Supporting Information

Stimuli-Responsive Hydrogel Microcapsules for the Amplified Detection of MicroRNAs

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Table S1. The nucleic acid sequences used in this study.

No.	Sequence (5'→3')
1	/5AmMC6/ TTTTTTAGCTGATAACACTG
2	/5Acryd/TTTTTTTTTAACACTGtCCATCTTTACCAGACAGTGTTATCAGCT
3	/5Acryd/ TGCTCTAGATCTGGTTG
4	AAGATGGACAGTGAGCTGATAACACTGTCAACCAGATCTAGAGC
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-200a	UAACACUGUCUGGUAACGAUGU
miR-200b	UAAUACUGCCUGGUAAUGAUGA
miR-21	UAGCUUAUCAGACUGAUGUUGA
*rfb*E	GGCCAAGGATTAGCTGTACAT
HP	TCCACTCTCGACCTGTCCATCTTTACCAGACAGTGTTATCAGCTGAGGTCGAGAGTGGCTT
ASHP	GAGAGTGCCACTCTCGACC

*MiR-200a, miR-200b, and miR-21 were used in the sequence selectivity tests. Underline indicates the nucleotides which are different from miR-141.

Determination of the Ratio of Acrylamide/Acrydite-Nucleic Acids

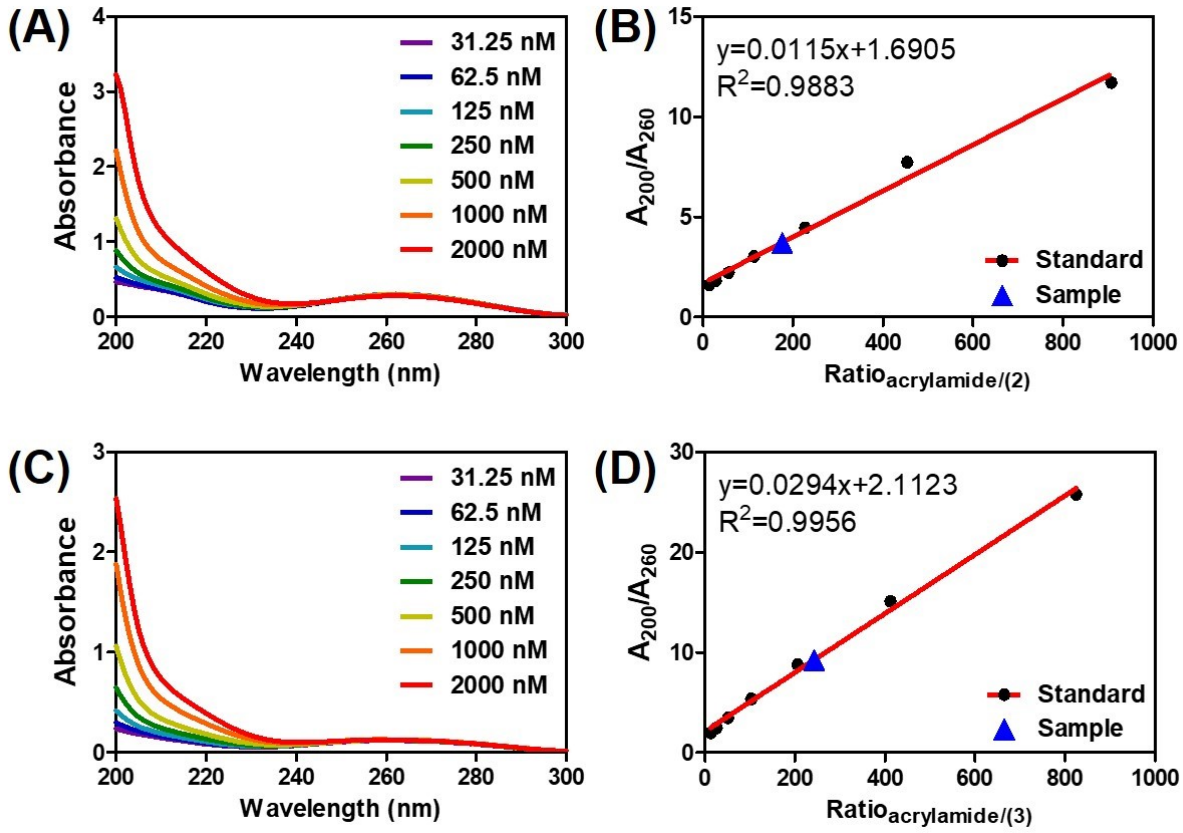

Figure S1. (A) Absorbance spectra of a fixed 5 μM nucleic acid (**2**) with different concentrations of acrylamides. (B) Calibration curve from absorbance ratio of acrylamide (150 kDa, *ca*. 2110 monomers) to nucleic acid (A_{200}/A_{260}) under different ratios of acrylamide/nucleic acid (**2**). The ratio of acrylamide units to nucleic acid (**2**) was determined spectroscopically to be 176. (C) Absorbance spectra of a fixed 5 μM nucleic acid (**3**) with different concentrations of acrylamides. (D) Calibration curve from absorbance ratio of acrylamide (150 kDa, *ca*. 2110 monomers) to nucleic acid (A_{200}/A_{260}) under different ratios of acrylamide/nucleic acid (**3**). The ratio of acrylamide units to nucleic acid (**3**) was determined spectroscopically to be 243.

