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论文题目：控制胰岛素按需释放的温敏微针用于糖尿病精准治疗

Thermosensitive microneedles capable of on demand insulin release for precise diabetes treatment

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Contents

Abstract

As a novel painless and minimally invasive transdermal drug delivery method, microneedles have solved the challenges of microbial infection and tissue necrosis associated with multiple subcutaneous injections for drug delivery in diabetic patients. However, common soluble microneedles are difficult to switch drug release on and off at any time according to the patient's needs during long-term use, which is one of the most critical elements of diabetes treatment. Herein, we have designed an insoluble insulin thermosensitive microneedle (ITMN) that can control the release of insulin at any time by adjusting the temperature, enabling precise treatment of diabetes. The thermosensitive microneedles were produced by in situ photopolymerisation of the temperature-sensitive compound N-isopropylacrylamide (NIPAm) with the hydrophilic monomer N-vinylpyrrolidone (NVP), which was encapsulated with insulin inside and bound to a mini heating membrane outside. *In vitro* and *in vivo* experiments have demonstrated that the ITMN we prepared has good mechanical strength and temperature sensitivity, can release significantly different insulin doses at different temperatures, and effectively regulates blood glucose in type I diabetic mice. Therefore, ITMN provides a possibility for intelligent and convenient on-demand drug delivery for diabetic patients, and when combined with blood glucose testing devices, it has the potential to form an integrated and precise closed-loop treatment for diabetes, which is of great importance in diabetes management.

Keywords: Thermosensitive microneedles; Drug delivery; Temperature-controlled release; Diabetes treatment

摘 要

微针作为一种新型的无痛、微创经皮给药方式，已经解决了糖尿病患者多次皮下注射所导致的一些问题，如微生物感染或组织坏死。然而，普通的可溶性微针在长期使用过程中，很难根据患者的需要“开关式”释放药物，而这却是糖尿病治疗成功与否的关键。因此，为了突破上述瓶颈，我们设计了一种不溶性胰岛素温敏微针（ITMN），它可以通过调节温度随时控制胰岛素的释放，从而使糖尿病得以精准治疗。温敏微针是通过温敏化合物N-异丙基丙烯酰胺（NIPAm）与亲水单体N-乙烯基吡咯烷酮（NVP）原位光聚合产生，其内部包裹着胰岛素，外部结合到微型的加热膜上。体内外实验表明，我们制备的ITMN微针具有良好的机械强度和温敏特性，可在不同温度下释放不同剂量的胰岛素，并且能够有效地调节 I 型糖尿病小鼠的血糖。总的来说，ITMN为实现糖尿病患者智能、按需给药提供了可能。当与血糖仪结合时，有潜力形成一种综合、精准的糖尿病闭环治疗方式，在糖尿病治疗管理中具有重要意义。

关键词：温敏微针；药物递送；温控释放；糖尿病治疗

Body Text

1. Introduction

Compared to drug release in response to endogenous stimuli, external stimuli are not necessary to take into account differences in disease processes between patients, thus enabling easier and accurate spatiotemporal control of drug release [1-3]. Among external stimuli, temperature is one of the most frequently investigated stimulus signals due to the merits of simple control and convenient application to biological and other fields [4-6]. Temperature-sensitive polymers such as poly-N-isopropylacrylamide (PNIPAm), methylcellulose, hydroxypropylcellulose (HPC), and polyvinyl alcohol-vinyl acetate copolymer (PVA) are well-studied drug delivery materials, which perform conformational transformations according to temperature changes, as changing from the swollen form to the contracted form, thus expelling the drug encapsulated within them. Meanwhile, this conformational transformation process is reversible, which subsequently causes a halt in drug release [7-10]. Therefore, thermosensitive polymers are capable of "on-off" drug release through temperature changes for on-demand drug delivery.

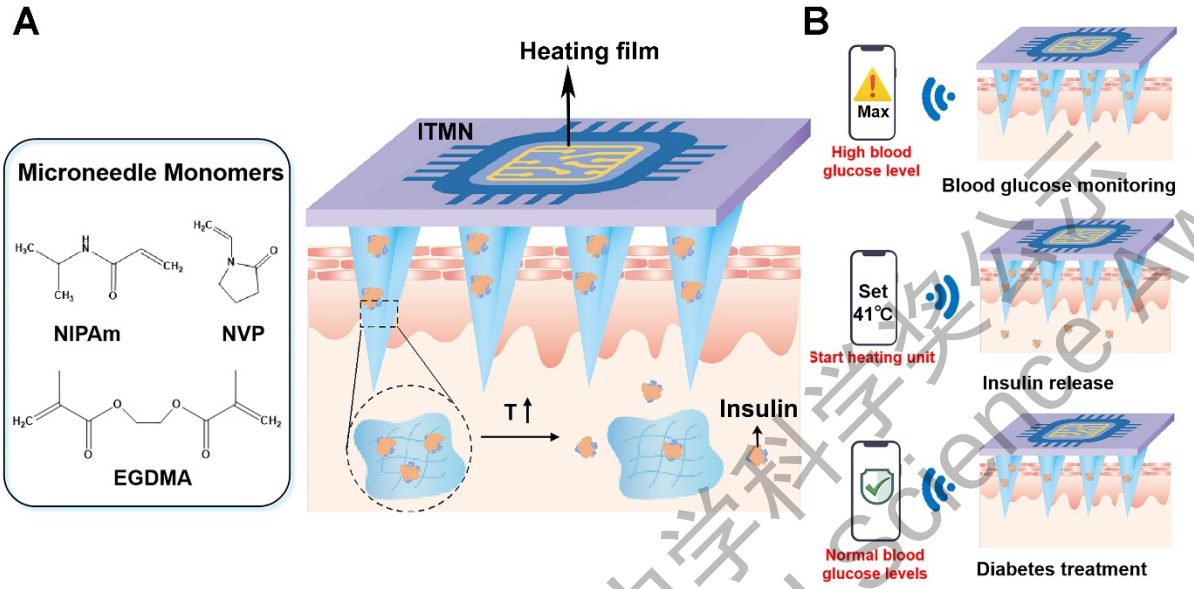
As a novel transdermal drug delivery approach, microneedles present an innovative perspective for the transdermal delivery of protein drugs [11,12]. Microneedles between 50-900 μm in length can painlessly penetrate the cutaneous stratum corneum and reach the epidermis or dermis at a predetermined depth, where the drug is then rapidly absorbed by diffusion or lymph-mediated uptake, and the resulting micropore channels can also repair themselves after drug delivery [13,14]. Compared to oral, transpulmonary and nasal drug delivery, microneedle delivery avoids chemical and enzymatic degradation in the digestive tract, and allows protein-based drugs with a relatively large molecular weight to break through the skin barrier and enter the capillary network. Furthermore, the convenience of its administration increases compliance in patients, thus facilitating disease control [15-17].

It is clear from the above that if a microneedle with temperature-sensitive properties that will effectively achieve subcutaneous “on-off” release of macromolecular drugs. Diabetes mellitus (DM) is a metabolic disease that causes an increased prevalence of several diseases and could be

life-threatening in severe cases [18,19]. As a common drug for the treatment of diabetes mellitus, insulin has a short half-life. Diabetic patients are required to inject insulin subcutaneously by themselves several times a day and frequently adjust the dose administered according to the blood glucose value, which is highly susceptible to various side effects such as microbial infection, local tissue necrosis and improper glycemic control [20,21]. Therefore, exploring microneedle delivery modalities that can replace subcutaneous injection for minimally invasive transdermal delivery of insulin is clinically attractive for diabetes treatment. However, most of the current delivery microneedles are soluble microneedles with poor performance in effectively improving bioavailability while controlling drug release [22-24]. Controlling the dose of insulin is critical during the implementation of a treatment plan for patients with diabetes. Inadequate doses of insulin make it difficult to lower blood glucose to normal levels, while excessive insulin uptake can cause hypoglycaemia, which in severe cases can lead to coma or even death [25,26]. Thus, there is an urgent need to develop an insoluble thermosensitive microneedle patch to achieve on-demand insulin release in order to both conveniently and precisely meet the therapeutic needs of diabetic patients.

Herein, we have designed insulin thermosensitive microneedles (ITMN) that enables the release of insulin to be controlled by temperature changes, which are expected to achieve precise diabetes treatment by adjusting insulin release at any time according to blood glucose values. The thermosensitive microneedles comprise the temperature-sensitive compound N-isopropylacrylamide (NIPAm) and the hydrophilic monomer N-vinylpyrrolidone (NVP), which are produced by in situ photopolymerisation and then encapsulated with insulin and combined with mini heating membranes, resulting in insoluble microneedles with temperature-controlled insulin release (**Scheme 1A**). Experiments have demonstrated that the ITMN has excellent mechanical strength and temperature sensitivity, and can penetrate the skin stratum corneum to achieve effective drug delivery and responsive release, as well as showing effective glycemic control in *in vivo* studies. Once combined with a blood glucose detection device, a closed loop intelligent diabetes treatment system could be established with integrated diagnosis and treatment. When the blood glucose is monitored above the set value, the mini heating membrane is triggered to release insulin in response to the thermosensitive microneedles; when the blood glucose level returns to the normal range, the mini heating membrane is switched off, thus effectively avoiding the risk of

hypoglycaemia due to over-release of the drug (**Scheme 1B**). Thus, our construction of this thermosensitive microneedle confers the possibility for personalised and precise diabetes treatment with highly potential clinical applications.



Scheme 1. A schematic diagram of the preparation process of an insulin temperature-sensitive microneedle (ITMN) and its combination with a blood glucose detection device for the precise treatment of diabetes. (A) Constructing monomers and mechanism of action of ITMN. (B) The action process of ITMN combined with blood glucose detection mobile terminal.

2. Materials and Methods

2.1 Materials

N-isopropylacrylamide (NIPAm), N-vinylpyrrolidone (NVP), ethylene glycol dimethacrylate (EGDMA), fluorescein isothiocyanate (FITC) and 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (photoinitiator 2959) were purchased from Aladdin (Shanghai, China). Insulin (from porcine pancreas) was purchased from Macklin (Shanghai, China). Bovine serum albumin (BSA) was obtained from Labgic Technology (Hefei, China) and PBS buffer was obtained from Servicebio Corporation (Wuhan, China). Trypan Blue was purchased from SunShine Biotechnology (Nanjing, China). PDMS microneedle negative moulds (15 × 15 arrays, needle body diameter 380 μm, needle body height 850 μm, needle tip distance 700 μm) were prepared by Weixin Technology (Taizhou, China). The polyimide heating film was custom made by Yousheng Electronics Ltd (Suzhou, China).

2.2 Cell culture and animals

L929 cells (mouse epithelioid fibroblasts) were cultured in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and placed in an incubator at 37°C with 5% CO_2. ICR mice (male, 20-30 g) were supplied by the Experimental Animal Centre of Yangzhou University (Yangzhou, China). All animal experiments were performed in accordance with ethical approval by the ethical committee of China Pharmaceutical University.

