Supporting Information

Microneedles combined with sticky and heatable hydrogel for local painless anesthesia

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EXPERIMENTAL SECTION

Chemicals and reagents: Sodium hydroxide(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), dopamine hydrochloride (purchased from SaEn Chemical Reagent Technologies Co, Ltd. ShangHai, China),

acrylamide(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), ammonium persulfate(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), N,N-methylenebisacrylamide(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), tetramethylethylenediamine(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), chloroauric acid(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), trisodium citrate(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), CCK (WST) (purchased from Beijing Zoman Biotechnology Co, Ltd. BeiJing, China), sodium alginate(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), calcium carbonate(purchased from Shanghai Macklin Biochemical Co, Ltd. ShangHai, China), gluconolactone(purchased from SaEn Chemical Reagent Technologies Co, Ltd. ShangHai, China)

EXPERIMENTAL SECTION

Preparation Preparation of PDA-PAM-AuNps hydrogel patch

Preparation of spherical Au nanoparticals (AuNps): The AuNps was composited by the classic citric acid reduction method: we dissolved 3 ml 0.029 M chloroauric acid into 200 ml ddH_2O and heated to boil (about 160-170 ℃) at 450 rpm, then 7 ml 1% wt trisodium citrate solution was added and continued to heat for 15 minutes and stirred for 30 minutes. Then we cooled it down to room temperature. After centrifuging at 11000 rpm and removing the supernatant, the solution was washed twice with ddH_2O and finally retained 10 ml as final AuNps solution.[22]

Preparation of PDA-PAM-AuNps hydrogel: We dissolved a certain amount of sodium hydroxide in ultrapure water and adjusted the pH to about 11, then we took 10 ml of the solution and added 0.02g of dopamine hydrochloride for stirring for 12 h. Until the mixture solution became completely black, 2.5 g of acrylamide was added and continued stirring for 2 h, then 0.25 g of ammonium persulfate was added and stirred for 1 h. Then, 0.003 g N,N-methylenebisacrylamide was added and stirred for 30 min. Then, we added 10 μL tetramethylethylenediamine to react for 10 s to form a hydrogel system [23], 2 ml AuNps solution was added immediately.

Preparation of local anesthetic patch: First, we poured the above solution into a mold and a medical sponge was placed in the center. Second, after the hydrogel was solidified, we took it out and added the local anesthetic drug (lidocaine, mepivacaine, etc.) to the medical sponge.

Morphology and elemental analysis of PDA-PAM-AuNps hydrogel patch

After vacuum-drying the prepared PDA-PAM-AuNps hydrogel(PPAh) for 24 h, the morphology of the PPAh was observed by SEM(ZEISS/Sigma 300). The elemental composition of the hydrogel was analyzed by EDS(ZEISS/Sigma 300).

Tensile strength and sticky strength of hydrogel patch

The prepared PDA-PAM-AuNps hydrogels(PPAh), PDA-PAM hydrogels(PPh) were subjected to tensile and sticky strength tests and compared with hyaluronic acid-catechol (HA-CA) hydrogels.

Infrared heating effect of the prepared hydrogel local anesthesia patch

The prepared PDA-PAM-AuNps and PDA-PAM hydrogels were placed on a 37 °C thermostat (simulating human body temperature), using near-infrared (NIR) light (AC220/50HZ) to irradiate them for 5 min, 10 min, and 15 min respectively. The infrared camera (CE0682) was used to record the temperature.

Formability of hydrogel local anesthesia patch

Various shapes of models were designed by 3Ds Max software, 3d printing(Aurora 603s) was applied here to obtain the designed resin mold. Poured the prepared hydrogel solution into mold and we got various shapes of hydrogels after solidification.

Anesthetic effect of hydrogel local anesthesia patch

The frog's legs will shake after being stimulated by pain. Based on this, syringe, microneedle, microneedle + local anesthesia patch + syringe were used to stimulate the legs separately, and the frequency of the leg shaking was recorded by a fixed camera. We used 3 frogs to do this experiment and obtained the finial statistical results.

Rat grimace scale (RGS).

When the rats were stimulated by different degrees of pain, a series of changes would take place in the face, such as the tightening of the eye socket, the protrusion of the nose, the bulge of the cheeks, the change of the position of the ears and the change of

the whiskers.Which represent different RGS scores (scale from 0-2), and videos were taken to obtain the static photos to evaluate the pain.