Characterization of the Microparticles by SEM and EDX Analysis

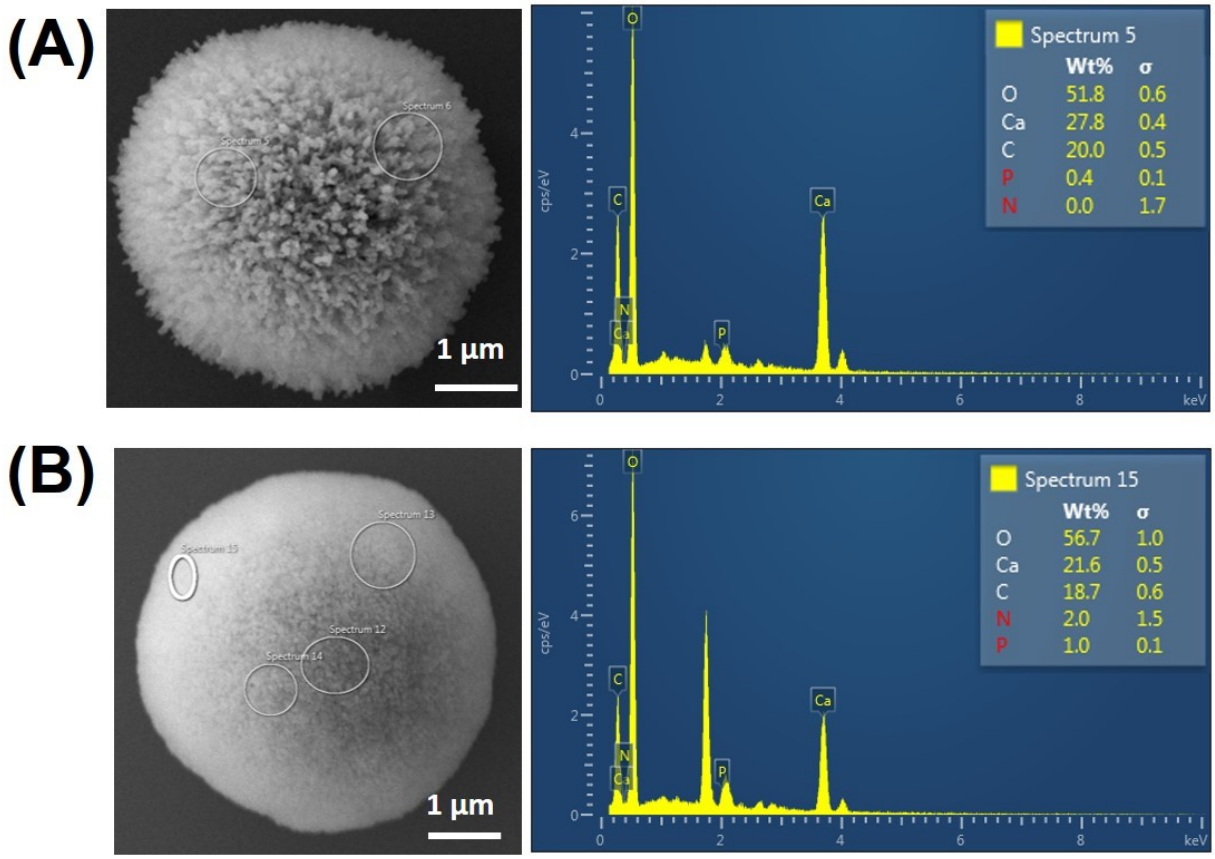

Figure S2. SEM images and EDX results of uncoated $CaCO_3$ microparticles (A) and DNA–acrylamide hydrogel-coated $CaCO_3$ microparticles (B). Scale bars: 1 µm. The EDX results shown higher N and P percentages on DNA–acrylamide hydrogel coated $CaCO_3$ microparticles indicates the modification of DNA hydrogel on particles surface.

Characterization of the DNA–Acrylamide Hydrogel Microcapsules by SEM

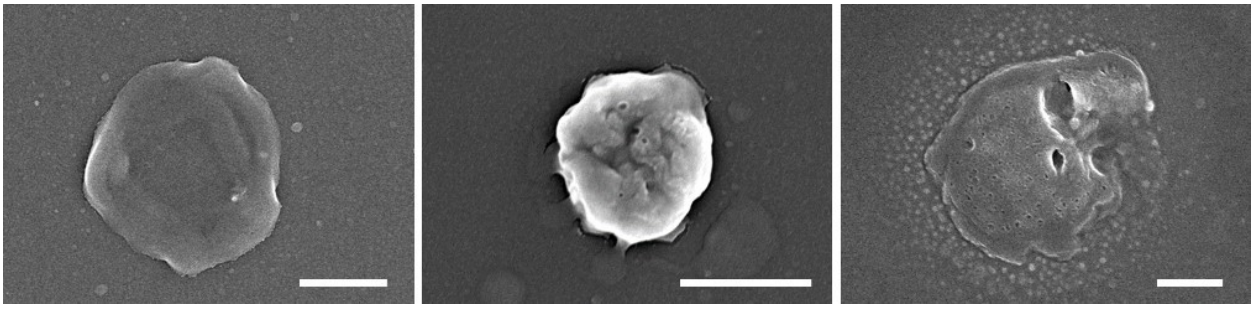

Figure S3. Representative SEM images of DNA–acrylamide hydrogel microcapsules after the etching of the $CaCO_3$ core. Scale bar: 1 μm.