2.3 Synthesis and characterization of the thermosensitive material NIPAm-NVP (NEN)

100 mg of NIPAm and 2 mg of photoinitiator Irgacure 2959 were added into a centrifuge tube, into which 2 mL DI water, 100 μL NVP and 2 μL crosslinker EGDMA were added and mixed to obtain clear and transparent solution. The solution was irradiated with UV light (365 nm 7W) for 30 min under ice bath, so that the monomer NVP and NIPAm reacted by free radical polymerisation to obtain the microneedle material NIPAm-NVP (NEN).

The obtained thermosensitive material NEN was dialyzed overnight at room temperature to remove unreacted monomers, and then lyophilized for subsequent characterization. The ^{1}H NMR spectrum of EGDMA were as follows: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.14(d, 2H) , 5.60(d, 2H), 4.41(t, 4H), 1.96(d, 6H); ^{1}H NMR spectrum of NVP: ^{1}H NMR (500 MHz, D_2O) δ 6.86(dd, 1H),

4.58(t, 2H), 3.54(t, 2H), 2.47(t, 2H), 2.04(m, 2H); ^{1}H NMR spectrum of NIPAm: ^{1}H NMR (500 MHz, D2O) δ 6.10(d, 1H), 5.65(d, 1H), 5.63(d, 1H), 3.90(m, 1H), 1.09(d, 6H); ^{1}H NMR spectrum of NEN: ^{1}H NMR (500 MHz, D_2O) δ 4.70, 3.79, 3.73, 3.58, 3.55, 3.53, 3.19, 2.32, 2.04, 1.93, 1.50, 1.04.

Meanwhile, appropriate amounts of NEN, NVP and NIPAm were taken respectively for attenuated total reflection (ATR) infrared spectroscopy (BRUKER TENSOR 27, Germany) with a spectral scan range of 400-4000 cm^{-1}.

2.4 Investigation of the swelling properties of the thermosensitive material NEN

100 mg of monomer NIPAm and 2 mg photoinitiator Irgacure 2959 were added into a centrifugal tube, then 100 μL deionized water, 100 μL monomer NVP and crosslinker EGDMA (1%, 4% or 8% of the total mass of the two monomers) were added into the tube to obtain transparent mixed solution. The mixed solution was irradiated with UV light (365 nm 7W) for 30 min under ice bath to obtain the thermosensitive material NEN. m_0 (g) of NEN was placed in 10 mL pH 7.4 PBS at 33°C, and removed from the PBS at different time points, then carefully wiped the surface water and recorded precisely the mass m_i (g) until the swelling reached equilibrium. Calculate swelling ratio by the following formula:

$$\text{swelling ratio} = \frac{m_i - m_0}{m_0} \times 100\%$$

The swelling rate and saturation swelling ratio were used to evaluate the swelling ability of the NEN with different crosslinker ratio.

At the same time, NEN was placed in PBS under water bath at 33℃ (60 min) and 41℃ (20 min) alternately, removed from PBS at the corresponding time points, and carefully wiped the surface water and weighed precisely to calculate the swelling ratio. The temperature response ability of NEN was estimated by the change of swelling ratio.

2.5 Formulation screening of NIPAm to NVP ratio in NEN

Materials were added as shown in the table 1 to lead the mass ratios of NIPAm and NVP to be 5:4, 1:1 and 5:6, respectively. After homogenization, clear and transparent solution of thermosensitive monomer material was obtained. Then 160 μL of the solution was mixed with 40 μL BSA-FITC (50 mg/mL). 20 μL of each mixture was placed in a 1.5 mL centrifuge tube and

irradiated by UV light for 30 min under ice bath to produce the thermosensitive material NEN.

Materials	Ratio		
	5:4	1:1	5:6
NIPAm	100 mg	100 mg	100 mg
Irgacure 2959	2 mg	2 mg	2 mg
DI water	100 μL	100 μL	100 μL
EGDMA	2 μL	2 μL	2 μL
NVP	80 μL	100 μL	120 μL

Table 1: The dosage of all materials in formulation screening.

2.6 Safety examination of NEN

The MTT method was utilized to investigate the safety of the microneedle material NEN on L929 cells as follows: cells in logarithmic growth were digested and inoculated in 96-well plates. The 96-well plates were incubated at 37°C and 5% CO_2 for 24 h. A series of concentration gradients of 1 ng/mL, 10 g/mL, 10^2 ng/mL, 10^3 ng/mL, 10^4 ng/mL, 10^5 ng/mL, 10^6 ng/mL, 10^7 ng/mL of NEN was added and blank medium was used as the control group. The 96-well plates were incubated for 24 h before 20 μL of 5 mg/mL MTT solution was added and incubated for 4 h. The supernatant medium was then removed from the plates, 150 μL of DMSO solution was added and gently shaken to dissolve the purple crystals, and the UV absorbance of each well was measured by an enzymatic digitizer (Synergy2, Bio Tek, USA) at a detection wavelength of 490 nm. The safety of NEN was evaluated by the Relative Growth Rate (RGR) of L929 cells, which was calculated using the following formula:

$$RGR = \frac{OD_T - OD_0}{OD_C - OD_0}$$

Where, OD_c: absorbance of the control group, OD_T: absorbance of the experimental group, OD_0: absorbance of the blank control group.

2.7 Preparation and characterisation of insulin thermosensitive microneedles (ITMN)

100 mg NIPAm and 2 mg photoinitiator Irgacure 2959 were added into a centrifuge tube, then 2 mL DI water, 100 μL NVP and 2 μL crosslinker EGDMA were added into the tube and mixed to

obtain clear and transparent monomer solution. The insulin was dissolved in 0.1 M dilute hydrochloric acid to form high concentration insulin stock solution according to the actual required dose. 40 μL of insulin stock solution was mixed with 160 μL of monomer solution. The mixture is then transferred to a microneedle mould and centrifuged at 5000 rpm for 30 min to fill the cavities of the mould. After the removal of the surplus liquid, the microneedle mould is exposed to UV light (365 nm 7W) for 15 min under ice bath to produce the microneedle bodies (**Fig. 1**).

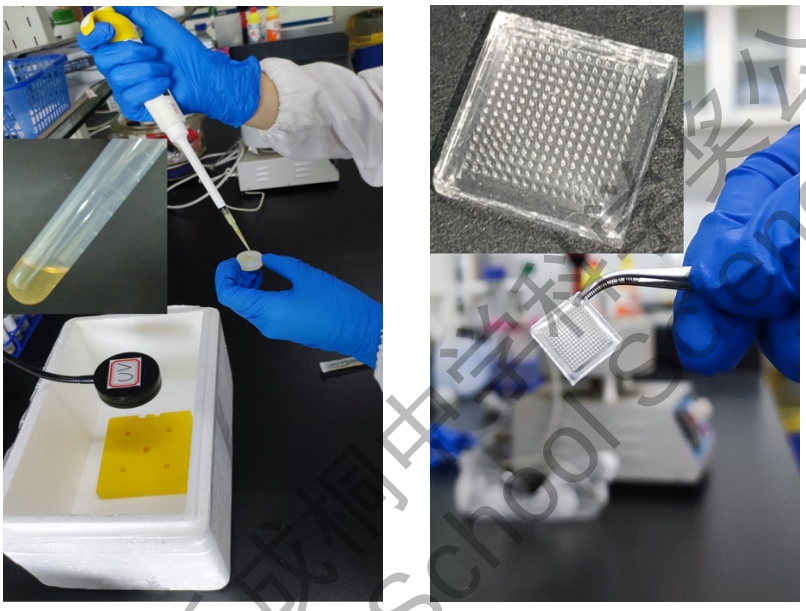


Fig.1 Preparation of insulin-loaded thermosensitive microneedles (ITMN)

Next, 2 mg of photoinitiator Irgacure 2959 was weighed into a 1.5 mL centrifuge tube. 80 μL DI water, 120 μL NVP and 2 μL cross-linker EGDMA were also added into the tube, then mixed well to form clear and transparent monomer solution to fabricate the back of microneedles. The solution was added to the microneedle mould and centrifuged at 5000 rpm for 10 min, after which it was placed under ice bath and irradiated with UV light (365nm 7W) for 15 min to cure the backing. The completed microneedles were dried overnight in a desiccator and carefully demoulded to obtain insulin thermosensitive microneedles ITMN. The surface morphology of the microneedles was characterised by Scanning Electron Microscope (Hitachi Regulus-8100, Japan) and digital camera (Canon, Japan).

Meanwhile, a control group of non-thermosensitive microneedles NVP-MN was prepared in a similar way to ITMN. 2 mg Irgacure 2959 was weighed into a 1.5 mL centrifuge tube, then 100 μL of DI water, 100 μL NVP and 2 μL crosslinker EGDMA were added into the tube and mixed well to obtain clear and transparent solution. The mixture of non-thermosensitive microneedle

monomer solution and the appropriate amount of insulin stock solution was transferred to a microneedle mould for further centrifuging and UV-irradiating to obtain the microneedle body. The microneedle backing was fabricated in the same way as described above. After drying and demoulding, the intact NVP-MN can be further employed as a negative control for thermosensitive microneedles.

During microneedle preparation, the amount of insulin lost during UV curing after centrifugation is negligible and therefore the amount of insulin encapsulated in the microneedle can be calculated by the following equation:

$$m_i = \frac{V_1 \times N \times C \times V_2}{200}$$

where, m_i is the amount of insulin encapsulated in each microneedle patch, mg; V_1 is the volume of each needle body, μL; N is the number of needles bodys; C is the concentration of the insulin stock solution, mg/mL, and V_2 is the volume of insulin stock solution per 200 μL of needle solution, mL.

2.8 Evaluation of the mechanical strength of microneedles

Thermosensitive microneedles with crosslinker contents of 1%, 4% and 8% were fabricated respectively and the fracture force of the microneedles was measured by an electronic universal testing machine (Chuan Testing, China) and force-displacement curves were gained. The displacement tested was 0.35 mm, the preload force was 0.01 N and the velocity was 0.1 mm/s.

2.9 Morphology characterisation of microneedles encapsulated with BSA-FITC

To characterise the morphology of the microneedles by fluorescence photography, the encapsulated insulin was substituted with fluorescein isothiocyanate (FITC)-labelled bovine serum albumin (BSA-FITC). BSA-FITC was synthesized as follows: 200 mg of BSA powder was dissolved in 5 mL of sodium carbonate-sodium bicarbonate buffer (pH 9) to form 40 mg/mL solution of BSA. In addition, 10 mg of FITC powder was dissolved in 100 μL of dimethyl sulfoxide (DMSO) to form 100 mg/mL FITC solution. The FITC solution was added dropwise into the BSA solution with stirring and continue to stir for 12 h at room temperature in the dark. The reaction product was then dialyzed in pure water for 2 days to obtain clear orange liquid, which was lyophilized to obtain BSA-FITC.

20 μL of 50 mg/mL BSA-FITC solution was mixed with 180 μL of thermosensitive or non-thermosensitive monomer solution, and the mixture was transferred into a microneedle mold. The BSA-FITC-loaded microneedles were placed on slides and imaged by inverted fluorescence microscopy (EVOS FL, Life Technologies) to characterise their morphology.