The permeability of different administation modes.

Firstly, we prepared the sodium alginate hydrogel: added 0.1 g of CaCO3 into 4.9 g 2%wt sodium alginate solution and stirred evenly, then 0.2 g gluconolactone was added and stirred for 10 s to gel formation. Secondly, syringe, microneedle + local anesthesia patch and microneedle + local anesthesia patch + NIR were respectively applied here to transfer the anesthetics into sodium alginate hydrogels for diffusion. The effects of different delivery methods on the penetration range of local anesthetics in the gel were observed.

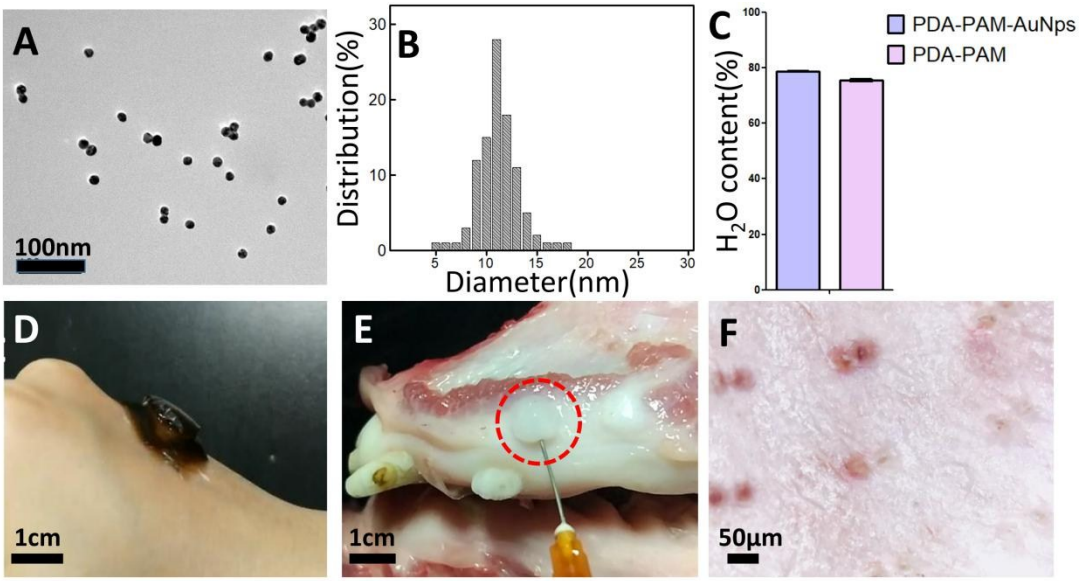
Cytotoxicity and toxicity of hydrogel local anesthesia patch on rat skin

1 g PDA-PAM- AuNps hydrogel was immersed in 10 ml PBS buffer for 48 h at 37 °C. Then, the concentration gradient of the extract was used for human fibroblast toxicity test (CCK8). The PDA-PAM-AuNps and PDA-PAM hydrogels were applied to the skin of SD rats for 10 days, and the skin tissues were taken for HE staining to observe whether the two hydrogels had damage to them.

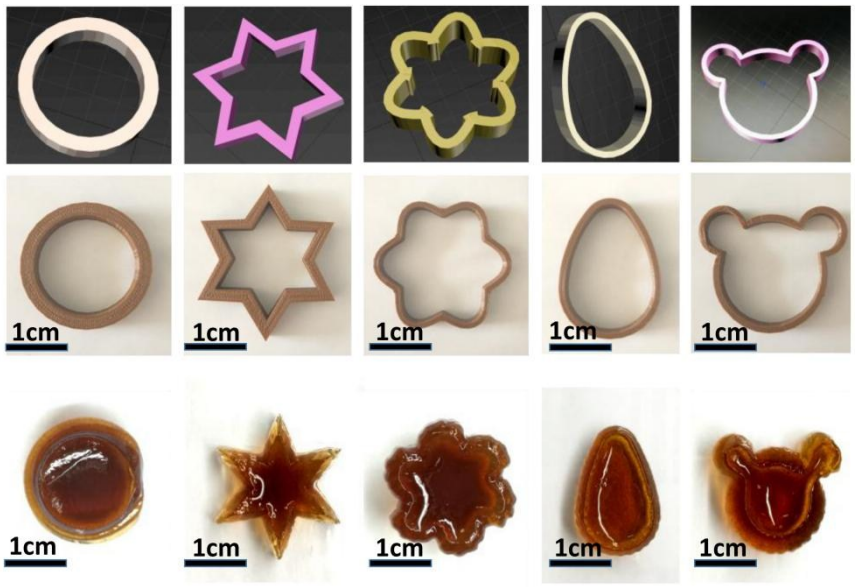
Comparison of self-healing ability of rat skin after using microneedle or syringe