Sequences Design for Isothermal Strand Displacement Polymerization/Nicking Amplification Machinery (SDP/NA)

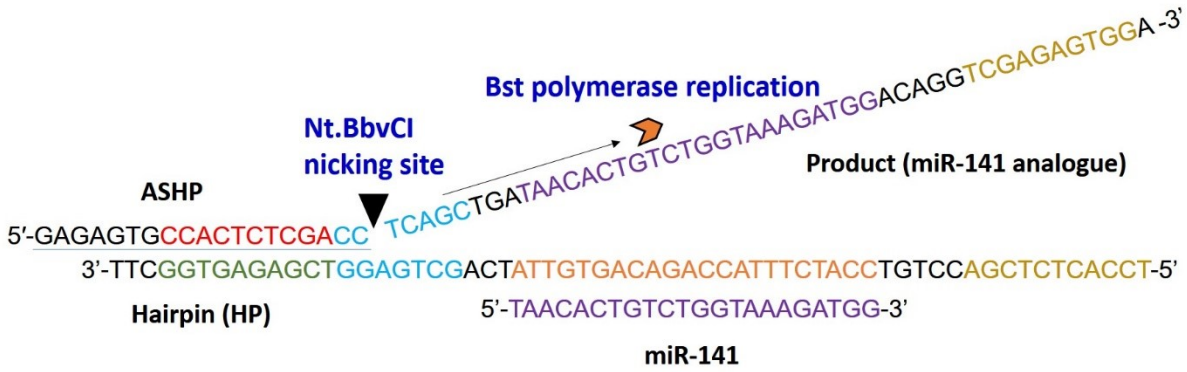

Figure S4. Schematic representation of the sequence replicated by the *Bst* polymerase and the nicking site of the Nt.BbvCI nicking endonuclease involved in the isothermal strand displacement polymerization/nicking amplification machinery (SDP/NA). The sequence with underline represents an auxiliary hairpin DNA strand (ASHP), it can bind to the open hairpin (HP) and acts as a primer for polymerase replication. The symbol (▼) indicates Nt.BbvCI nicking site. MiR-141 analogue is the product of strand-displacement amplification.