2.10 In vitro release studies

Thermosensitive microneedles (BSA-TMN) and non-thermosensitive microneedles (BSA-NVP-MN) encapsulated with 145 μg of BSA-FITC were prepared according to the method described in 2.5, where the mass ratio of NIPAm and NVP in thermosensitive microneedles was 5: 4.

Insulin-FITC was synthesized as one of the microneedle-loaded drugs for release study. Insulin-FITC was synthesized as follows: 20 mg of insulin powder was dissolved in 5 mL of sodium carbonate-sodium bicarbonate buffer (pH 9) to form 4 mg/mL insulin solution. In addition, 4 mg of FITC powder was dissolved in 100 μL of dimethyl sulfoxide (DMSO) to prepare 40 mg/mL of FITC solution. The FITC solution was then added dropwise to 4 mg/mL of insulin solution with stirring, and the reaction was stirred at room temperature for 12 h under light-proof conditions.

To investigate the release behaviour of the microneedles, thermosensitive microneedles (ITMN) and non-thermosensitive microneedles (NVP-MN) encapsulated with insulin or insulin-FITC were fabricated and their release behaviour at different temperatures was investigated. The above microneedles were immersed in PBS buffer (pH 7.4) at 33°C or 41°C. 50 μL of sample was taken from the release medium and placed in a 96-well plate at 2 h, 4 h, 6 h, 8 h, 10 h, 12 h and 24 h. Meanwhile, equivalent volume of release medium was replenished. The sample solution was topped up to a volume of 200 μL with PBS buffer (pH 7.4). The fluorescence absorption of insulin-FITC was measured using a Microplate Reader at 485 nm excitation wavelength and 528 nm emission wavelength, while the amount of insulin released from the insulin-coated microneedles was measured using Coomassie brilliant blue.

To study the release behaviour of temperature-sensitive microneedles when place at two temperatures alternatively, each microneedle was immersed in 1 mL of PBS buffer (pH 7.4). The release system was heated to 41°C for 20 min, 50 μL was sampled, and an equal volume of release

medium was replenished; subsequently, the temperature was cooled to 33°C for 60 min, 50 μL was sampled, and the release medium was replenished. The aforementioned heating to 41°C and cooling to 33°C was one cycle, seven cycles were performed. The sample solution was topped up to a volume of 200 μL with PBS buffer (pH 7.4). The fluorescence absorption of FITC-insulin was measured using a Microplate Reader at 485 nm excitation wavelength and 528 nm emission wavelength, while the amount of insulin released from the insulin-coated microneedles was measured using Coomassie brilliant blue.

2.11 Ex vivo skin penetration studies

Microneedles BSA-TMN and BSA-NVP-MN encapsulated with BSA-FITC and microneedles ITMN and NVP-MN encapsulated with insulin-FITC were inserted into the dorsal skin of isolated mice and the microneedle-skin conjugate was subsequently immobilized on a transdermal diffusion cell. The recipient cell was filled with 8 mL pH 7.4 PBS buffer. The transdermal diffusion cells were placed on a transdermal diffusion tester (TK-12B, SHANGHAI KAIKAI TECHNOLOGY TRADING CO., LTD.) and the recipient cells solution was set at a constant temperature of 37°C.

Afterwards, the transdermal release of the microneedles was investigated under the temperature cycling condition, where the microneedles were heated to 41°C for 20 min, 150 μL liquid was sampled from the recipient cells and replenished with equal volume of PBS buffer; subsequently, the microneedle was set at 33°C for 60 min, 150 μL liquid was sampled and replenished with equal volume of PBS. Seven cycles were performed for fluorescence quantification in total experiment.

2.12 ITMN skin penetration assay

Firstly, 40 mg of trypan blue powder was dissolved in 1 mL distilled water to obtain 4% trypan blue solution and stored at 4℃. Before using, the solution was diluted 10 times by PBS （pH 7.4） to obtain a final concentration of 0.4%. After unhairing dorsal skin of the mice with hair removal cream (Vetin), ITMN was applied vertically to the back of the mice with slight force for 15 min. Then removed the microneedles completely and stained the applied sites with 0.4% trypan blue solution for 15 min, and residual trypan blue solution was gently wiped with pH 7.4 PBS buffer

so as to subsequently photograph and observe.

After photographing, the microneedles applied sites skin was carefully peeled from the mice, rinsed with saline and immersed in 4% paraformaldehyde solution for fixation. Then H&E staining was performed to investigate mechanical strength and safety of the microneedles.

2.13 Skin safety evaluation of ITMN

After unhairing the back of mice, ITMN was applied vertically to the dorsal skin the mice with slight force for 30 min. The microneedles applied sites were photographed, at 0, 10 and 30 min after completely removing the microneedles, to observe whether the application of microneedles would induce damage to dorsal skin of mice, which would initially assess the safety of the ITMN.

2.14 In vivo therapy evaluation

To investigate the therapy effect of ITMN on diabetic mice, the type I diabetic ICR mice model was constructed: male ICR mice were fasted without water for 12 h, followed by intraperitoneal injection of 120 μg/kg of Streptozotocin. Blood glucose level was measured at 7 days and 14 days after the injection, and the both blood glucose value above 200 mg/mL means successfully obtaining the model.

The type I diabetic ICR mice were separated into five groups: ITMN+Heating (temperature-sensitive microneedle with heating film), ITMN+RT (temperature-sensitive microneedle without heating film), NVP-MN (non-temperature-sensitive microneedle), SC insulin (subcutaneous injection insulin) and Non-treated Healthy (control). The different microneedles were applied to the back skin of mice in each group (**Fig. 2**). The dose of insulin was 0.45 mg in ITMN+Heating, ITMN+RT and NVP-MN groups, and insulin injected subcutaneously was 0.05 mg. Tail vein blood glucose values were measured each hour for 10 h by blood glucose meter (Yuyue, China) in each group. The microneedles were heated at 41°C for 20 min in ITMN+Heating group, then the blood glucose level in tail vein was measured after 10 min. If the blood glucose level was above 200 mg/mL, the microneedles would be heated for another 20 min and stop heating if the blood glucose value return back to normal level.

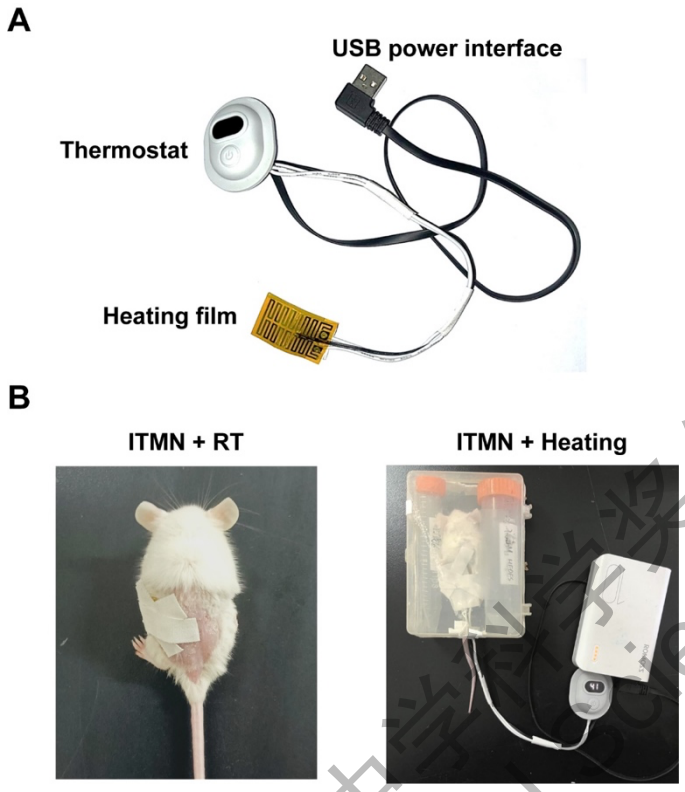


Fig. 2 (A) Physical image of polyimide heating film. (B) Schematic diagram of the experimental setup for the *in vivo* efficacy of ITMN group.

3 Results and discussion

3.1 Synthesis and chemical characterization of needles in ITMN patches

Poly(N-isopropylacrylamide) (PNIPAm) and its derivatives are of interest in thermosensitive hydrogel drug delivery systems with a low critical solubility temperature (LCST): below LCST, the polymer is in solution as a helical hydrophilic chain; above LCST, its structure changes to a tight spherical conformation, facilitating the expulsion of water molecules and reducing its size [27]. As the LCST of NIPAm is about 32°C, it is lower than the normal body surface temperature of the human body. The introduction of hydrophilic monomers was found to be an effective strategy to increase its LCST [28]. Therefore, to make it better suitable for *in vivo* application, NIPAm was copolymerised with the hydrophilic monomer NVP to make its LCST slightly higher than the normal body surface temperature. In this study, we applied NIPAm as a temperature-sensitive material and EGDMA as a cross-linking agent to produce the needles of the ITMN patch, NIPAm-NVP (NEN), by copolymerisation with the hydrophilic monomer NVP under UV light irradiation in the presence of the photoinitiator Irgacure 2959. As shown in **Fig. 3A**, the crosslinker EGDMA, which contains a double bond at both ends, enables crosslinking of NIPAm and NVP via an addition polymerisation reaction to produce microneedles.

The NMR hydrogen spectra of the monomeric materials and the NEN are shown in **Fig. 3B**. The chemical shifts of the hydrogen on the double bonds in the monomers NVP, NIPAm and the cross-linker EGDMA were all above 4 ppm, but the characteristic peaks of the NEN was mainly concentrated in the range of 1-4 ppm, which indicated that there were almost no double bonds on the NEN and most of the double bonds had been polymerised, proving that the monomers were successfully polymerised into temperature-sensitive materials.

Then, the monomeric and temperature sensitive materials were characterized by attenuated total reflection (ATR) infrared spectroscopy and the results are shown in **Fig. 3C**. The IR spectra of NIPAm showed characteristic peaks at 1656 cm^{-1} and 1620 cm^{-1} attributed to carbonyl (C=O) and carbon-carbon double bond (C=C) stretching vibrations, respectively. The IR spectra of the monomer NVP showed absorption peaks at 1693 cm-1 and 1625 cm-1 attributed to stretching vibrations of the carbonyl group (C=O) and the carbon-carbon double bond (C=C). Both monomers show stronger peaks in the 800-1000 cm^{-1} range (around 840 cm^{-1} and 980 cm^{-1}

respectively), which is consistent with the previous literature [29] reporting a stronger peak for CH^2=CHR in the 800-1000 cm^{-1} range. At the same time, absorption peaks associated with secondary amides were also found in the infrared spectrum of NIPAm, respectively an in-plane bending vibration (β_{NH}) at 1544 cm^{-1} and an out-of-plane bending vibration (γ_{NH}) at 3284 cm^{-1}. In contrast, only the carbonyl (C=O) absorption peak at 1643 cm^{-1} is found in the polymer NEN, and the characteristic peaks of the carbon-carbon double bond disappear, as does the peak of CH_2=CHR at 800-1000 cm^{-1}, which indicates that the carbon-carbon double bond (C=C) of the monomers NVP and NIPAm has undergone a radical polymerisation reaction to become a single bond. In addition, the infrared spectrum of NEN exhibited absorption peaks associated with seconal amides at 1538 cm^{-1} and 3294 cm^{-1}. These results demonstrate the successful synthesis of the temperature-sensitive material NEN.

