Supporting Information for

In Situ Enrichment and Ultrasensitive Analysis of Interstitial Fluid miRNA Enabled by Hydrogel Microneedles Coupled with DNA-Gated Metal-Organic Frameworks

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Materials and methods

Materials

All DNA oligonucleotides used in this experiment were purchased from Sangon Biotech Co., Ltd. (Shanghai, China) and purified by high-performance liquid chromatography (HPLC), and the sequences were listed in Tab. S1. Zirconium chloride, anhydrous ($ZrCl_4$), 2-Aminoterephthalic Acid (BDC-NH_2), Gold (III) chloride solution ($HAuCl_4$), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (NHS) and sodium citrate were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). N,N-dimethyformamide (DMF), Methanol absolute were purchased from Kemiou Chemical Reagent Co, Ltd. (Tianjin, China). Gelatin (Gel) was purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Methacrylic anhydride (MA) and Rhodamine 6G (R6G) were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). Ethanol absolute was purchased Yuanli Chemical Co., Ltd. (Tianjin, China). N,N,N', N'-Tetramethylethylenediamine (TEMED), ammonium persulfate (APS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

Instruments

Transmission electron microscopy (TEM) measurements were measured on HITACHI H-8100 EM and JEOL JEM-2100 F. Scanning electron microscope (SEM) images were performed on a Hitachi SU8010 scanning electron microscope. Powder X-ray diffraction (PXRD) patterns were recorded using a Rigaku SmartLab 9 kW. Fourier infrared spectra (FT-IR) were recorded on Thermo Nicolet is5. 1H-NMR spectra were collected on a Bruker ACF400 (400 MHz) supplied by Bruker Biospin. The Zeta potential of the sample was measured on a Malvern Panalytical Zetasizer Nano ZS90. Fluorescence spectra were performed using an Edinburgh FS5 steady-state transient fluorescence spectrometer. The mechanical property of cross-linked GelMA MN patches was examined using a universal testing machine SHIMADZU AGS-X. The SERS Spectra were obtained from Thermo DXR2xi Raman Imaging Microscope.

Cell viability assay was measured on a Tecan Infinite E Plex.

Preparation of GelMA

GelMA was synthesized according to the method reported previously.[1] The modification mechanism of gelatin by methacrylic anhydride to form GelMA is illustrated in Fig. S15. Initially, 10 g of gelatin was dissolved in 100 mL of PBS and stirred at 60℃ for 1 h. Subsequently, 8 mL of methacrylic anhydride was added dropwise at a rate of 1 mL per minute and vigorously stirred at 50℃ for 3 h. Finally, 100 mL of PBS (50℃) was added to terminate the reaction, and it was dialyzed in 50℃ distilled water using a 12 kDa-14 kDa dialysis bag for 5 days.

Preparation of MOF

The MOF was synthesized according to a previously reported method.[2] 240 mg of $ZrCl_4$, 220 mg of terephthalic acid, and 3.8 g of benzoic acid were added to 20 mL of DMF and sonicated for approximately 3 min to dissolve. The mixture was then transferred to a Teflon-lined vessel and maintained at 120℃ for 20 h. Afterward, the mother liquor was cooled to room temperature and centrifuged at 8000 rpm for 10 min to obtain the precipitate. The resulting solid was washed with DMF and anhydrous ethanol to remove residual reaction precursors, with the washing process repeated three times. Finally, the solid was dried under reduced pressure at 100℃ overnight for subsequent use.

Preparation of DNA-gated MOF

First, 10 μL of 100 μM DNA1 was added to 990 μL of a solution containing 400 mM EDC and 100 mM NHS, and the mixture was stirred for 1 h. Next, 1 mg of MOF was added to the solution and allowed to react at room temperature for 3 h. The resulting mixture was then centrifuged, and the precipitate was washed three times with PBS buffer (10 mM, pH 7.4) to obtain DNA1@MOF. The MOF modified with DNA1 was redispersed and used for the loading of the signaling molecule R6G, resulting in DNA1@R6G@MOF. Specifically, 50 μL of 10 mM R6G was added to DNA1@MOF, and the mixture was diluted with PBS buffer (10 mM, pH 7.4) to a final volume of 1 mL. The solution was then stirred for 12 h to load R6G. Next, 10 μL of 100 μM DNA2 was added to the solution and incubated at 37°C for 3 h to form the DNA-gated MOF,

