milstien, S., Spiegel, S., 2019. Ormdl3 and allergic asthma: from physiology to pathology. *J. Allergy Clin. Immunol.* 144 (3), 634–640.
- Lee, H.S., Ryu, H.R., Roh, J.Y., Park, J.-H., 2017. Bleomycin-Coated microneedles for treatment of warts. *Pharm. Res.* 34 (1), 101–112. <https://doi.org/10.1007/s11095-016-2042-x>.
- Lee, K., Goudie, M.J., Tebon, P., Sun, W.J., Luo, Z.M., Lee, J., Zhang, S.M., Fetah, K., Kim, H.J., Xue, Y.M., Darabi, M.A., Ahadian, S., Sarikhani, E., Ryu, W., Gu, Z., Weiss, P.S., Dokmeci, M.R., Ashammakhi, N., Khademhosseini, A., 2020. Non-transdermal microneedles for advanced drug delivery. *Adv. Drug Deliv. Rev.* 165–166, 41–59. <https://doi.org/10.1016/j.addr.2019.11.010>.
- Lhernould, M.S., Deleers, M., Delchambre, A., 2015. Hollow polymer microneedles array resistance and insertion tests. *Int. J. Pharm.* 480 (1–2), 152–157. <https://doi.org/10.1016/j.ijpharm.2015.01.019>.
- Lin, Y.F., Shen, W.D., 2015. An analysis of the origin and modern application of sanfu tie theory. *Jiangsu J. Tradit. Chin. Med.* 47, 10–12.
- Liu, D.P., Yu, B., Jiang, G.H., Yu, W.J., Zhang, Y., Xu, B., 2018. Fabrication of composite microneedles integrated with insulin-loaded CaCO₃ microparticles and PVP for transdermal delivery in diabetic rats. *Mater. Sci. Eng. C* 90, 180–188. <https://doi.org/10.1016/j.msec.2018.04.055>.
- Liu, F., Yu, J.E., Bai, L., Xue, Z., Zhang, X.G., 2015. Pingchuan formula improves asthma via restoration of the Th₁₇/Treg balance in a mouse model. *BMC Complement. Altern. Med.* 15, 234. <https://doi.org/10.1186/s12906-015-0755-8>.
- Liu, S., Quan, Y.S., Shenshan, W.N., Fang, L., Shanben, C., 2010. Preparation and characterization of novel hyaluronic acid microneedles for insulin transdermal delivery. *J. Shenyang Pharm. Univ.* 27 (6–10), 75.
- Li, X., Wang, Y.L., Guo, X.P., 2021. Advances in research on hyaluronic acid applied in topical delivery system. *Chin. J. Aesthetic Med.* 30, 177–180.
- Li, Y.X., Zhong, M., Wang, Y., Ma, W.H., 2020. Establishment of common animal model of asthma. *Chin. J. Comp. Med.* 30, 97–101.
- Lopez-Ramirez, M.A., Kupor, D., Marchiori, L., Soto, F., Rueda, R., Reynoso, M., Narra, L.R., Chakravarthy, K., Wang, J., 2021. Combinatorial microneedle patch with tunable release kinetics and dual fast-deep/sustained release capabilities. *J. Mater. Chem. B* 9 (9), 2189–2199. <https://doi.org/10.1039/D1TB00141H>.
- Luo, Z.M., Sun, W.J., Fang, J., Lee, K., Li, S., Gu, Z., Dokmeci, M.R., 2019. Khademhosseini, A. Biodegradable gelatin methacryloyl microneedles for transdermal drug delivery. *Adv. Healthcare Mater.* 8 <https://doi.org/10.1002/adhm.201801054>.
- Panda, A., Matadh, V.A., Suresh, S., Shivakumar, H.N., Murthy, S.N., 2021. Non-dermal applications of microneedle drug delivery systems. *Drug Delivery Transl. Res.* <https://doi.org/10.1007/s13346-021-00922-9>.
- Pukfukdee, P., Banlunara, W., Rutwaree, T., Limcharoen, B., Sawutdechakul, P., Pattarakankul, T., Sansureerungsikul, T., Toprangkobin, P., Leelahavanichkul, A., Panchaprateep, R., Asawanonda, P., Palaga, T., Wanichwecharungruang, S., 2020. Solid composite material for delivering viable cells into skin tissues via detachable dissolvable microneedles. *ACS Appl. Bio Mater.* 3 (7), 4581–4589. <https://doi.org/10.1021/acsabm.0c00498>.