The thermosensitive microneedles designed in this study are swelling-only but not dissolving microneedles, which rely on the structural contraction of the thermosensitive polymer to expel the drug during release. Hence, the swelling ability of the temperature-sensitive material NEN at 33°C was investigated at different cross-linker ratios. Since NEN swells and dissolves quickly in water when the cross-linker ratio is below 1% and is not suitable for microneedle preparation, we investigated the swelling capacity of NEN at 1%, 4% and 8% cross-linker ratios, and the results are shown in **Fig. 3D-i**. The swelling capacity of NEN with 1%, 4% and 8% crosslinkers varied significantly, with swelling rates of about 144.9%, 70.8% and 35.2% at 40 min and 236.2%, 103.7% and 57.2% at 100 min, respectively. The 1% cross-linker NEN reached swelling equilibrium at approximately 16 h with an equilibrium swelling rate of 417%, while the 4% and 8% NEN reached swelling equilibrium at approximately 8.5 h with equilibrium swelling rates of 149% and 84.6%. The swelling rate and maximum swelling capacity of the 1% cross-linker NEN were obviously higher than those of the other two groups, indicating that the higher the percentage of cross-linker in this temperature-sensitive material, the weaker the swelling capacity.

The results of cyclic swelling of NEN alternately placed at 33°C (simulating human epidermal temperature) for 60 min and 41°C (simulating microneedle heating temperature) for 20 min are shown in **Fig. 3D-ii**. The NEN with 1% cross-linking agent showed the most obvious stepwise change in swelling rate under alternating temperature changes, while the remaining two

groups showed very little change, which correlated with the results of swelling rate. It was also proved that the NEN with 1% cross-linker undergoes a highly evident crumpling of volume when the temperature rises to 41°C, which facilitates the rapid release of the drug, while when the temperature decreases to 33°C, the NEN reabsorbs water and expands rapidly in volume, slowing down the release of the drug. Based on the above swelling capacity results, the NEN polymer containing 1% crosslinker was finally selected for the synthesis of thermosensitive microneedles in this study.

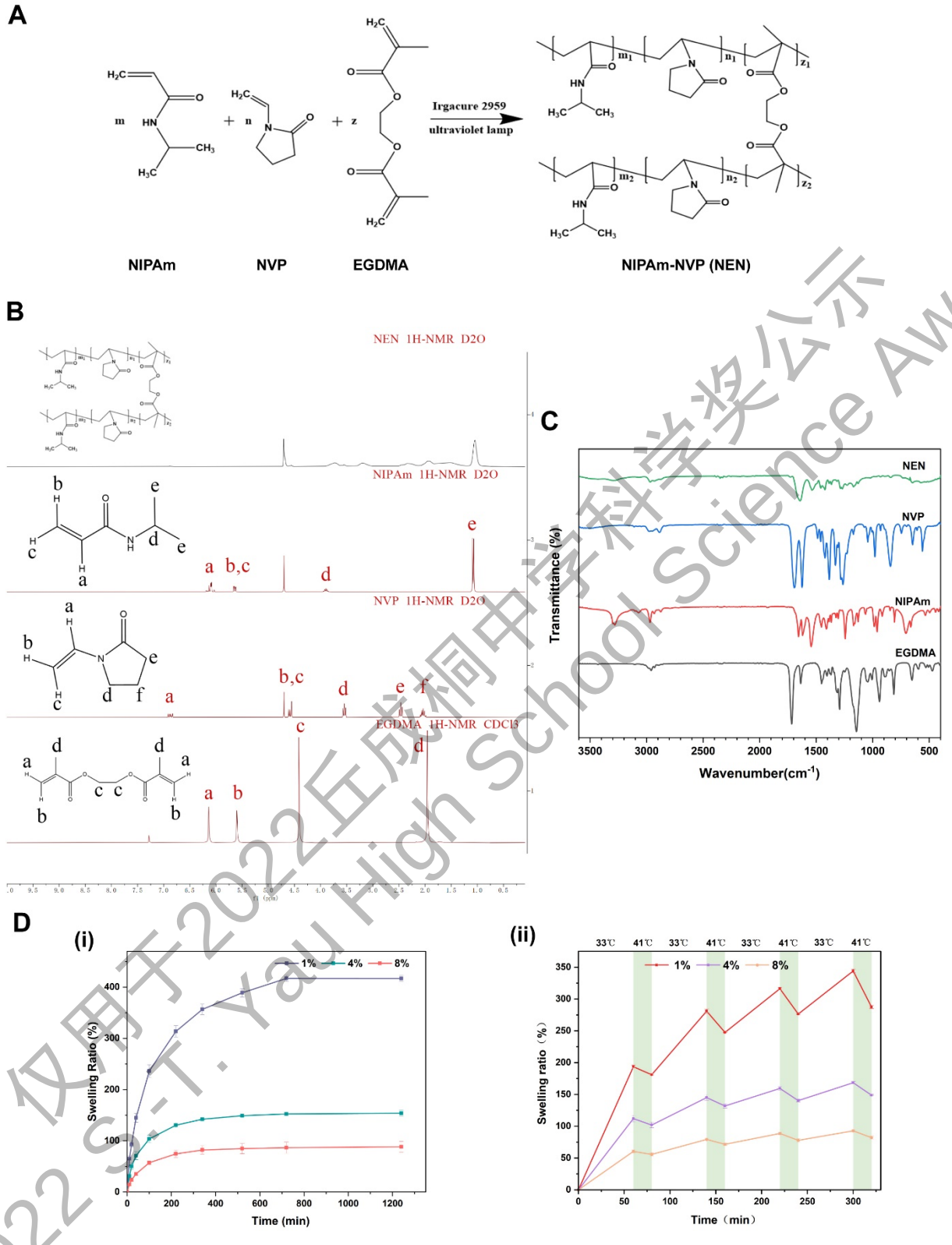


Fig.3 Synthesis and chemical characterization of materials for the needles of thermosensitive microneedles. (A) Synthesis process of NEN. (B) The NMR hydrogen spectra of NEN, NIPAm, NVP and EGDMA. (C) Attenuated total reflection infrared spectra of NEN, NIPAm, NVP and EGDMA. (D) Examination of the swelling properties of NEN containing different ratios of crosslinkers.

3.2 Formulation screening and cytotoxicity study of NEN

To screen superior temperature-sensitive release ratios of NEN, samples with different mass ratios of NIPAm to NVP: NIPAm:NVP=5:4, NIPAm:NVP=5:5 and NIPAm:NVP=5:6 were prepared for investigation on the premise that NIPAm is soluble in a solvent mixture of NVP and water. The above samples were placed in 1.5 mL EP tubes and subsequently placed in three different temperature water baths (25, 33 and 41°C), where 25°C simulated room temperature conditions, 33°C simulated human epidermal temperature and 41°C was the heating temperature for ITMN application. As shown in **Fig. 4A**, the sample with a NIPAm: NVP mass ratio of 5: 4 released the highest amount of BSA-FITC at a temperature of 41°C, reaching 48.9% at 12 h, which was distinctly different from the amount released at 33°C (19.2%). The release behaviour was similar when the NIPAm: NVP mass ratios were 5: 5 and 5: 6, with 33.0% and 30.0% released at 41°C for 12 h. The difference in release between these two samples at 41°C and 33°C was smaller compared to the sample with a NIPAm: NVP mass ratio of 5: 4. In addition, compared to the temperature-sensitive formulation, the release of the non-temperature-sensitive formulation was quite similar at different temperatures, with 38.7% release at 12 h at 41°C and 33.3% release at 12 h at 33°C. Therefore, taking into account the solubility of NIPAm and the release of the drug, the NEN composed of NIPAm: NVP mass ratio of 5: 4 was chosen as the needle for ITMN for the subsequent study.

To further evaluate the safety of the needles of ITMN patch, different concentrations of NEN were subjected to MTT experiments on L929 cells. In the range of NEN concentrations from 1 ng/mL to 10^7 ng/mL, there was no obvious apoptosis, indicating that the needles of ITMN were virtually non-toxic to mouse epithelial-like fibroblasts (**Fig. 4B**).

