Supporting Information

Single cell multi-miRNAs quantification with hydrogel microbeads for liver cancer cell subtypes discrimination

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**Experimental Section**

**Materials & Reagents**

SG-2506 borosilicate glass was purchased from Changsha Shaoguang Chrome Blank Co., Ltd. Dechroming liquid contained 20% (m/v) Ce(NH$_4$)$_2$(NO$_3$)$_6$ and 3.5% (v/v) CH$_3$COOH. Etching solution contained 1.86% (m/v) NH$_4$F, 4.64% (v/v) HNO$_3$ and 5.00% (v/v) HF. SYLGARD™ 184 silicone elastomer kit for polydimethylsiloxane (PDMS) was purchased from Dow Corning (Midland, MI). Hexadecane was purchased from 3A Chemicals Co., Ltd. (Shanghai, China). Phi29 DNA polymerase, ssDNA/RNA CircLigase™ were purchased from HaiGene Biotech Co., Ltd. (Haerbin, China). Exonuclease I and dNTPs were purchased from New England Biolabs Ltd. (UK). Poly(ethylene glycol) diacrylate (MW 700) (PEG-DA-700) and 2-hydroxy-2-methyl-propiophenone (photo initiator) were purchased from Sigma-Aldrich (U.S.A.). Azide functionalized PEG-acrylate (N$_3$-PEG-acrylate, MW 2000) and poly(ethylene glycol) diacrylate (MW 3400) (PEG-DA-3400) were purchased from ToYong Biotech. Inc. (Shanghai, China). Tris buffer was prepared as aqueous solution containing 10 mM Tris-HCl, 5 mM MgCl$_2$, 100 mM KCl, and 10 mM NaCl at pH = 8.0. MES buffer was prepared as aqueous solution containing 30 mM MES and 0.05% (v/v) Tween 20 at pH = 6.0. Phosphate buffered saline (PBS) was prepared as aqueous solution containing 150 mM NaCl, 3 mM KCl, 10 mM Na$_2$HPO$_4$, 1.5 mM KH$_2$PO$_4$ and 5 mM MgCl$_2$ at pH = 7.4. Cell lysis buffer was prepared as aqueous solution containing 0.05% Tween-20 (v/v), 2% (w/v) sodium dodecyl sulfate (SDS), 200 μg/mL proteinase K, 350 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA at pH = 8.0. All other reagents were of analytical grade. All aqueous solutions were prepared using ultrapure water ($\geq$ 18 MΩ, Milli-Q, Millipore). The microRNAs were obtained from GenePharma Co., Ltd. (Shanghai, China). All oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and purified by high-performance liquid chromatography. Sequences of the microRNAs and oligonucleotides were listed as follows. DEPC (Diethy pyrocarbonate) treated water was used in the throughout process of miRNA operation.