denoted as DNA1@DNA2@R6G@MOF. To obtain purified DNA-gated MOFs, the product was collected by centrifugation at 10,000 rpm and washed at least 5 times to remove excess R6G and DNA. Finally, the DNA-gated MOFs was redispersed in PBS buffer (10 mM, pH 7.4) for further applications. To quantify the amount of DNA loaded onto the MOF, the absorbance at 260 nm was measured for both the initial DNA solution (A_0) and the residual DNA solution in the supernatant after MOF modification (A). By comparing the initial DNA concentration (C_0) with the residual DNA concentration, the DNA loading capacity of the MOF was determined. The DNA loading capacity was calculated using the following formula:

$$DNA\ loading\ capacity = (A_0\text{-}A) / A_0 \times C_0 \times 10^{-3}$$

Preparation of the FL-SERS Dual-Mode Biosensor

First, 0.1 mg of the solid sample was dispersed in 900 μL of PBS buffer solution (pH 7.4). Then, 100 μL of the target analyte at different concentrations was added to the dispersion, and the mixture was stirred and incubated at room temperature for 1 h. After incubation, 200 μL of the resulting sample was taken and centrifuged at 8000 rpm for 3 min to remove the solid particles. The supernatant obtained was then diluted with 800 μL of PBS (pH 7.4) for subsequent analysis.

For fluorescence analysis, the fluorescence of Rho 6G was excited at 525 nm, and the emission was recorded at 553 nm. For SERS analysis, a gold colloid was deposited onto a clean silicon wafer as the SERS substrate. The sample was then added dropwise onto the gold substrate. SERS signals were measured using a laser with a wavelength of 785 nm, an excitation power of 10 mW, and an integration time of 10 s.

Preparation of GelMA MNs

The polydimethylsiloxane (PDMS) microneedle mold was purchased from Taizhou Microchip Pharmaceutical Technology Co., Ltd. (Zhejiang, China). To prepare the GelMA hydrogel precursor solution, 25 mg of lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was added as a photoinitiator to 10 mL of deionized water. Then, 1.5 g of GelMA prepolymer was dissolved in the LAP-containing solution at 60°C to obtain the GelMA hydrogel precursor solution. This solution was subsequently filled into the PDMS mold and centrifuged at 3000 rpm for

3 min to ensure complete filling of the mold. The filled mold was then exposed to 500 mW of ultraviolet (UV) light for 30 s. Afterward, the mold was stored in a dark environment for 24 h to allow the GelMA microneedles to dry.

Swelling Capacity of GelMA MNs

The swelling capacity of the GelMA MNs was evaluated according to an established protocol. Initially, the dry weight of the MNs was recorded as M_0. The MN patch was then immersed in phosphate-buffered saline (PBS, pH 7.4). After soaking, excess surface water was removed, and the swollen weight of the MNs was recorded as M_t. The swelling capacity was calculated using the following formula:

$$S\% = (M_t - M_0)/M_0 \times 100\%$$

Mechanical Properties and Skin Penetration of GelMA MN Patches

The mechanical properties of GelMA MN patches were evaluated using a universal testing machine, as described in previous studies.[3] Briefly, the patches were placed on a stainless plate with the tips facing upward. A sensor applied a vertically oriented force at a rate of 1 mm min^{-1}, and the correlation between the applied force and the compression of the microneedles was recorded.

To assess the skin penetration capability, GelMA MN patches were applied to the skin of a mouse cadaver for 1 min. After removing the patches, the treated tissue was stained with trypan blue to highlight areas of penetration. Excess trypan blue was washed off with PBS, and the skin was imaged. The presence of blue spots indicated successful penetration of the stratum corneum.