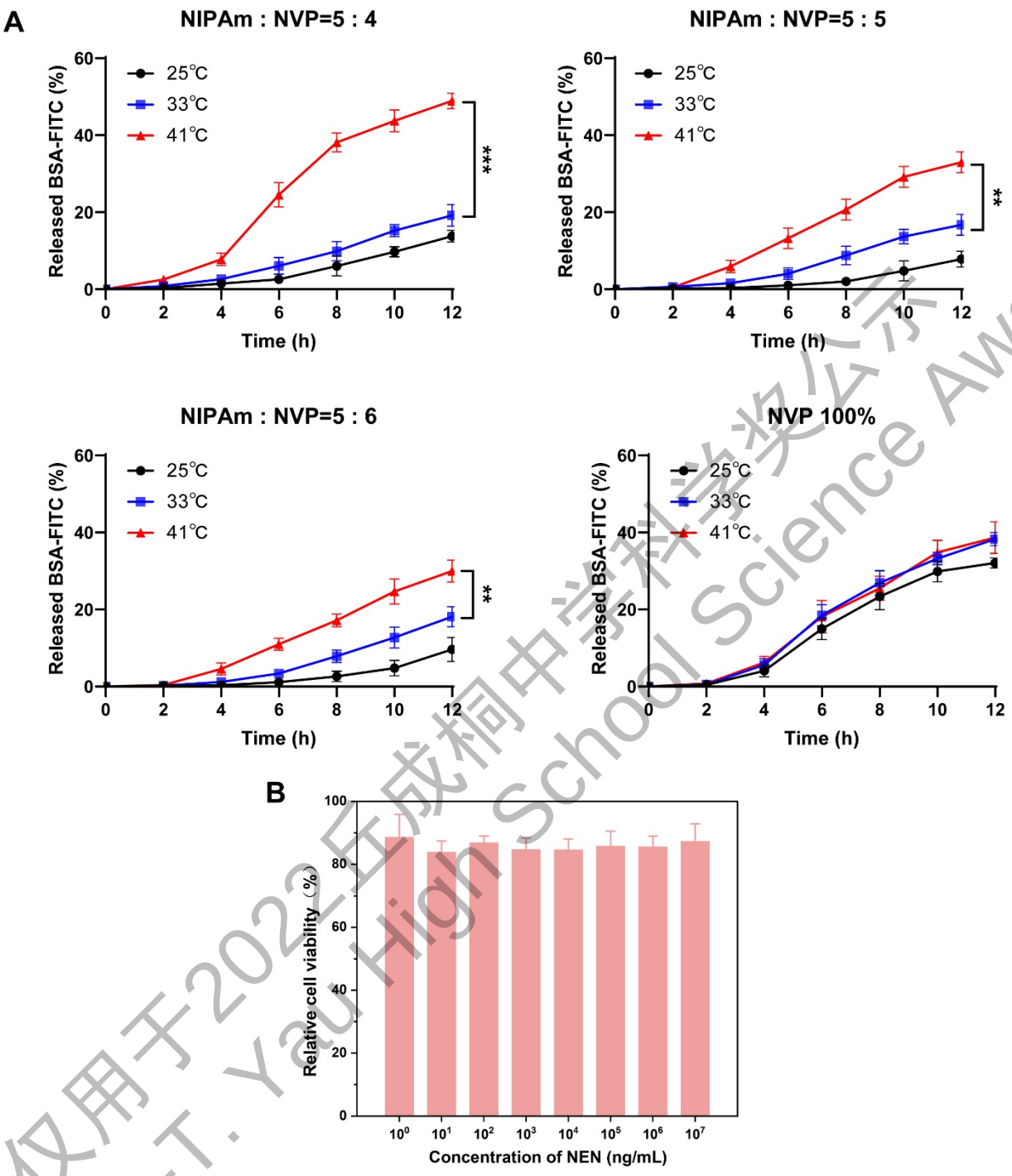


Fig.4 Formulation screening and cytotoxicity study of NEN. (A) *In vitro* BSA-FITC release of several thermosensitive and non-thermosensitive polymers at different temperatures (n = 3). (B) MTT assay to detect cytotoxicity of NEN (n = 3). Data are shown as mean ± SD; n indicates the number of biologically independent samples. *P < 0.05, **P < 0.01, ***P < 0.001.

3.3 Preparation and characterization of ITMN

The process of preparation of the ITMN patch is shown in **Fig. 5A**. First, the NEN solution is adequately filled into the pores of the microneedle mould by centrifugation, and then the drug-containing temperature-sensitive needles is produced by UV light irradiation. Next, a microneedle base solution without the temperature-sensitive material and drug is added, followed by UV irradiation and drying to produce the complete microneedle.

As a novel form of transdermal drug delivery, microneedles are able to overcome the barrier of the stratum corneum and penetrate the dermis at a certain depth, which allows the drug to be rapidly absorbed through local capillary and lymphatic networks [30]. Therefore, having a certain mechanical strength to penetrate the skin is essential for microneedles to be effective. To evaluate the mechanical strength of ITMN, insulin thermosensitive microneedles with 1%, 4% and 8% of cross-linker were prepared and the fracture force of the microneedle patch was measured by an electronic universal testing machine to obtain the force-displacement curve. The results are shown in **Fig. 5B**. The increased percentage of crosslinker increased the stiffness of the microneedle but weakened the strength of the microneedle. By comparison, the thermosensitive microneedles with 1% cross-linking agent had the highest strength and were the least likely to cause fracture of the needles in application, with a fracture force of approximately 0.30 N/needle, which is greater than the 0.058 N/needle required to penetrate the skin [31,32]. This result reaffirms that the choice of 1% crosslinker for the preparation of thermosensitive microneedles results in better physical properties.

The morphological characterization of the microneedles was then performed. The ITMN images taken with a digital camera are shown in **Fig. 5C**, which shows that the prepared microneedle patches are transparent and have a regular array structure. Meanwhile, the microneedles were imaged by scanning electron microscopy (**Fig. 5D**), showing a neat array of ITMN with conical shaped needles and a sharp tip, which is beneficial for penetrating the skin and improving drug utilization. Subsequently, the encapsulated drug was replaced with BSA-FITC to produce microneedles with fluorescent needles, and photographed by fluorescence microscopy. As shown in **Fig. 5E,** both temperature-sensitive and non-temperature-sensitive microneedles were over 600 μm in length, and the drug was successfully loaded into the needle body and deposited

mostly at the tip, probably due to centrifugation, which coincided with the efficient penetration of the drug through the skin stratum corneum.

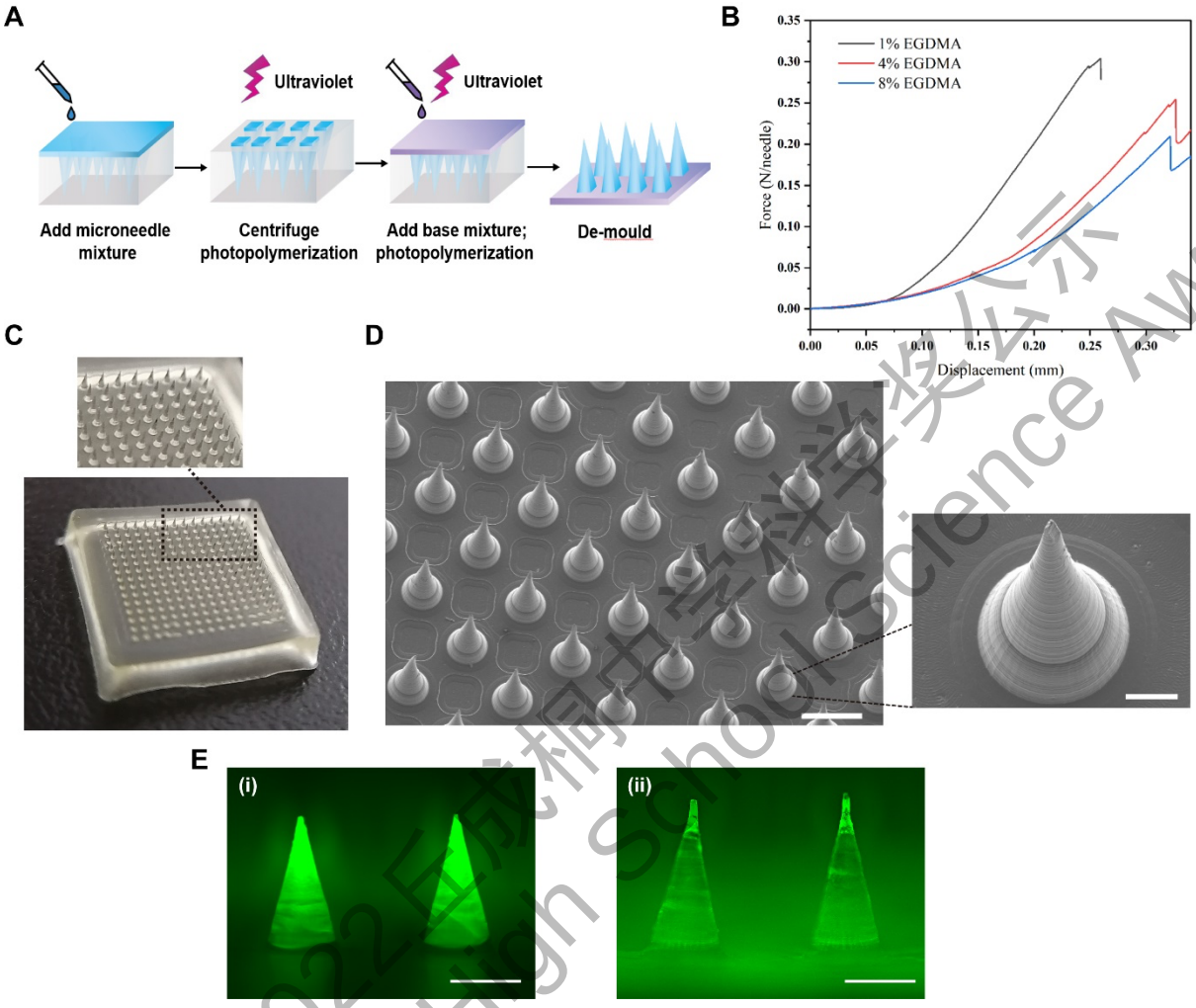


Fig.5 Preparation and characterization of thermosensitive microneedles. (A) Schematic representation of ITMN prepared by in situ photopolymerization strategy. (B) Mechanical strength assay of ITMN. (C) Digital camera photograph of ITMN. (D) Scanning electron microscope image of ITMN. Scale bars, 500 μm (left panel), 100 μm (right panel). (E) Fluorescence microscopy images of a BSA-FITC-loaded thermosensitive microneedles (i) and non-thermosensitive microneedles (ii). Scale bars, 400 μm.

3.4 In vitro release of BSA-FITC thermosensitive microneedles

After investigating the *in vitro* temperature-sensitive release behaviour of samples with different mass ratios of NIPAm to NVP, microneedles with the best release ratio (NIPAm: NVP mass ratio of 5:4) were selected for subsequent release studies. The *in vitro* release behaviour of the thermosensitive microneedles was first examined by encapsulating the model drug BSA-FITC. BSA-FITC thermosensitive microneedles (BSA-TMN) and BSA-FITC non-temperature-sensitive microneedles (BSA-NVP-MN) were placed in PBS solution and both groups were divided into

two groups heated at 33°C and 41°C for 24 h. The release of BSA-FITC was measured in this period. As shown in **Fig. 6A**, BSA-TMN released 46.1% of BSA-FITC in PBS solution at 41°C for 24 h, while only 19.7% of BSA-FITC was released at 33°C, showing a large difference in release at different temperatures. In contrast, the non-temperature sensitive control BSA-NVP-MN released similar amounts at 41°C and 33°C, both at around 42% (**Fig. 6B**). The above results indicate that the prepared temperature-sensitive microneedles had excellent temperature-dependent drug release compared to non-temperature-sensitive microneedles.

Typically, insulin has an onset time of 15-30 min *in vivo* [33,34]. Based on this, we placed the microneedles in PBS solution and set them to be heated at 41°C for 20 min and subsequently lowered to 33°C for 60 min as a temperature cycle to simulate the release behaviour of the microneedles during actual use. The results are shown in **Fig. 6C**. The BSA-TMN release profile showed a stepwise pattern in the first five temperature cycles, with a higher release rate at 41°C and a flatter release at 33°C, which proved that the drug release of this formulation was sensitive to temperature response and reached 39.0% after seven cycles for the temperature-sensitive formulation. Instead, the BSA-FITC in BSA-NVP-MN was released at a certain rate of gentle release (**Fig. 6D**) and the effect of temperature on its release rate was weak.

To further simulate the *in vivo* release of the drug, the transdermal diffusion cell was employed to examine the *in vitro* skin penetration of BSA-TMN and BSA-NVP-MN. The microneedles were first pierced into the skin of mice, and then the microneedles and mouse skin were fixed in the transdermal diffusion cell and subjected to seven temperature cycles of 41°C (20 min)-33°C (60 min). The results are shown in **Fig. 6E**. The transdermal release of the BSA-TMN group was stepwise and clearly temperature dependent, releasing approximately 10.9% of BSA-FITC after 560 min. Whereas the release of the BSA-NVP-MN group did not vary with temperature, with approximately 14% of BSA-FITC released after seven cycles (**Fig. 6F**).