<table>
<thead>
<tr>
<th>Oligonucleotides</th>
<th>Oligonucleotide sequences (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA-21</td>
<td>UAG CUU AUC AGA CUG AUG UUG A</td>
</tr>
<tr>
<td>H1-21</td>
<td>TCC TAA AGC ATG ACC TTC CGT TTC AAA A AA A AAA A AAA TCA ACA TCA GTC TGA TAA GCT AGT GGT GTA TAG CTT ATC AGC</td>
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<td></td>
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</tr>
<tr>
<td><strong>H2-21</strong></td>
<td>AGC TAT ACA CCA CTA GCT TAT CAG ACT GTG GTG TAA AAA AAA AAA AAC GTA GTT TCA AGC GCA GCC AGA TT</td>
</tr>
<tr>
<td><strong>miRNA-122</strong></td>
<td>UGG AGU GUG ACA AUG GUG UUU G</td>
</tr>
<tr>
<td><strong>H1-122</strong></td>
<td>TCC TAA AGC ATG ACC TTC CGT TTC AAA AAA AAA AAA CAA ACA CCA TTG TCA CAC TCC AGT GGT GTA TGG AGT GTG ACA GAC AC</td>
</tr>
<tr>
<td><strong>H2-122</strong></td>
<td>CTC CAT ACA CCA CTG GAG TGT GAC AAT GTG GTG TAA AAA AAA AAA AAC GTA GTT TCA AGC GCA GCC AGA TT</td>
</tr>
<tr>
<td><strong>miRNA-222</strong></td>
<td>AGC UAC AUC UGG CUA CUG GGU</td>
</tr>
<tr>
<td><strong>H1-222</strong></td>
<td>TCC TAA AGC ATG ACC TTC CGT TTC AAA AAA AAA AAA ACC CAG TAG CCA GAT GTA GCT GTG GTG TAA GCT ACA TCT GCC ATA A</td>
</tr>
<tr>
<td><strong>H2-222</strong></td>
<td>TAG CTT ACA CCA CAG CTA CAT CTG GCT AGT GTG GTA AAA AAA AAA AAA CGT AGT TTC AAG CGC AGC CAG ATT</td>
</tr>
<tr>
<td><strong>h1-21</strong></td>
<td>TCA ACA TCA GTC TGA TAA GCT AGT GTA TAG CTT ATC AGC CTC AGC</td>
</tr>
<tr>
<td><strong>h2-21</strong></td>
<td>AGC TAT ACA CCA CTA GCT TAT CAG ACT GTG GTG TA</td>
</tr>
<tr>
<td><strong>h1-122</strong></td>
<td>CAA ACA CCA TTG TCA CAC TCC AGT GGT GTA TGG AGT GTG ACA GAC AC</td>
</tr>
<tr>
<td><strong>h2-122</strong></td>
<td>CTC CAT ACA CCA CTG GAG TGT GAC AAT GTG GTG TA</td>
</tr>
<tr>
<td><strong>h1-222</strong></td>
<td>ACC CAG TAG CCA GAT GTA GCT GTG GTG TAA GCT ACA TCT GCC ATA A</td>
</tr>
<tr>
<td><strong>h2-222</strong></td>
<td>TAG CTT ACA CCA CAG CTA CAT CTG GCT AGT GTG GTA</td>
</tr>
<tr>
<td><strong>SQ-H2-21</strong></td>
<td>AGC TAT(-FAM) ACA CCA CTA GCT TAT CAG ACT GTG GTG TA(-BHQ1)A AAA AAA AAA AAA AAC GTA GTT TCA AGC GCA GCC AGA TT</td>
</tr>
<tr>
<td><strong>SQ-H2-122</strong></td>
<td>CTC CAT(-FAM) ACA CCA CTG GAG TGT GAC AAT GTG GTG TA(-BHQ1)A AAA AAA AAA AAA AAC GTA GTT TCA AGC GCA GCC AGA TT</td>
</tr>
<tr>
<td><strong>SQ-H2-222</strong></td>
<td>TAG CTT(-FAM) ACA CCA CAG CTA CAT CTG GCT AGT GTG GTA(-BHQ1)AAA AAA AAA AAA CGT AGT TTC AAG CGC AGC CAG ATT</td>
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<td><strong>FQ-H2-122</strong></td>
<td>CTC CAT(-TAMRA) ACA CCA CTG GAG TGT GAC AAT GTG GTG TA(-BHQ2)A AAA AAA AAA AAA AAC GTA GTT TCA AGC GCA GCC</td>
</tr>
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</table>
AGA TT

FQ-H2-222  TAG CTT(-Cy5) ACA CCA CAG CTA CAT CTG GCT AGT GGT GTA(-BHQ3) AAA AAA AAA AAA CGT AGT TTC AAG CGC AGC AGC CAG ATT

H1-21-FAM  TCC TAA AGC ATG ACC TTC CGT TTC AAA AAA AAA AAA TCA ACA TCA GTC TGA TAA GCT AGT GTA TAG CTT ATC AGC CTC AGC FAM