Self-healing tests were performed on the skin of SD rats by using microneedles, syringes, and microneedles + far infrared light (FIR) to stimulate. The skin was observed by electronic magnification camera, by which means, the self-healing time

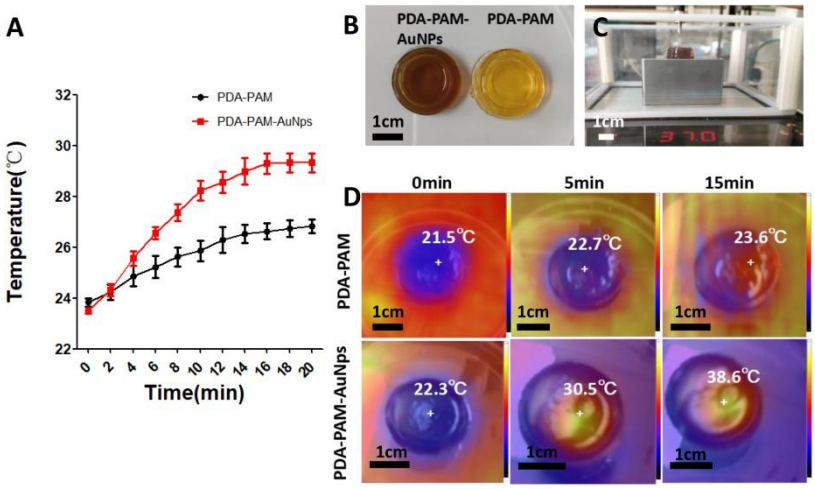
was recorded and analyzed.



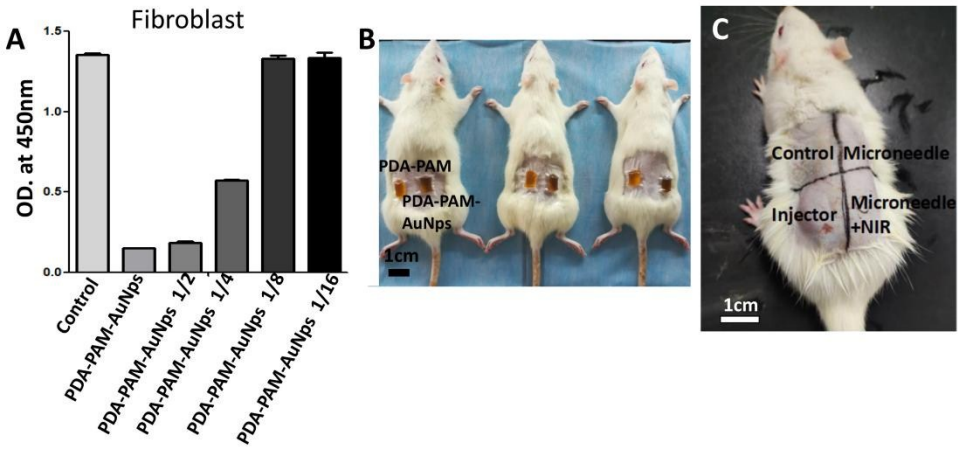
FigS1. A was the TEM image of gold nanoparticles, whose size distribution was shown in FigS1. B. FigS1. C was the water content of PPh and PPAh. FigS1. D was a rendering of applying the PPAh patch to human skin. FigS1. E was a partial swelling (red dotted area) of the pig gingival mucosa after anesthetic injection. FigS1.F was an enlarged micrograph of the gingival mucosa after applying microneedle.