From the above results, it was observed that the thermosensitive microneedle designed in this project has the property of temperature-controlled drug release, while the non-thermosensitive microneedle has no such property. In clinical practice, controlled release of insulin is essential for stabilizing blood glucose in diabetic patients, as uncontrolled over-release of insulin could lead to serious consequences such as hypoglycemic coma [25,35]. Therefore, we have designed a

thermosensitive microneedle to facilitate a safe and convenient option for the treatment of diabetic patients.

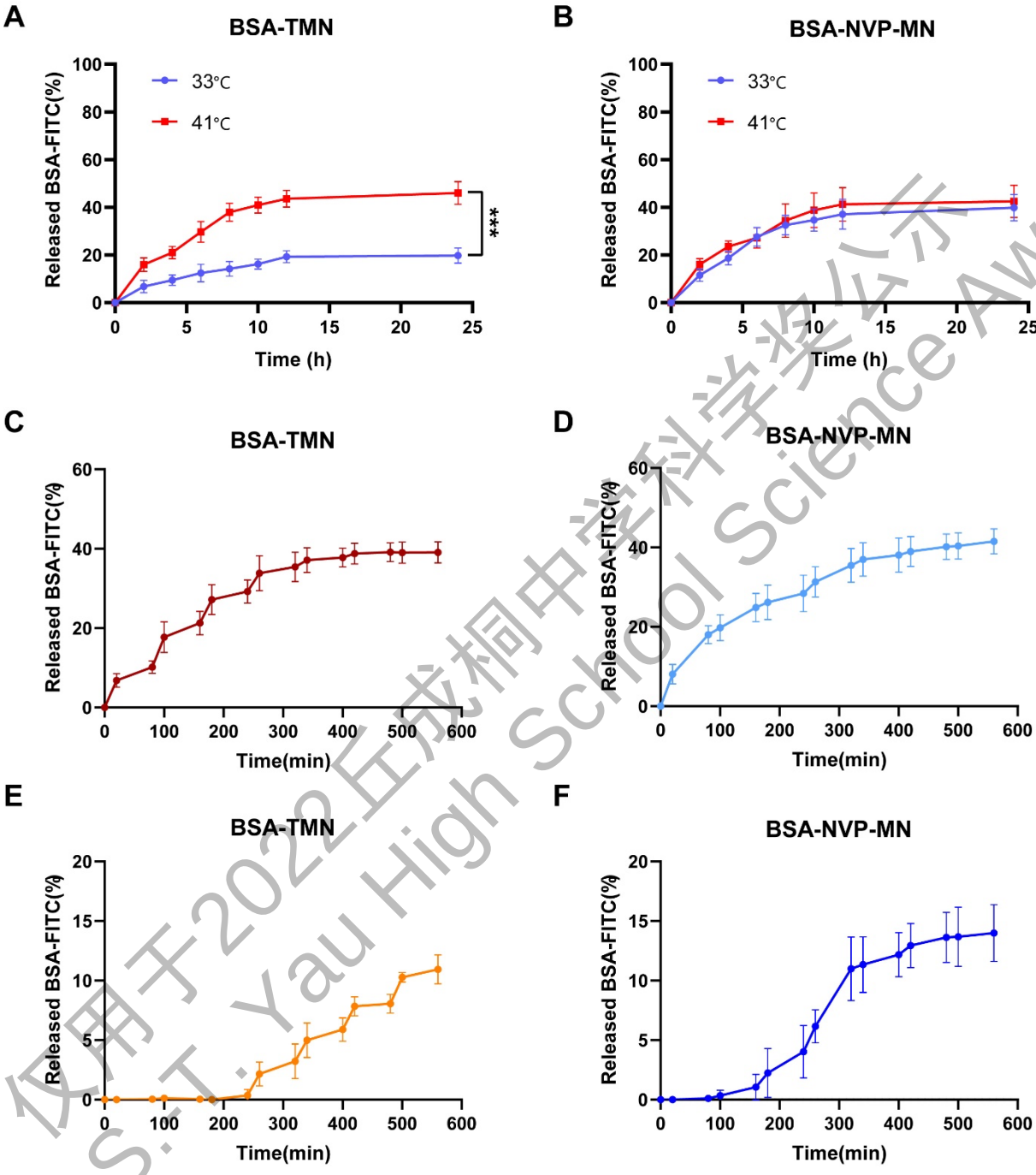


Fig.6 *In vitro* and *ex vivo* skin penetration studies of drug release from thermosensitive microneedles encapsulating the BSA-FITC in PBS solution. (A) BSA-FITC release from BSA-TMN in PBS solution at 41°C and 33°C for 24 h (n = 3). (B) BSA-FITC release from BSA-NVP-MN in PBS solution at 41°C and 33°C for 24 h (n = 3). (C) BSA-FITC release from BSA-TMN in PBS solution subjected to seven 41°C-33°C temperature cycles (n = 3). (D) BSA-FITC release from BSA-NVP-MN in PBS solution subjected to seven 41°C-33°C temperature cycles (n = 3). (E) BSA-FITC release from BSA-TMN during seven 41°C-33°C temperature cycles in a transdermal diffusion cell (n = 3). (F) BSA-FITC release from BSA-NVP-MN during seven 41°C-33°C temperature cycles in a transdermal diffusion cell (n = 3). Data are shown as mean ± standard deviation; n indicates the number of biologically independent samples. *P < 0.05, **P < 0.01, ***P < 0.001.

3.5 In vitro release of the ITMN patch

Following the study of the release behaviour of BSA-FITC within microneedles, insulin encapsulation was continued to verify the *in vitro* release of thermosensitive microneedles. First, thermosensitive microneedles (ITMN) and non-thermosensitive microneedles (NVP-MN) loaded with insulin or insulin-FITC were placed in PBS solution, set at 33°C and 41°C for 24 h. The results of the fluorescence quantification are shown in **Fig. 7A, 7B**. In 24 h, ITMN released 49.8% of insulin-FITC in PBS at 41°C and only 22.7% of insulin-FITC at 33°C. NVP-MN, which has no temperature-sensitive properties, released similar amounts of insulin at 41°C and 33°C, about 45.7%. The results of the insulin release measured by the Kormas Brilliant Blue method showed the similar results. ITMN released 54.4% of insulin at 41°C and 23.7% at 33°C, while NVP-MN released almost the same amount of insulin at both temperatures (**Fig. S1A, S1B**). Thus, the results once again demonstrated a pronounced temperature dependence of drug release from the prepared thermosensitive microneedles compared to the non-thermosensitive group.

The temperature cycling of 41°C (20min)-33°C (60min) was further performed to simulate the release behaviour of the microneedles during actual application. The results of the fluorescence quantification are shown in **Fig. 7C-D**. The release rate of insulin-FITC encapsulated in ITMN was higher at 41°C and flatter at 33°C. After seven cycles, the release of insulin-FITC reached 40.2%. On the other hand, the release of NVP-MN was remained smooth and the effect of temperature on its release rate was less, which was also consistent with the release of insulin measured by the Kormas Brilliant Blue method (**Fig. S1C, S1D**).

Next the ex vivo skin penetration of encapsulated insulin-FITC microneedles was examined. ITMN and NVP-MN were punctured into the skin of mice and then subjected to seven temperature cycles of 41°C (20 min)-33°C (60 min) in a transdermal diffusion cell. As shown in **Fig. 7E-F,** the rate of transdermal release of insulin-FITC from ITMN in the first four cycles was stepwise, with a clear temperature dependence, and approximately 14.8% of insulin-FITC was released after seven cycles, while the release of NVP-MN was not stepwise, indicating that it had no control behaviour on insulin release. The above results confirm again that the thermosensitive microneedle in this subject is capable of temperature-controlled drug release and is a novel non-soluble microneedle with great application value.

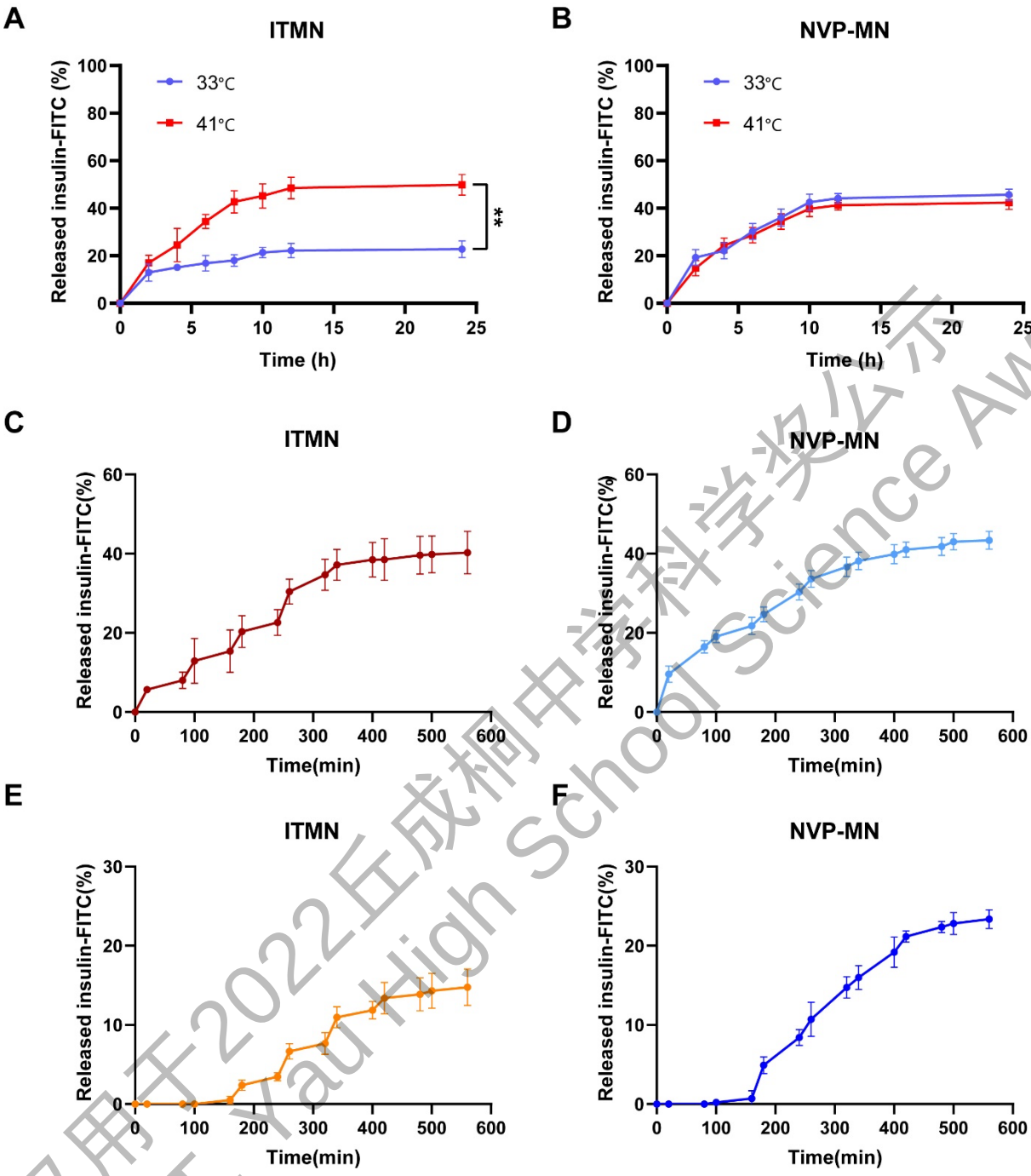


Fig.7 *In vitro* release of thermosensitive microneedles encapsulating insulin-FITC in PBS solution and *ex vivo* skin penetration studies. (A) Release of insulin-FITC from ITMN in PBS solution at 41°C and 33°C for 24 h (n = 3). (B) Insulin-FITC release from NVP-MN in PBS solution at 41°C and 33°C for 24 h (n = 3). (C) Insulin-FITC release from ITMN in PBS solution for seven 41°C-33°C temperature cycles (n = 3). (D) Insulin-FITC release from NVP-MN in PBS solution for seven temperature cycles of 41°C-33°C (n = 3). (E) Insulin-FITC release from ITMN during seven 41°C-33°C temperature cycles in a transdermal diffusion cell (n = 3). (F) Insulin-FITC release from NVP-MN during seven 41°C-33°C temperature cycles in a transdermal diffusion cell (n = 3). Data are shown as mean ± standard deviation; n indicates the number of biologically independent samples. *P < 0.05, **P < 0.01, ***P < 0.001.