H2-21-Cy5  Cy5-AGC TAT ACA CCA CTA GCT ACT GTG GTG TAA AAA AAA AAA AAC GTA GTT TCA AGC GCA GCC AGA TT

DNA nanowire  AAT CTG GCT GCC CTT GAA ACT ACG AAA AAA AAA AAA GAA ACG GAA GGT CAT GCT TTA GGA AAA AAA AAA AAA

DNA nanowire-Cy3  Cy3-AAT CTG GCT GCC CTT GAA ACT ACG AAA AAA AAA AAA GAA ACG GAA GGT CAT GCT TTA GGA AAA AAA AAA AAA

DBCO-DNA nanowire  AAT CTG GCT GCC CTT GAA ACT ACG AAA AAA AAA AAA GAA ACG GAA GGT CAT GCT TTA GGA AAA AAA AAA AAA

pre-circular21  P'-GT TCT TGC TGA GGC TGT TAT CTT GCT GAG GCT GAC GCA CCT TGC TGA GCC TGA TAG

reporter-21-TAMRA  TAMRA-CTT GCT GAG GCT G

pre-circular122  P'-CAT TTT GCA TAT AGT ACT CTG GTA TTT ATT GTC ATA TAG TGT CTA CGC ACT TGC ATA TAG TGT CTG TCA

reporter-122-FAM  FAM-TGT CAT ATA GTG TCT

pre-circular222  P'-TGT TAC GCA GTA TTA TGG TTA TAC GCA GTA TTA TGG ACG CAC ACG CAG TAT TAT GG

reporter-222-Cy5  Cy5- ACG CAG TAT TAT G

miRNA-21-M1  TAG CTT ATC AGA CTA ATG TTG A

miRNA-21-M3  TAG CTT ACC AGA CTA ATG TAG A

miRNA-122-M1  TGG AGT GCG ACA ATG GTG TTT G

miRNA-122-M3  TGG GGT GGC ACA ATG GTA TTT G

miRNA-222-M1  AGC TAC ATC TGG CTG CTG GGT

miRNA-222-M3  AGC AAC ACC TGG CTG CTG GGT

miRNA-21-FAM  FAM-TAG CTT ATC AGA CTG ATG TTG A

miRNA-155-TAMRA  TAMRA-TTA ATG CTA ATT GTG ATA GGG GT

AF405  acrydite-AAA AAA AAA AAA-Alexa Fluor 405

The hybridization regions of MCP components, H1-21(-122/-222), H2-21(-122/-222), and DNA nanowire were marked with the same color. The anchoring regions of pre-circular21(122/222) to
H1-21(-122/-222) were in italic, and the repeating units in pre-circular21(122/222) were underlined. The mismatched bases were in bold. SQ-H2-21, SQ-H2-122, SQ-H2-222, FQ-H2-122 and FQ-H2-222 were used to prepare self-quenched miRNAs capture probes (SQ-MCPs).

**Apparatus**

Gel electrophoresis was performed on PowerPac™ Basic electrophoresis analyzer (Bio-Rad, U.S.A.) and imaged on Biorad ChemiDoc XRS facility (Bio-Rad, U.S.A.). Fluorescence spectra were measured on F-7000 spectrometer (HITACHI, Japan). MALDI-TOF mass spectra were measured with a 4800 plus MALDI TOF/TOF analyzer (AB sciex, U.S.A.), and the data analysis was performed with a Data Explorer™ Software from AB Sciex (U.S.A.). FTIR spectra were measured with a Nicolet iS50 FT-IR Spectrometer (Thermo Fisher Scientific, U.S.A.) with the KBr pellet technique. SEM images were obtained by JSM-7800F Schottky Field Emission Scanning Electron Microscope (JEOL Ltd., Japan) at 5 kV. Glass slides were cleaned and activated by PDC-MG Oxygen Plasma Cleaner (Chengdu MINGHENG Science & Technology Co., Ltd., China). Microdroplets were generated by a home-made PDMS microfluidic chip pumped by FLOW-EZ (Fluigent, France). UV-induced polymerization of microdroplets was achieved via a UVLED (AVENTK, Shanghai, China). The bright field images of hydrogel microbeads were captured by an Ti-U inverted fluorescence microscope (Nikon, Japan). Quantification of miRNAs in hydrogel microbeads was performed via confocal microscopy (Leica TCS SP5, 63× objective with 1.4 numerical aperture, oil immersion). Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed with Applied Biosystems StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, U.S.A.).