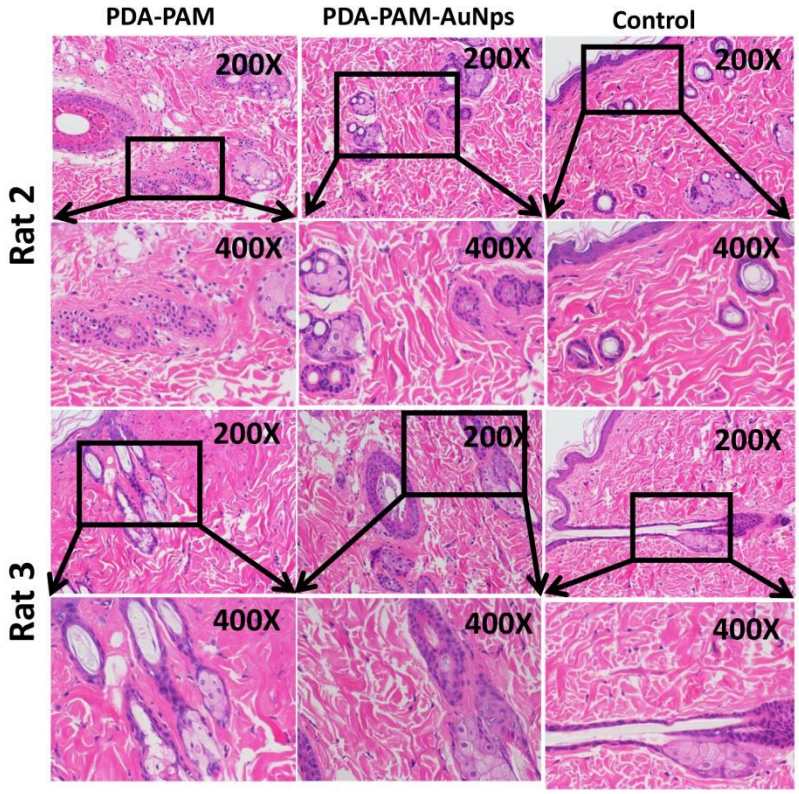
FigS2. The picture showed various models printed by 3D printer, and various shapes of hydrogels were made by those molds.



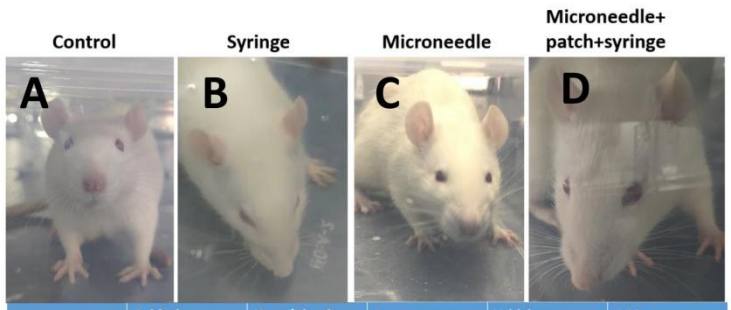
FigS3. A was the temperature rise curve of PPh and PPAh after using NIR, and FigS3. B was the physical pictures of PPAh and PPh. In FigS3. C, the hydrogel was placed on a 37 ℃ constant temperature plate (simulating the human body temperature) and then irradiated by NIR, then the temperature changes of PPh and PPAh were recorded as infrared images .



FigS4. A showed the cytotoxicity of human fibroblasts after co-cultured with PPAh extract (diluted with different multiples). FigS4. B was the schematic diagram of pasting PPh and PPAh on rats for 10 days. For assessing the skin's self-healing ability, microneedle, syringe and microneedle plus NIR were separately applied to the rat skin as well as control(FigS4. C).



FigS5. The figure showed the HE staining results of another two rats after pasting PPh and PPAh for 10 days.



	E	Orbital	Nose/Cheek	Ears	Whiskers	RGS
Rat1	Syringe	2	2	2	1	1.75
	Microneedle	0	2	0	1	0.75
	Microneedle+patch+Syringe	0	1	0	0	0.25

	F	Orbital	Nose/Cheek	Ears	Whiskers	RGS
Rat2	Syringe	2	2	2	2	2
	Microneedle	1	2	0	0	0.75
	Microneedle+patch+Syringe	0	1	0	0	0.25

	G	Orbital	Nose/Cheek	Ears	Whiskers	RGS
Rat3	Syringe	2	2	2	1	1.75
	Microneedle	0	1	0	1	0.5
	Microneedle+patch+Syringe	0	0	0	0	0

Fig. S6A-D were the static photos of the facial expressions of rats with different stimulation methods, FigS6. E-F was the RGS scale score of each group of rats.