3.6 In vivo application study of ITMN in mice

Based on the validation that thermosensitive microneedle release *in vitro* is temperature-controlled a study was conducted on the application of ITMN patch in mice. Typan blue is a cell-active dye that is unable to penetrate the cuticle under normal physiological conditions; when the cuticle is broken, it is able to stain the internal tissue through the break [36]. We applied ITMN vertically to the dorsal skin of mice treated with hair removal, and subsequently stained the area with 0.4% Typan blue solution. The results are shown in **Fig. 8A**, where clear and neat pinholes are visible on the surface of the skin after application of the microneedles.

In addition, the mechanical strength and safety of the microneedles were further investigated by H&E staining of the mouse skin. As shown in **Fig. 8B-i**, the skin of mice showed microneedle-like depressions and partial fracture of the stratum corneum after ITMN patch application, whereas the untreated skin surface of mice was smooth and coherent (**Fig. 8B-ii**). Meanwhile, no inflammatory reactions were observed in the skin of the mice after ITMN application, which has a high safety profile. The above results confirm that the prepared thermosensitive microneedles have satisfactory mechanical strength, capable of effectively breaching the barrier effect of the skin stratum corneum and improving the efficiency of transdermal drug delivery.

ITMN was then applied vertically to the dorsal skin of the depilated mice and the application sites were photographed at 0, 10 and 30 min after removal of the microneedles. As shown in **Fig. 8C**, when the microneedles were first removed, the marks were clearly visible; by the 10th minute, the marks had disappeared; and by the 30th minute, the skin of the mice had basically returned to normal. These results demonstrate that the prepared thermosensitive microneedles have excellent safety and are non-allergenic.

Finally, the therapeutic effect of ITMN in diabetic mice was examined. ICR mice with type I diabetes were divided into the ITMN+Heating group, ITMN+RT (ITMN unheated room temperature group), NVP-MN, sc insulin (subcutaneous insulin injection group) and Non-treated Healthy group and applied to the skin of the back of the mice (**Fig. 8D**). In the ITMN+Heating group, the ITMN was first heated for 20 min and then the blood glucose was measured after 10 min: if the blood glucose was excessive, the heating was continued for 20 min; if the blood glucose was normal, the heating was stopped. The above process was continued for 10 hours. Following

the treatment of each group of diabetic mice, the mice in the ITMN+Heating group were able to maintain normal blood glucose levels for about 6 hours between 2 and 7 hours, while the mice in the other microneedle preparation groups did not have a significant decrease in blood glucose and were still in a hyperglycemic state. Furthermore, the mice in the subcutaneous insulin injection group were only allowed to maintain normal blood glucose levels for a relatively short period of time. Therefore, the *in vivo* treatment results proved that ITMN, when used in combination with the blood glucose testing device, can precisely restore blood glucose in diabetic mice by temperature-initiated insulin release during hyperglycemia, and stop the release of large amounts of insulin during normoglycemia, ultimately achieving stable blood glucose for a longer period of time, providing a safe and long-lasting convenient treatment for diabetic patients (**Fig. 8E**).

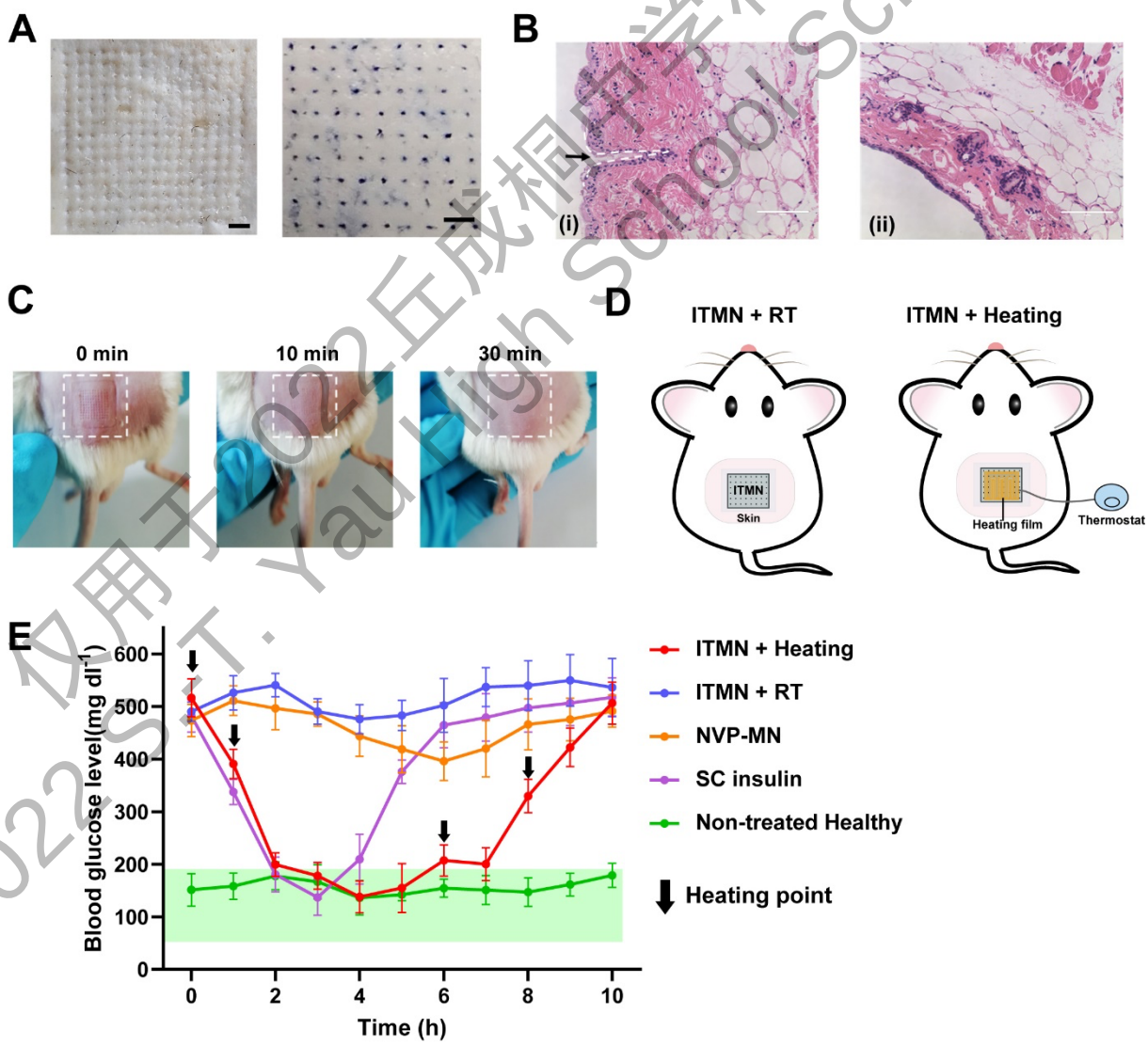


Fig.8 *In vivo* efficacy validation of ITMN. (A) The dorsal skin of mice before and after the application of

ITMN after trypan blue staining. Scale bar, 1 mm. (B) H&E staining of mouse skin after ITMN treatment (i) and without treatment (ii). Scale bar, 100 μm. (C) Dorsal skin of mice after 0, 10 and 30 min of ITMN application. (D) Schematic representation of ITMN administration in diabetic mice. (E) Plot of *in vivo* blood glucose changes in mice after administration of each group of microneedles (n = 3). RT = room temperature. SC = subcutaneous injection. Data are shown as mean ± standard deviation; n indicates the number of biologically independent samples.

4. Conclusion

In summary, we have produced insulin thermosensitive microneedles with promising mechanical strength and safety by a simple in situ photopolymerization method, which demonstrated excellent thermosensitive release performance *in vitro* and *in vivo*, and effectively avoided various adverse effects caused by insufficient or excessive drug release, and precisely controlled blood glucose in type I diabetic mice. In this study, insulin was encapsulated in the thermosensitive microneedle in a simple and gentle manner, and its encapsulation rate was close to 100%, while the protein-based drug activity was successfully retained. Additionally, the thermosensitive microneedle only swells but does not dissolve when applied, avoiding both allergic reactions to the skin and the problem of sudden drug release caused by the dissolution of the microneedle. Finally, when combined with a blood glucose detection device, it enables a closed-loop diabetes diagnosis and treatment function in real time, which holds the promise of exploring precise and personalized diabetes therapy.

References

[1] S. Hossen et al., Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review. J Adv Res 15, 1-18 (2019).

[2] T. Alejo, L. Uson, M. Arruebo, Reversible stimuli-responsive nanomaterials with on-off switching ability for biomedical applications. J Control Release 314, 162-176 (2019).

[3] C. M. Wells et al., Stimuli-Responsive Drug Release from Smart Polymers. J Funct Biomater 10 (2019).

[4] Z. Jin et al., A PTX/nitinol stent combination with temperature-responsive phase-change 1-hexadecanol for magnetocaloric drug delivery: Magnetocaloric drug release and esophagus tissue penetration. Biomaterials 153, 49-58 (2018).

[5] B. Lei, M. Sun, M. Chen, S. Xu, H. Liu, pH and Temperature Double-Switch Hybrid Micelles for Controllable Drug Release. Langmuir 37, 14628-14637 (2021).

[6] M. Amin, T. Lammers, T. L. M. Ten Hagen, Temperature-sensitive polymers to promote heat-triggered drug release from liposomes: Towards bypassing EPR. Adv Drug Deliv Rev 189, 114503 (2022).

[7] B. Jeong, S. W. Kim, Y. H. Bae, Thermosensitive sol-gel reversible hydrogels. Adv Drug Deliv Rev 54, 37-51 (2002).