**Preparation of MCPs-PEG-acrylate**

microRNA-21 (miRNA-21) specific capture probe (MCP-21) was prepared via DNA self-assembly. 5 μM miRNA-21 responsive hairpin H1 (H1-21), the corresponding hairpin H2 (H2-21) for catalytic hairpin assembly (CHA) reaction, and DNA nanowire were annealed by heating to 95°C for 4 min, and slowly cooled to room temperature over 2 h respectively. Equal molar of H1-21, H2-21 and DNA nanowire were then mixed and incubated at 37°C for 2 h to obtain MCP-21. DBCO labelled DNA nanowire (DBCO-DNA nanowire) were hybridized with H1-21, H2-21 to
obtain DBCO-MCP-21. 100 μL 14 mM N3-PEG-acrylate aqueous solution was mixed with 360 μL as-prepared DBCO-MCP-21 and incubated at 4°C for 1 h to conjugate DBCO-MCP-21 to N3-PEG-acrylate via click reaction, and obtained MCP-21 functionalized PEG-acrylate (MCP-21-PEG-acrylate). MCP-122-PEG-acrylate, MCP-222-PEG-acrylate were prepared respectively according to the same procedure with H1/H2-122, H1/H2-222, DBCO-DNA nanowire, and N3-PEG-acrylate.

Polyacrylamide gel electrophoresis (PAGE) analysis

10% native polyacrylamide gel was prepared using 5× TBE buffer and loaded with 3 μL DNA sample containing 0.5 μL 6× loading dye. The gel electrophoresis was run at 110 V for 85 min in 1× TBE buffer, and subsequently stained with SYBR™ Gold and scanned with Biorad ChemiDoc XRS facility (Bio-Rad, USA).

Agarose Gel Electrophoresis analysis

400 mg agarose powder was dissolved in 40 mL 1× TBE buffer and heated to prepare 1% agarose solution. The heated agarose solution was poured into a gel mold and cooled to room temperature to form a 1% agarose gel. The gel was transferred to an electrophoresis chamber bathed in 1× TBE buffer and loaded with 5 μL DNA sample containing 0.5 μL 6× loading dye. The gel electrophoresis was run at 100 V for 60 min in 1× TBE buffer, and subsequently stained with SYBR™ Gold and scanned with Biorad ChemiDoc XRS facility (Bio-Rad, USA).

Cell culture

Human liver cancer cell lines HepG2, HCCLM3, and MHCC-97L were obtained from KeyGEN Biotech (Nanjing, China) and cultured at 37 °C in Dulbecco’s modified Eagle’s medium (DMEM, KeyGEN KGM12800-500) supplemented with 10% FBS in a humidified incubator containing 5% CO2 and 95% air. Human normal liver HHL-5 cells were purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China) and cultured at 37 °C in Dulbecco’s modified Eagle’s medium (DMEM, GIBCO C1965500BT) supplemented with 10% FBS in a humidified incubator containing 5% CO2 and 95% air. Cell numbers were determined with a Petroff-Hausser cell counter (U.S.A.).
qRT-PCR for miRNA-21, 122, and 222 quantification from cell lysis

Total RNAs were extracted from HepG2, HCCLM3, MHCC-97L and HHL-5 cells respectively using RNAiso Plus Trizol reagent (TaKaRa, China). cDNAs were prepared following cDNA synthesis kit operation instruction and amplified with PCR. Standard calibration curves were constructed with cycle threshold (Ct) values of different concentrations of synthetic miRNA-21, miRNA-122 and miRNA-222, and the expression levels of cellular extracted miRNA-21, miRNA-122 and miRNA-222 were calculated by comparing with their respective standard calibration curve.