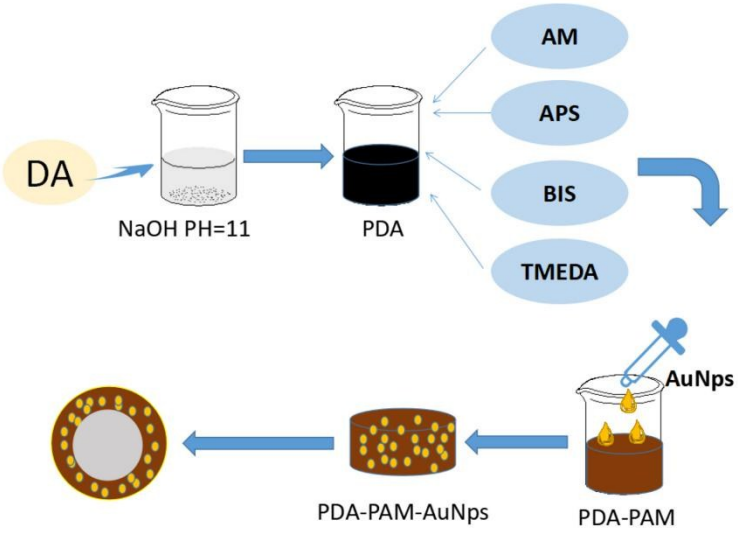


Fig. S7.The schematic diagram of the preparation process of PPAh.

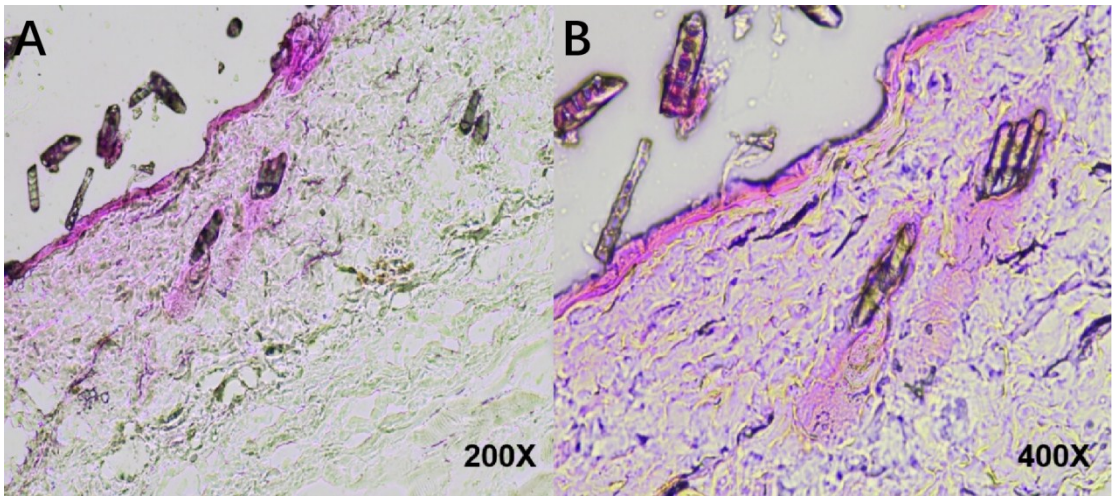


Fig S8. Fluorescence microscope images of rat skin treated with microneedles and stained with Rhodamine B(A:200x, B:400x)

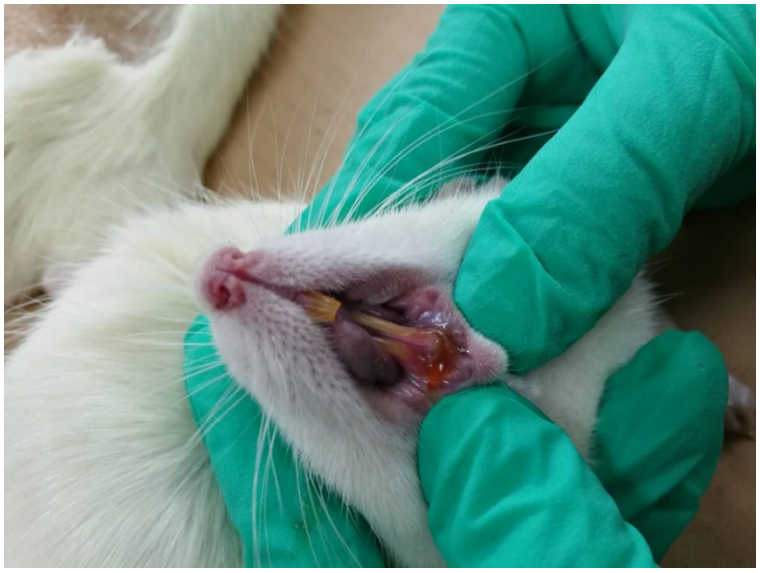


Fig S9. Hydrogel can be attached to the oral mucosa of rats.