- Shoji, K., Bradley, J.S., Reed, M.D., Anker, J.N.V.D., Domonoske, C., Capparelli, E.V., 2016. Population Pharmacokinetic Assessment and Pharmacodynamic Implications of Pediatric Cefepime Dosing for Susceptible-Dose-Dependent Organisms. *Antimicrob. Agents Ch.* 60, 2150–2156. <https://doi.org/10.1128/aac.02592-15>.
- Sun, Y., Du, L., Liu, Y., Li, X., Li, M., Jin, Y., Qian, X., 2014. Transdermal delivery of the in situ hydrogels of curcumin and its inclusion complexes of hydroxypropyl-β-cyclodextrin for melanoma treatment. *Int. J. Pharm.* 469 (1), 31–39. <https://doi.org/10.1016/j.ijpharm.2014.04.039>.
- Suriyaamporn, P., Rangsimawong, W., Ngawhirunpat, T., 2021. Fabrication of dissolvable hyaluronic acid microneedle patches for intraocular fluorescein sodium delivery.

- Tezel, A., Fredrickson, G.H., 2008. The science of hyaluronic acid dermal fillers. *J. Cosmet. Laser Ther.* 10 (1), 35–42. <https://doi.org/10.1080/14764170701774901>.
- Wang, H., Yuan, Y.X., Qiu, L., Feng, B.M., Wang, H.G., Jiang, G., Shi, L.Y., Tang, L., Wang, Y.Q., Liu, D., Cao, X.M., 2011. Effect of sinapine on relieving asthma and its mechanism. *Chin. Tradit. Herb. Drugs.* 42, 134–136.
- Wu, L., Shrestha, P., Iapichino, M., Cai, Y., Kim, B., Stoeber, B., 2021. Characterization method for calculating diffusion coefficient of drug from polylactic acid (PLA) microneedles into the skin. *J. Drug Delivery Sci. Technol.* 61, 102192. <https://doi.org/10.1016/j.jddst.2020.102192>.
- Wu, X.-Q., Peng, J., Li, G.-Q., Su, H.-P., Liu, G.-X., Liu, B.-Y., 2016. Association between skin reactions and efficacy of summer acupoint application treatment on chronic pulmonary disease: a prospective study. *Chin. J. Integr. Med.* 22 (4), 284–292.
- Wu, Y.Q., Tian, S., Bai, M., Miao, M.S., 2020. New Thinking on Drug Transdermal Absorption Based on Skin Functional Characteristics. *World Chinese Med.* 15 (361–363), 368. <https://doi.org/10.3969/j.issn.1673-7202.2020.03.012>.
- Xie, A., 2013. Pharmacokinetic of Cycloserine in Healthy Rat Lungs and Brains by Microdialysis. Soochow University.
- Xu, J., Danehy, R., Cai, H., Ao, Z., Pu, M., Nusawardhana, A., Rowe-Magnus, D., Guo, F., 2019. Microneedle patch-mediated treatment of bacterial biofilms. *ACS Appl. Mater. Interfaces* 11 (16), 14640–14646. <https://doi.org/10.1021/acsami.9b02578>.
- Yenkoidiok-Douti, L., Barillas-Mury, C., Jewell, C.M., 2021. Design of dissolvable microneedles for delivery of a pfs47-based malaria transmission-blocking vaccine. *ACS Biomater. Sci. Eng.* 7 (5), 1854–1862. <https://doi.org/10.1021/acsbomaterials.0c01363>.
- Yu, W.J., Jiang, G.H., Liu, D.P., Li, L., Chen, H., Liu, Y.K., Huang, Q., Tong, Z.Z., Yao, J.M., Kong, X.D., 2017a. Fabrication of biodegradable composite microneedles based on calcium sulfate and gelatin for transdermal delivery of insulin. *Mater. Sci. Eng. C* 71, 725–734. <https://doi.org/10.1021/10.1016/j.msec.2016.10.063>.
- Yu, W.J., Jiang, G.H., Liu, D.P., Li, L., Tong, Z.Z., Yao, J.M., Kong, X.D., 2017b. Transdermal delivery of insulin with bioceramic composite microneedles fabricated by gelatin and hydroxyapatite. *Mater. Sci. Eng. C* 73, 425–428. <https://doi.org/10.1016/j.msec.2016.12.111>.
- Zhang, J., Ma, F.S., Zhan, H.H., Huang, Y.C., 2017. Matrix materials and their composites for dissolvable microneedle construction: a review. *Mater. Rep.* 31 (129–134), 161. <https://doi.org/10.11896/j.issn.1005-023X.2017.019.018>.