In *vitro* Simulation Extraction and miRNA Detection

To simulate in vitro ISF extraction, various concentrations of miRNA were incorporated into a 1% (m/v) agarose hydrogel. A GelMA microneedle patch was then pressed into the agarose hydrogel. After 30 min, the microneedle patch was gently removed and transferred to a centrifuge tube containing 200 μL PBS (pH 7.4). Following centrifugation at 12,000 rpm for 10 min, the microneedle patch was discarded. Subsequently, 800 μL of PBS (pH 7.4) and 0.1 mg of probe were added. The samples were then analyzed by both fluorescence (FL) and surface-enhanced Raman scattering (SERS) methods, as previously described.

In *vitro* Cell Viability Assessment

Cell viability was evaluated using the MTT assay as previously described. NCTC clone 929 cells (L929) were seeded in a 96-well plate at a density of 10,000 cells per well and cultured to confluence. GelMA MNs were incubated in the culture medium for 12 and 24 h to obtain extracts, which were then incubated with the cells for an additional 24 h. L929 cells cultured in a medium without MN extracts served as the negative control. After incubation, 50 μL of 1 g/L MTT solution was added to each well and incubated for 4 h. The absorbance of the samples was measured at 490 nm using a microplate reader.

Skin Insertion Experiment

The volunteer was informed and signed informed consent before the experiment, and the experimental research process was in accordance with the Declaration of Helsinki. Microneedle patch was applied to the volunteer's skin, and the skin condition was recorded at different time points after using the microneedle patch.

In *vivo* ISF Sampling and miRNA Detection

All the animal experiments were conducted following the guidelines of the Tianjin Committee of Use and Care of Laboratory Animals. All animal procedures were approved by the Animal Care and Use Committee at Nankai University. Six mice were randomly divided into two groups (three mice per group) for different treatments. Both the experimental and control groups were fed under the same conditions. The experimental group received a subcutaneous injection of 100 μL of 100 nM target miRNA solution into the dorsal skin of each mouse, resulting in mice with high miRNA levels in ISF. The control group received a subcutaneous injection of 100 μL of saline into the dorsal skin of each mouse. After a waiting period of 30 min to allow the target miRNA to diffuse into the skin ISF, microneedle patches were pressed into the mouse skin and secured with medical tape for 30 minutes. Subsequently, the microneedle patches were transferred to centrifuge tubes for centrifugation. After removing the patches, probes were added for subsequent analysis.