[8] P. N. Le, C. K. Huynh, N. Q. Tran, Advances in thermosensitive polymer-grafted platforms for biomedical applications. Mater Sci Eng C Mater Biol Appl 92, 1016-1030 (2018).

[9] Z. T. Lin et al., Thermosensitive and Conductive Hybrid Polymer for Real-Time Monitoring of Spheroid Growth and Drug Responses. ACS Sens 6, 2147-2157 (2021).

[10] A. Kasiński, M. Zielińska-Pisklak, E. Oledzka, M. Sobczak, Smart Hydrogels - Synthetic Stimuli-Responsive Antitumor Drug Release Systems. Int J Nanomedicine 15, 4541-4572 (2020).

[11] J. Yu et al., Microneedle-array patches loaded with hypoxia-sensitive vesicles provide fast glucose-responsive insulin delivery. Proceedings of the National Academy of Sciences 112, 8260-8265 (2015).

[12] S. H. Bariya, M. C. Gohel, T. A. Mehta, O. P. Sharma, Microneedles: an emerging transdermal drug delivery system. Journal of Pharmacy and Pharmacology 64, 11-29 (2012).

[13] Y.-C. Kim, J.-H. Park, M. R. Prausnitz, Microneedles for drug and vaccine delivery. Advanced drug delivery reviews 64, 1547-1568 (2012).

[14] M. R. Prausnitz, R. Langer, Transdermal drug delivery. Nature biotechnology 26, 1261-1268 (2008).

[15] J. Yu et al., Glucose-responsive insulin patch for the regulation of blood glucose in mice and minipigs. Nature biomedical engineering 4, 499-506 (2020).

[16] K. J. Lee et al., A practical guide to the development of microneedle systems–In clinical trials or on the market. International journal of pharmaceutics 573, 118778 (2020).

[17] J. Yang, X. Liu, Y. Fu, Y. Song, Recent advances of microneedles for biomedical applications: drug delivery and beyond. Acta Pharmaceutica Sinica B 9, 469-483 (2019).

[18] A. D. Association, Diagnosis and classification of diabetes mellitus. Diabetes care 36, S67 (2013).

[19] Wang et al., Core–shell microneedle gel for self-regulated insulin delivery. ACS nano 12, 2466-2473 (2018).

[20] M. L. Misso, K. J. Egberts, M. Page, D. O'Connor, J. Shaw, Cochrane review: Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus. Evidence-Based Child Health: A Cochrane Review Journal 5, 1726-1867 (2010).
[21] R. Hanas, J. Ludvigsson, Experience of pain from insulin injections and needle-phobia in young patients with IDDM. Practical Diabetes International 14, 95-99 (1997).
[22] K. Lee, C. Y. Lee, H. Jung, Dissolving microneedles for transdermal drug administration prepared by stepwise controlled drawing of maltose. Biomaterials 32, 3134-3140 (2011).
[23] S. Abdelghany et al., Nanosuspension-based dissolving microneedle arrays for intradermal delivery of curcumin. Pharmaceutics 11, 308 (2019).
[24] S. Liu et al., The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin. Journal of controlled release 161, 933-941 (2012).
[25] R. Ajjan, D. Slattery, E. Wright, Continuous glucose monitoring: a brief review for primary care practitioners. Advances in therapy 36, 579-596 (2019).
[26] N. den Braber et al., Requirements of an application to monitor diet, physical activity and glucose values in patients with type 2 diabetes: The diameter. Nutrients 11, 409 (2019).
[27] M. Karimi et al., Temperature-responsive smart nanocarriers for delivery of therapeutic agents: applications and recent advances. ACS applied materials & interfaces 8, 21107-21133 (2016).
[28] G. Fu, W. Soboyejo, Swelling and diffusion characteristics of modified poly (N-isopropylacrylamide) hydrogels. Materials Science and Engineering: C 30, 8-13 (2010).
[29] J. I. Ngadaonye, L. M. Geever, M. O. Cloonan, C. L. Higginbotham, Photopolymerised thermo-responsive poly (N, N-diethylacrylamide)-based copolymer hydrogels for potential drug delivery applications. Journal of Polymer Research 19, 1-15 (2012).
[30] L. Wei-Ze et al., Super-short solid silicon microneedles for transdermal drug delivery applications. International journal of pharmaceutics 389, 122-129 (2010).
[31] C. Lee et al., Evaluation of the anti-wrinkle effect of an ascorbic acid-loaded dissolving microneedle patch via a double-blind, placebo-controlled clinical study. International journal of cosmetic science 38, 375-381 (2016).
[32] Y. Liu, M. Pharr, G. A. Salvatore, Lab-on-skin: a review of flexible and stretchable electronics for wearable health monitoring. ACS nano 11, 9614-9635 (2017).
[33] P. Home, The pharmacokinetics and pharmacodynamics of rapid-acting insulin analogues and their clinical consequences. Diabetes, Obesity and Metabolism 14, 780-788 (2012).
[34] A. K. Sharma et al., Insulin analogs: Glimpse on contemporary facts and future prospective. Life sciences 219, 90-99 (2019).
[35] P. E. Cryer, Hypoglycemia in type 1 diabetes mellitus. Endocrinology and Metabolism Clinics 39, 641-654 (2010).
[36] F. Verbaan et al., Improved piercing of microneedle arrays in dermatomed human skin by an impact insertion method. Journal of controlled release 128, 80-88 (2008).

Supporting information

Thermosensitive microneedles capable of on demand insulin release for precise diabetes treatment

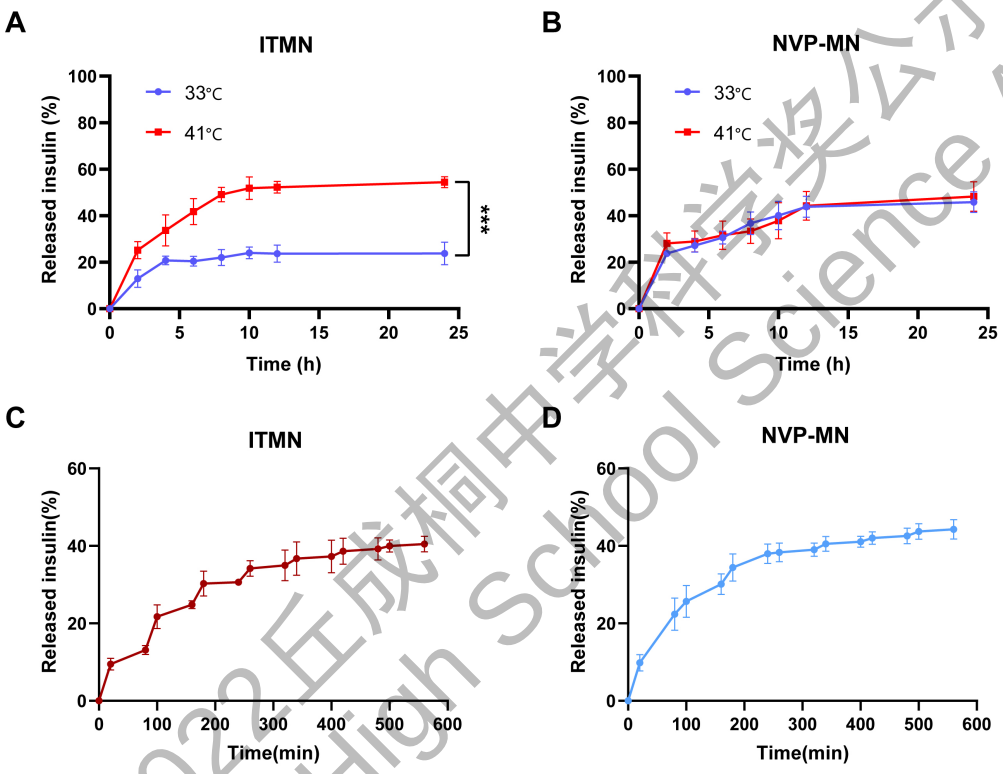


Fig.S1 *In vitro* release of insulin-loaded thermosensitive microneedles in PBS solution (measured by Coomassie brilliant blue method). (A) Insulin release from ITMN in PBS solution at 41°C and 33°C for 24 h (n = 3). (B) Insulin release of NVP-MN in PBS solution at 41°C and 33°C for 24 h (n = 3). (C) Insulin release from ITMN in PBS solution for seven temperature cycles from 41°C to 33°C (n = 3). (D) Insulin release profile of NVP-MN in PBS solution for seven temperature cycles of 41°C-33°C (n = 3). Data are shown as mean ± SD; n represents the number of biologically independent samples. *P < 0.05, **P < 0.01, ***P < 0.001.

Acknowledgement

Topic selection and research background of this paper

The topic of this paper was generated from the independent thinking of Xintong Shi and Sihao Jiang, based on the concern of the diabetes's life quality. After consulting the doctors, they finished the project design, experiment, data processing and paper writing under the guidance of their chemistry teacher Liangliang Xu. Of note, they synthesized temperature-sensitive polymer material and successfully applied it for the precise treatment of diabetes.

Authors contribution

Xintong Shi and Sihao Jiang conceive the study with Liangliang guidance. Xintong Shi completed the preparation, in vitro and in vivo evaluation of temperature-sensitive microneedles. Sihao Jiang completed the synthesis, physical and chemical property characterization and safety evaluation of temperature-sensitive microneedle materials. Xintong Shi and Sihao Jiang wrote the manuscript together.

The relationship between instructor and members, the role of instructor, and whether the guidance is paid

The instructor Xu Liangliang is the chemistry teacher of the team members. Mr Xu polished the paper. Mr.Xu guided the paper design, study and manuscript writing for free.

Data completed with the assistance of others

Yu Huiqiang and Tong Qing, technicians from the Modern Analysis Center of Nanjing University, assisted the characterization of temperature sensitive microneedle materials by nuclear magnetic resonance (NMR) and infrared spectroscopy (IR), which were the entrusted testing service.

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致谢

论文的选题来源、研究背景

本论文的选题是施欣彤和姜锶濠同学自主思考下产生的。出于对糖尿病人生活质量的关注，两位同学请教临床医生，在化学老师许亮亮的指导下，立足化学学科的聚合物化学领域，共同完成此课题的设计、实验、数据整理及论文撰写，合成了温度敏感高分子材料并用于糖尿病人血糖水平自适应的精准治疗。

每个队员在论文撰写中的承担的工作及贡献

许亮亮老师指导施欣彤及姜锶濠设计该研究课题. 施欣彤完成温敏微针的制备、体外评价及药效等体内研究，姜锶濠完成温度敏感微针材料的合成、理化性质表征及安全性研究。施欣彤、姜锶濠共同完成文稿的书写。

指导老师与学生的关系，在论文写作中的作用，及指导是否有偿

指导老师许亮亮是两位队员的化学老师，许亮亮老师完成论文的修改与优化。许老师无偿指导论文设计、实施与文稿写作。

他人协助完成的研究成果

南京大学现代分析中心的技术人员俞慧强、仝庆协助完成温度敏感微针材料的核磁共振 HNMR 及红外光谱 IR 的表征，属于委托测试服务。

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