Hydrogel microbeads preparation

The water-in-oil microdroplets were prepared with microfluidic chip. To prepare the microfluidic chip, a photomask (Shenzhen Newway Photomask Making Co., Ltd., China) was used to transfer the designed flow focused pattern (200 μm wide) onto the borosilicate glass substrate by 15 seconds UV exposure following soft lithography program. The glass substrate was sequentially immersed in developer (0.5% (w/w) NaOH aqueous solution) for 1 minute, dechroming liquid for 5 minutes and etching solution for 90 minutes at room temperature. After washing with ultrapure water and drying at 80°C for 1 h, the glass mould with designed microchannel pattern was achieved. A PDMS replica of the as-prepared mould was formed by mixing the PDMS oligomer and curing agent at a ratio of 10:1 (w/w) and curing the homogeneous, degassed mixture at 80°C for 90 min. The PDMS replica and a glass slide were then sealed using an Oxygen Plasma Cleaner. The water-in-oil microdroplets generation system contained oil phase of hexadecane with 4% (v/v) EM90 surfactant and two aqueous phases. One of the aqueous phases was composed of 10% (v/v) PEG-DA-700, 10% (v/v) of 20 mM PEG-DA-3400, 1% (v/v) photo initiator, and 300 nM MCP-21-PEG-acrylate, MCP-122-PEG-acrylate, and MCP-222-PEG-acrylate in DI water. Another aqueous phase contained various concentrations of miRNA-21, miRNA-122, and miRNA-222 or cell suspension. Two aqueous phases were converged in a flow focused microchannel and sheared into microdroplets by oil phase at the intersection point. The pressure for phases flow was set as 250 mbar for oil phase and 150 mbar for aqueous phases. In the droplet microenvironment, miRNA-21(122/222) activated MCP-21(122/222)-PEG-acrylate by triggering continuous CHA
reactions, and exposed single strand DNA region in MCPs for subsequent Rolling circle amplification (RCA) reactions. Water-in-oil microdroplets production was observed with an ECLIPSE TS100 microscope (Nikon) equipped with 4×, 10×, 20× and 40× objective lenses (air) and a FASTEC IL-5 high-speed camera, and recorded for analysis by Fastec FasMotion software. The as-prepared water-in-oil microdroplets were collected in a centrifuge tube and polymerized to get hydrogel microbeads under 50 s UV irradiation (365 nm, 75 mW/cm²). The miRNA activated MCP-21(122/222)-PEG-acrylate were covalently immobilized in the scaffolds of hydrogel microbeads during photo-polymerization process. After centrifugation to remove the supernatant, the as-obtained hydrogel microbeads were washed three times with 500 µL MES buffer and stored in 4°C for further signal amplification reaction.

Specific miRNA responsive signal amplification in hydrogel microbeads

Various concentration of miRNAs ranging from 100 fM to 50 nM were fused in the aqueous phase and encapsulated in the water-in-oil microdroplets. miRNA was repeatedly used in CHA reaction to activate MCP, which pre-amplified miRNA signal in water-in-oil microdroplets before the formation of hydrogel microbeads. RCA was performed for further signal amplification in hydrogel microbeads. To prepare circular template for RCA, 1 µL 10 µM phosphorylated pre-circular was added into a mixed solution of 15 µL H2O, 2 µL 10× CircLigase buffer™, 1 µL 50 mM MnCl2, and 1 µL single strand DNA circligase (100 U). The solution was kept at 60°C for 1 h followed by enzyme deactivation at 80°C for 10 min. 1 µL Exonuclease I (20 U), 3 µL 10× Exonuclease I buffer and 6 µL H2O were then added and incubated at 37°C for 1 h to remove the unreacted pre-circular followed by enzyme deactivation at 80°C for 20 min. The as-obtained circular template was confirmed by gel electrophoresis and stored at –20°C for further use.