Supporting tables and figures

Table S1. DNA sequences employed in DNA-gated MOF

Name	Sequence (5'-3')
DNA1	TTTTTGCTAATCGTGATAGGGGT
DNA2	ACCCCTATCACGATTAGCATTAA
miRNA	UUAAUGCUAAUCGUGAUAGGGGU

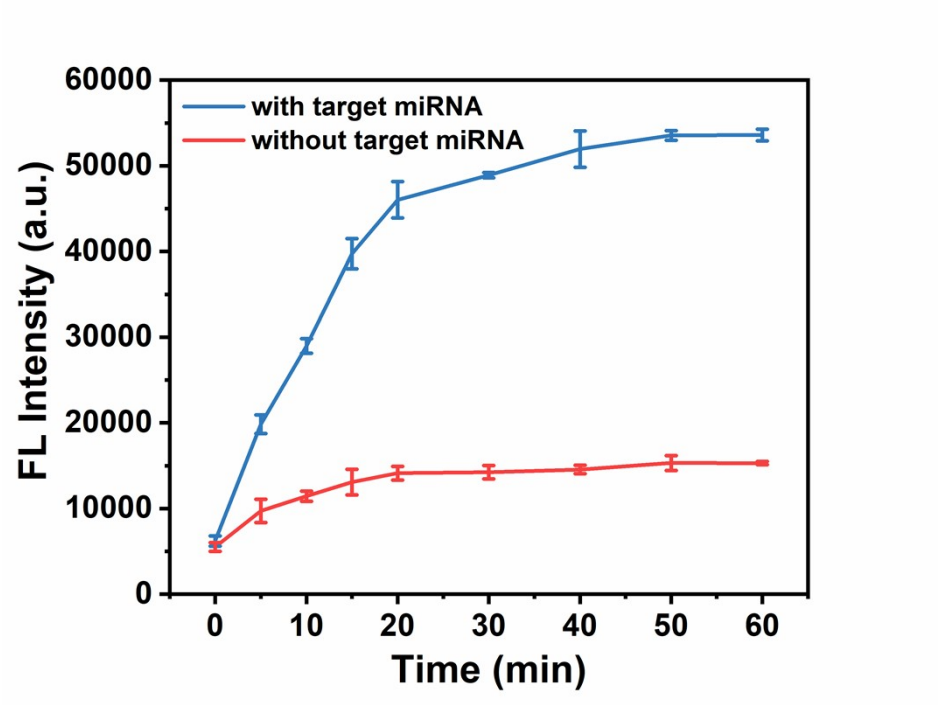

Fig. S1 Time-dependent fluorescence intensity release curves of DNA-gated MOFs probes with or without target miRNA.

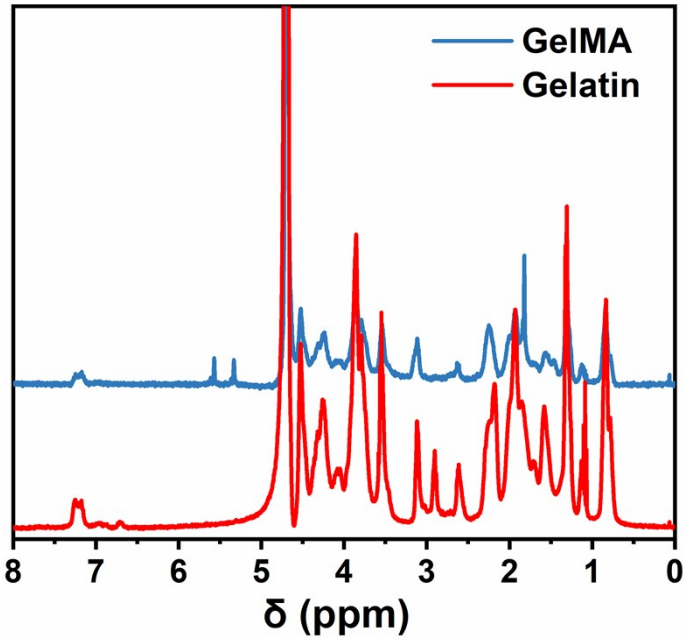

Fig. S2 ^{1}H-NMR spectrum of Gelatin and GelMA.

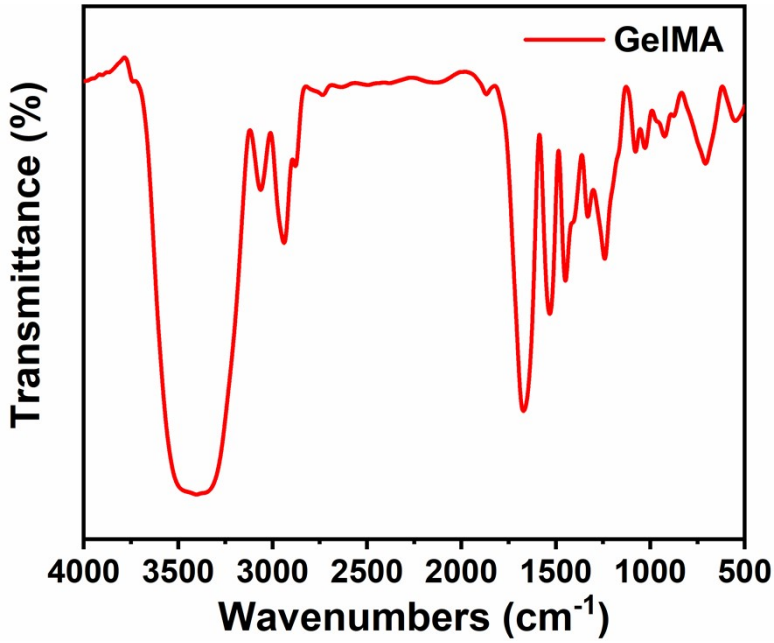

Fig. S3 FT-IR spectrum of GelMA.