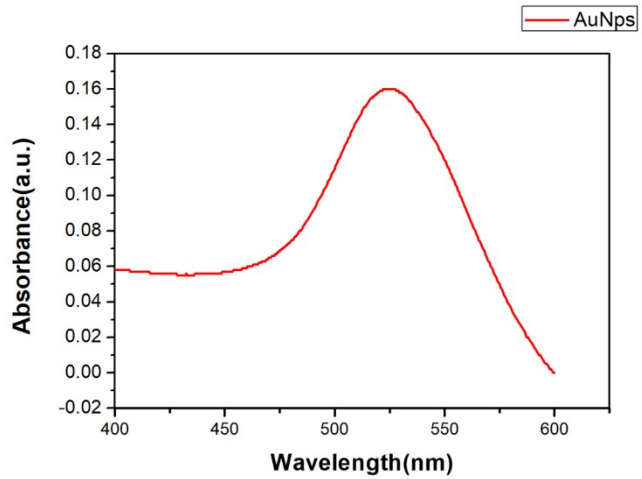


Fig S10. The absorption spectra of gold nanoparticles

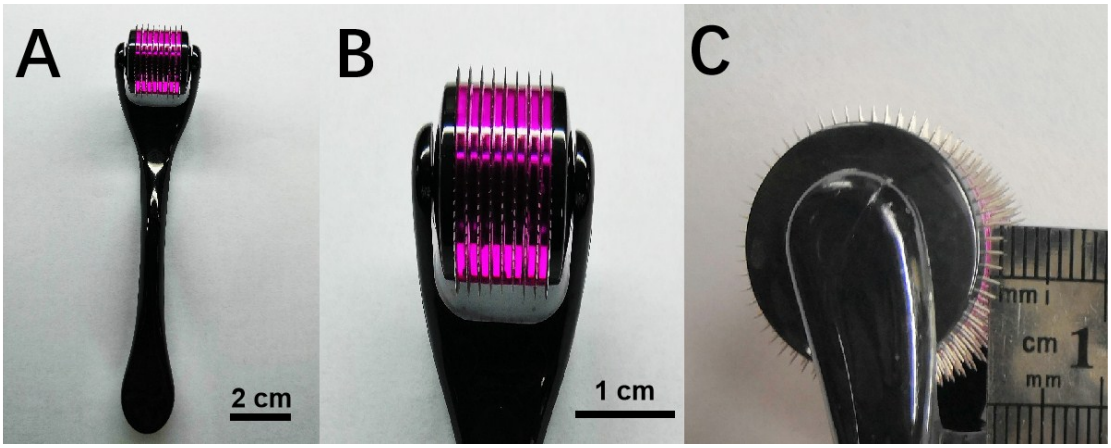


Fig S11. A-C were the pictures and specifications of microneedles used in the laboratory;