miRNA activated MCP and exposed a single strand DNA region which was used as primer for RCA reaction. 50 µL above obtained circular template was added to resuspend 10⁵ hydrogel microbeads and incubated at 37°C for 2 h to ligate to the exposed single strand DNA region in MCP. The hydrogel microbeads were washed three times with MES buffer, re-dispersed in 50 µL 1× phi29 buffer containing 0.2 mg/mL BSA, 1 mM dNTPs, 25 U phi29 DNA polymerase, and kept at 30°C overnight to perform RCA reaction inside hydrogel microbeads. After RCA reaction, the hydrogel microbeads were centrifuged and washed with Tris buffer for three times.
RCA products resulted from activated MCP-21, MCP-122, and MCP-222 were labelled with TAMRA, FAM, and Cy5 respectively by hybridizing with dyes labeled reporter DNA strands. 40 μL Tris buffer containing 1 μM reporter-21-TAMRA, reporter-122-FAM and reporter-222-Cy5 was mixed with the above-obtained hydrogel microbeads and incubated at 37°C for 2 h. The fluorophore labeled hydrogel microbeads were centrifuged, washed with Tris buffer three times, and stored at 4°C for further fluorescent measurement.

To quantify miRNAs from single cell encapsulated hydrogel microbeads, 200 μL cell lysis buffer was added to resuspend hydrogel microbeads and incubated for 90 min at 42°C at 400 rpm in a thermosthaker. The cells encapsulated in hydrogel microbeads were lysed and the intracellular miRNA-21, miRNA-122, miRNA-222 were simultaneously captured and retained in hydrogel microbeads by MCP-21, MCP-122, and MCP-222 which immobilized in the scaffold of hydrogel microbeads respectively. The corresponding fluorescence signal for miRNA-21, miRNA-122, miRNA-222 were then amplified according to the same reaction procedure described above.

To prove the capability of this method in cell sub-populations discrimination for mixture cells, liver cell lines HepG2, HCCLM3, MHCC97L and HHL-5 cells were pre-mixed equally and imported into one aqueous phase inlet of microfluidic chip for generation of single cell encapsulated hydrogel microbeads. After intra-beads signal amplification, the corresponding fluorescence signal for TAMRA, FAM and Cy5 were collected and converted to miRNA-21, miRNA-122, miRNA-222 copy numbers to present in 3D scatter profile with t-SNE distribution analysis.

**Imaging and quantification from hydrogel microbeads**

After performing signal amplification reaction in hydrogel microbeads, confocal microscopy (Leica TCS SP5, 63× objective with 1.4 numerical aperture, oil immersion) was performed to visualize and quantify miRNAs captured inside hydrogel microbeads. miRNAs were quantified by integrating fluorescence generated from reporter DNA strands in hydrogel microbeads. The hydrogel microbead was scanned with 2-μm z resolution to get a stack of 30 slices, the maximum intensity projection of the stacks was acquired by 3D process and quantified with ImageJ to obtain the integrated fluorescent intensity from the whole hydrogel microbead. TAMRA fluorescence was visualized from 555 to 605 nm under 543 nm excitation to quantify miRNA-21 amount. FAM
fluorescence was visualized from 500 to 540 nm under 488 nm excitation to quantify miRNA-122 amount. Cy5 fluorescence was visualized from 650 to 690 nm under 633 nm excitation to quantify miRNA-222 amount.