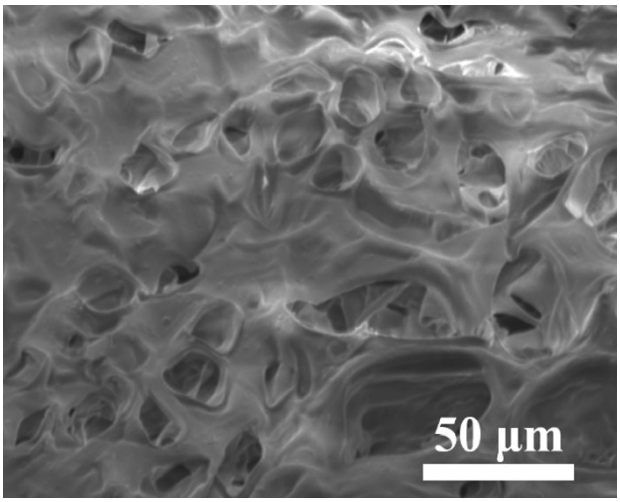

Fig. S4 SEM image of the freeze-dried GelMA hydrogel.

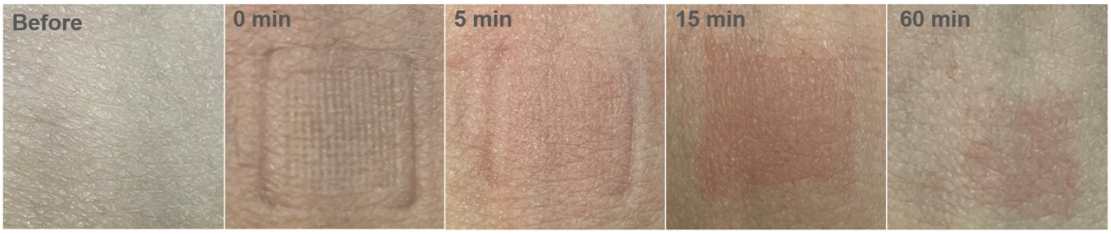

Fig. S5 Time-lapse images showing the condition of a volunteer's skin following the application of the MNs patch.

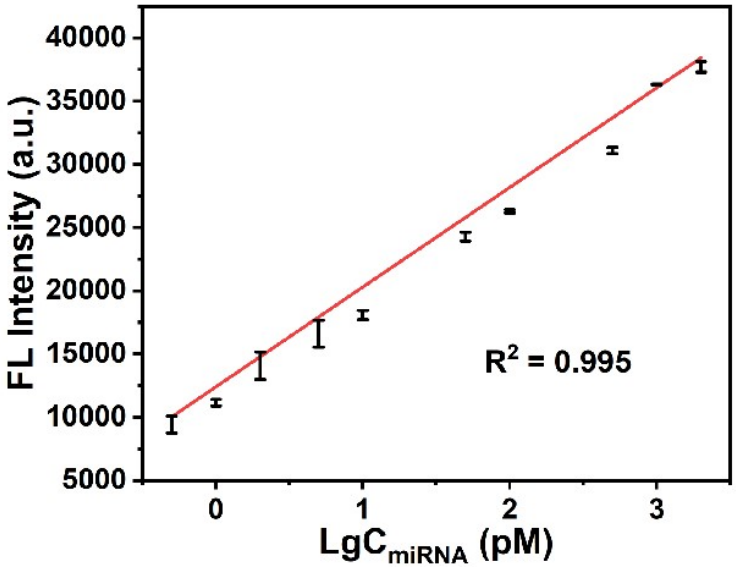

Fig. S6 The logarithmic calibration curves for miRNA concentration based on fluorescence intensity at 553 nm