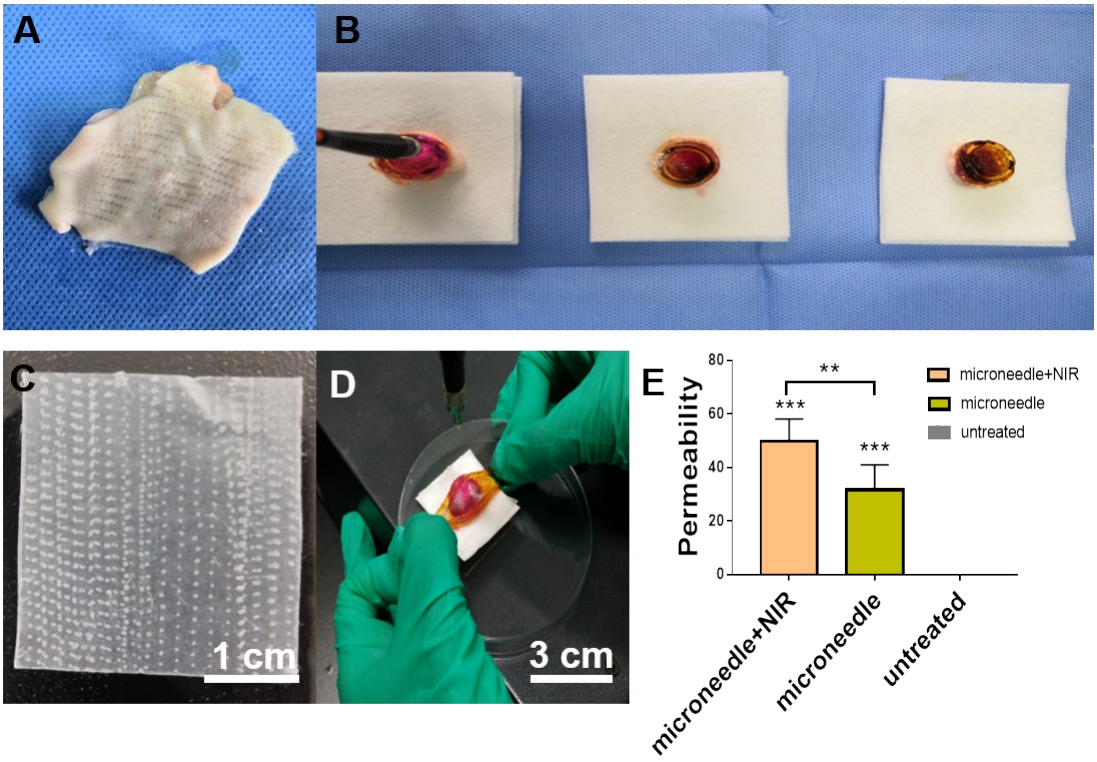
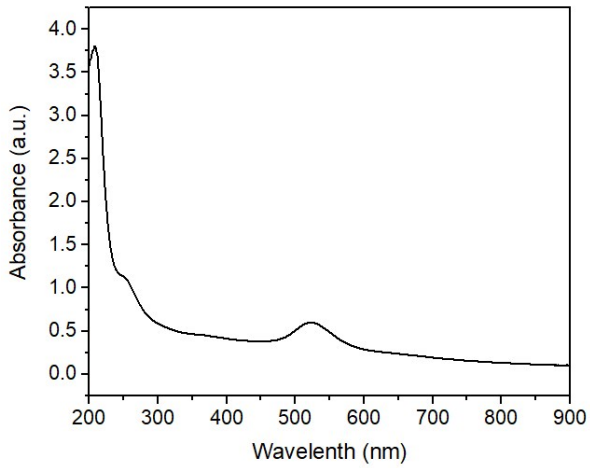


Fig S12. A. Images of rat skin treated by microneedles; B. Schematic diagram of penetration experiment 1; C. Seal film treated with microneedles; D. Schematic diagram of penetration experiment 2; E. Permeability of penetration experiment 2. (N=3. * P<0.05, ** P<0.01, ***p<0.001)



FigS13. The absorption spectra of AuNPs

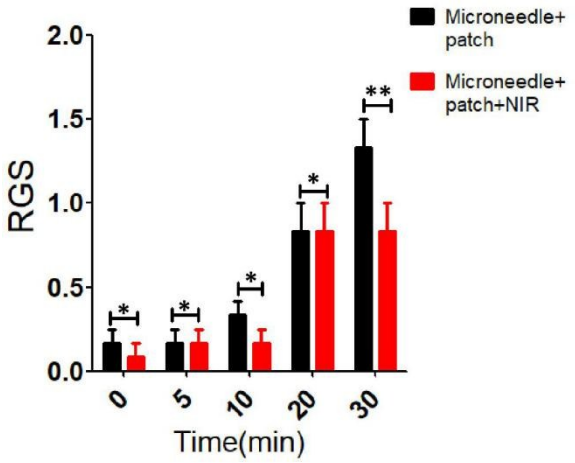


Fig.S14. The analgesic effect and duration of "microneedle + local anesthesia patch" and "microneedle + local anesthesia patch + NIR" delivered to the skin of rats (3 rats were selected for the two groups to prove the repeatability of the experiment).