**t-SNE analysis**

Single-cell multi-miRNAs quantifications were visualized in a two-dimensional map using t-Distributed Stochastic Neighbor Embedding (t-SNE) technique by python. Three-dimensional Euclidean distances between datapoints in 3D scatter profile were converted into conditional probabilities that represent similarities. As t-SNE is a variation of Stochastic Neighbor Embedding (SNE), a symmetrized version of the SNE cost function with simpler gradients was used. The Gaussian was replaced by the Student-t distribution to compute the similarity between two points in the low-dimensional space. Results were optimized by applying different learning rate (lr) and perplexity values in t-SNE analysis algorithm to get the best visualization of data points clustering in low-dimensional map.
Figure S1. (A) Design and (B) photograph of microfluidic chip with flow focused pattern, and (C) set up of microfluidic system with the microfluidic chip, FLOW-EZ pump for droplets generation, and microscope for observation.
Figure S2. Size distribution of hydrogel microbeads.
Figure S3. (A) Schematic illustration and (B) PAGE analysis of MCP synthesis. Lane 1-6 represent DNA nanowire, H1-21, H2-21, MCP-21, MCP-122 and MCP-222 respectively. (C) Standard calibration curves for DNA nanowire-Cy3, H1-21-FAM, and H2-21-Cy5. Inset: fluorescence spectra of tri-color MCP-21 at λ_ex of 525 nm, 470 nm, and 605 nm respectively. The data error bars indicated means ± S.D. (n=3).
Figure S4. Schematic illustration of SQ-MCP activation.
Figure S5. PAGE characterizations of circular templates preparations for RCA. lane 1-6: pre-circular21, circular-21, pre-circular122, circular-122, pre-circular222, circular-222.
Figure S6. Diameters of MCP-hydrogel microbeads dispersed in DI water and different reaction buffer of MES, Tris, and PBS over 8 days.
Figure S7. Fluorescence and bright images of SQ-MCP-21, SQ-MCP-122, SQ-MCP-222 co-functionalized hydrogel microbeads in response to 100 nM (A) miRNA-21, (B) miRNA-122, and (C) miRNA-222 respectively (scale bar: 10 μm).
Figure S8. Fluorescence images of single MCP functionalized hydrogel microbeads in response to 1 nM miRNA-21, miRNA-122, miRNA-222, 1-mismatched corresponding target miRNA (miRNA-M1) and 3-mismatched corresponding target miRNA (miRNA-M3) (scale bar: 10 μm).
Figure S9. (A) Fluorescence spectra and (B) Quantitative real-time fluorescence response profiling of supernatant collected from miRNA-21-FAM infused hydrogel microbeads functionalized with MCP-21 and unfunctionalized hydrogel microbeads, as well as the blank hydrogel microbeads without miRNA-21-FAM encapsulation. (C) Cy5 and FAM fluorescence intensities of MCP-21-Cy5 functionalized hydrogel microbeads (MCP-21-Cy5-microbeads) and MCP-21 functionalized hydrogel microbeads infused with miRNA-21-FAM (MCP-21-microbeads). The error bars were collected from 15 hydrogel microbeads of 3 different experiments and indicated means ± S.D. Inset: confocal image of MCP-21-Cy5-microbead (red) and 500 pM miRNA-21-FAM encapsulated MCP-21-microbead (blue) after co-incubation (scale bar: 20 μm).
Figure S10. Standard calibration curves for miRNA-155-TAMRA. Inset: fluorescence spectra of concentrated supernatant at λex of 525 nm. The data error bars indicated means ± SD (n=3).
Figure S11. Fluorescence and bright images of single cell encapsulated MCPs-functionalized hydrogel microbeads and unencapsulated beads after signal amplification (scale bar: 50 μm).
Figure S12. RT-PCR calibration curves of miRNA-21, miRNA-122 and miRNA-222. Quantitative real-time fluorescence response profiling of (A) miRNA-21, (C) miRNA-122 and (E) miRNA-222 at different concentrations. And plots of the $C_T$ values versus the logarithm value of concentrations of (B) miRNA-21, (D) miRNA-122 and (F) miRNA-222. The data error bars indicated means ± S.D. (n=3)
Figure S13. Quantitative real-time fluorescence response profiling of (A) miRNA-21, (B) miRNA-122 and (C) miRNA-222 intracellular expressions in HepG2, HCCLM3, MHCC97L, and HHL-5 cell lines. Comparisons of (D) miRNA-21, (E) miRNA-122, (F) miRNA-222 intracellular expressions determined from hydrogel microbeads and RT-PCR. The error bars for microbead were collected from 100 single cell encapsulated hydrogel microbeads and indicate means ± S.D. The error bars for RT-PCR indicated means ± S.D.
Figure S14. t-SNE clustering of single cell encapsulated hydrogel microbead for HepG2, HCCLM3, MHCC97L, and HHL-5 cell lines. Data from each cell line were collected individually. 100 single cell encapsulated hydrogel microbeads were analyzed per cell line.
Figure S15. t-SNE analysis results with different parameter sets of learning rate (lr) and perplexity values. Learning rate (lr) and perplexity set values were set at (A) 400, 50; (B) 450, 40; (C) 450, 60; (D) 500, 40; (E) 600, 40; (F) 650, 40; (G) 700, 55; (H) 750, 40 and (I) 900, 40.