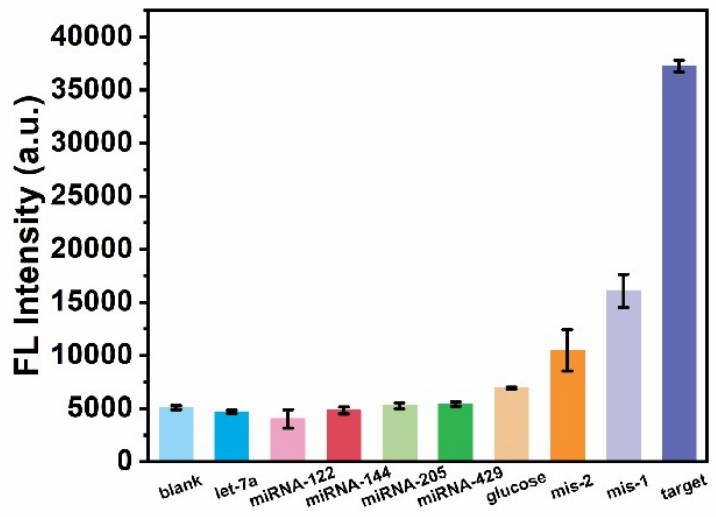

Fig. S7 Selectivity of the fluorescence-mode biosensor towards the target miRNA and other interferentst.

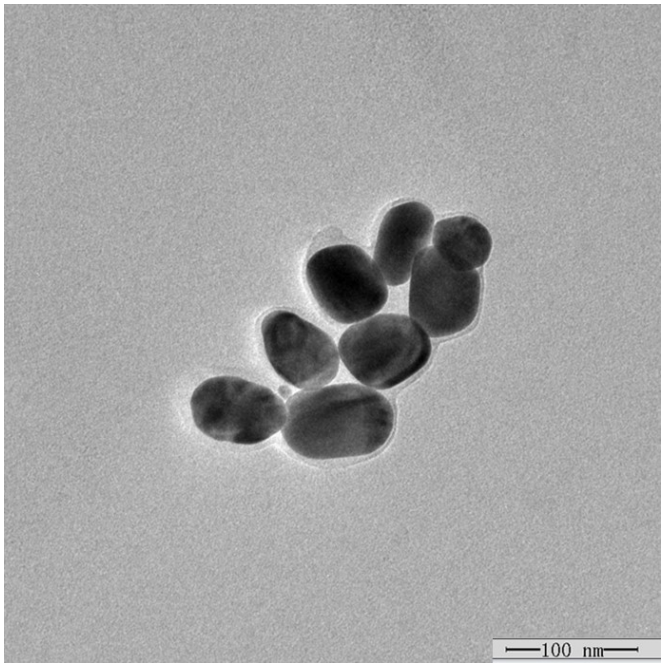

Fig. S8 TEM of the gold nanoparticles of the SERS substrate.

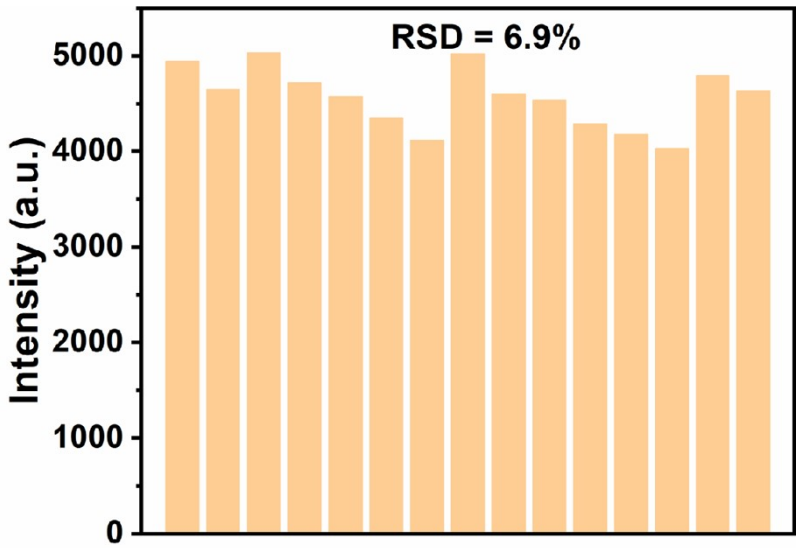

Fig. S9 SERS intensity of R6G and the RSD of the specific Raman peak at 1647 cm^{-1}.

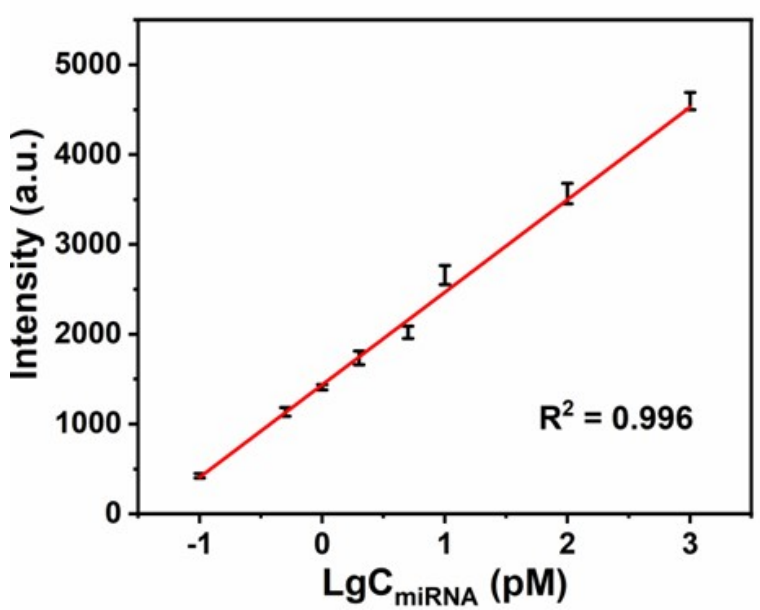

Fig. S10 The logarithmic calibration curves for miRNA concentration based on SERS intensity at 1647 cm^{-1}.

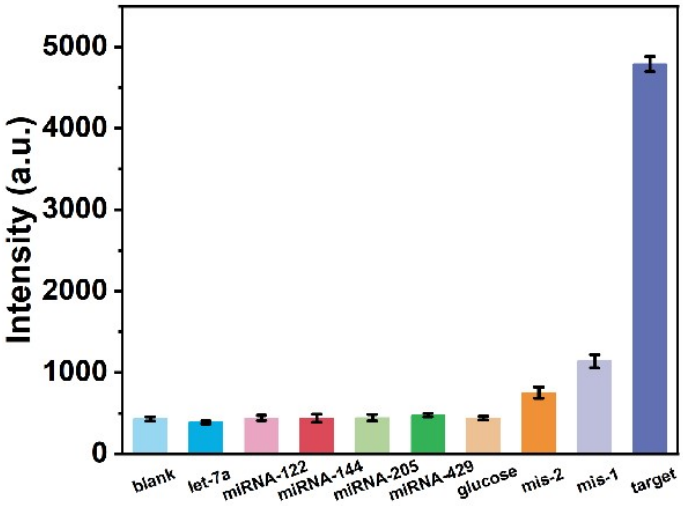

Fig. S11 Selectivity of the SERS-mode biosensor towards the target miRNA and other interferentst..

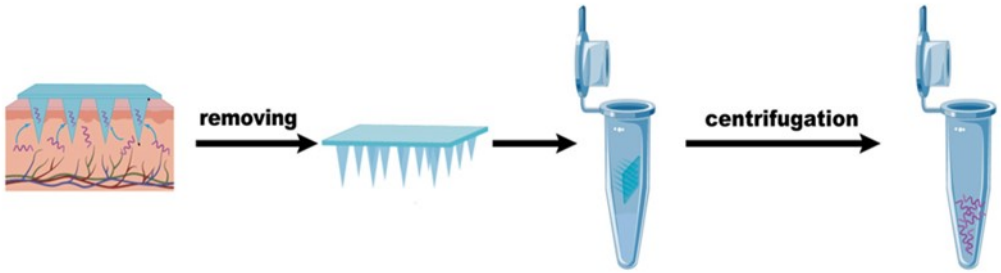

Fig. S12 Schematic illustration of microneedle-based miRNA extraction.

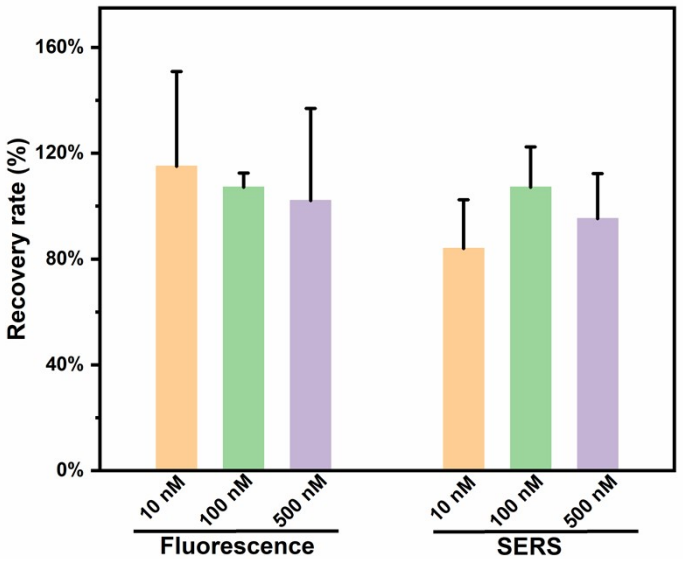

Fig. S13 Recovery rates of target miRNA in skin models under fluorescence-SERS detection.

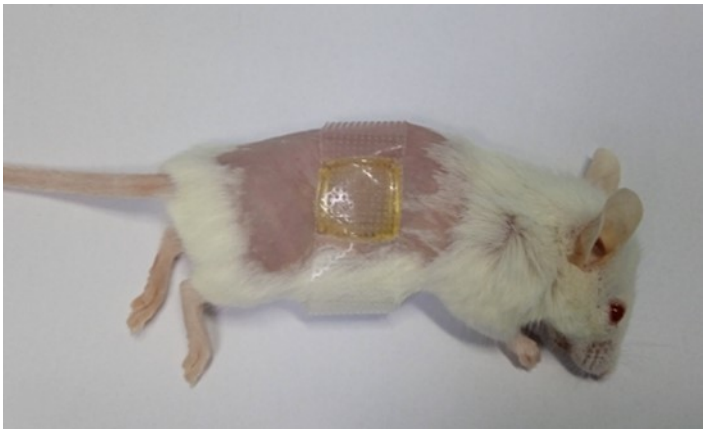

Fig. S14 Mice applied with microneedle patches.

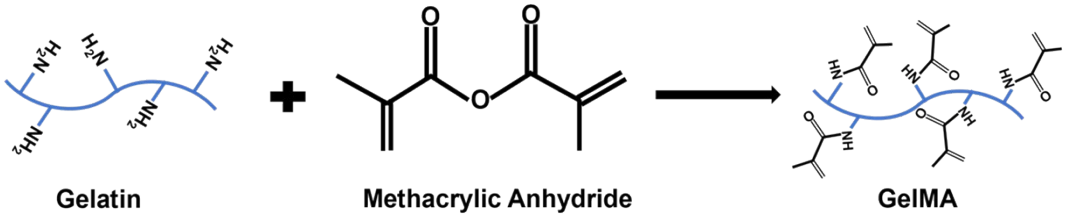

Fig. S15 The synthesis mechanism of GelMA.

References

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2. Y. Li, F. Zhang, W. Liu, M. Shao, Z. Hao, H. Zhang, R. Zhang, X. Li and L. Zhang, *Chem. Commun.*, 2023, **59**, 5431-5434.
3. J. Zhu, X. Zhou, H.-J. Kim, M. Qu, X. Jiang, K. Lee, L. Ren, Q. Wu, C. Wang, X. Zhu, P. Tebon, S. Zhang, J. Lee, N. Ashammakhi, S. Ahadian, M. R. Dokmeci, Z. Gu, W. Sun and A. Khademhosseini, *Small*, 2020, **16**, 1905910.