Characterization of the SDP/NA Products by Gel Electrophoresis

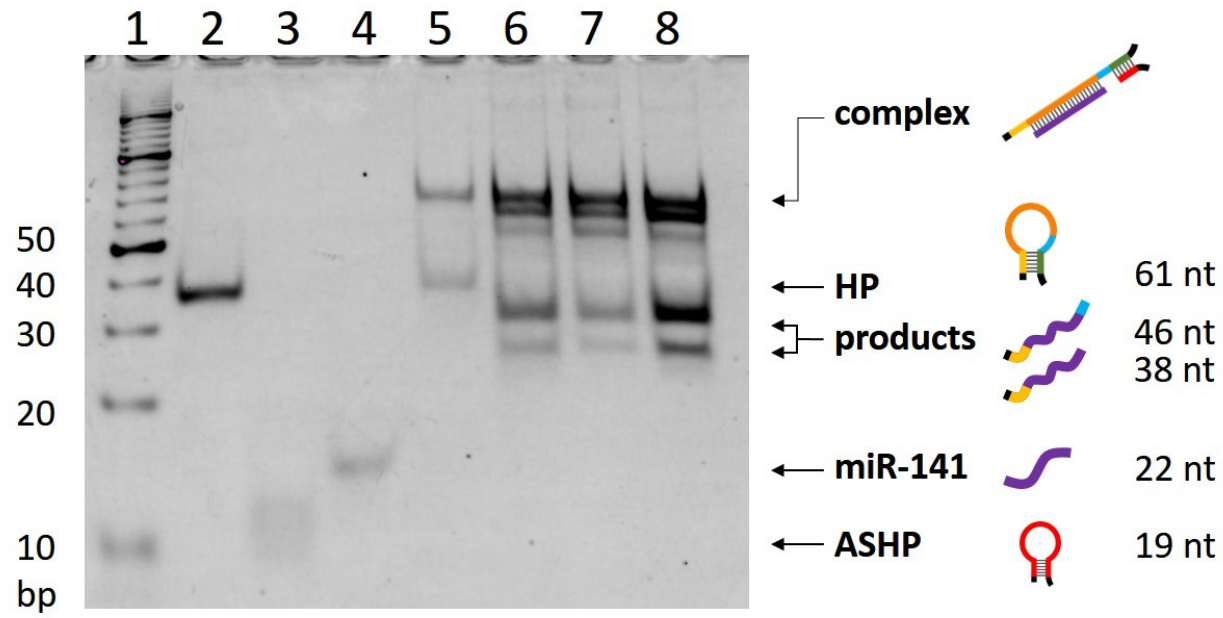

Figure S5. Native polyacrylamide gel electrophoresis image corresponding to isothermal strand displacement polymerization/nicking amplification machinery (SDP/NA) products. Lane 1: DNA marker; Lane 2: hairpin DNA (HP); Lane 3: auxiliary hairpin DNA strand (ASHP); Lane 4: mir-141; Lane 5: SDP/NA reaction 15 min without miR-141; Lane 6: SDP/NA reaction 15 min with 50 nM miR-141; Lane 7: SDP/NA reaction 30 min without miR-141; Lane 8: SDP/NA reaction 30 min with 50 nM miR-141. 12 % of native polyacrylamide gel.

Table S2. Comparison of different amplification mechanisms based on oligonucleotide for miRNA detection.

Target	Method	Amplification mechanism	Detection limit	Linear range	Assay time	Remarks	Ref.
miR-21 miR-141	Fluorescence	DNA polymerase/NEase-assisted signal amplification	9.8 pM (miR-21) 6.1 pM (miR-141)	2×10^{-11} to 5×10^{-10} M	22 h	Integration with silver nanoclusters (AgNCs) for simultaneously detecting dual targets; study in spiked serum samples.	1
miR-21	Fluorescence	Strand displacement reaction	4.4 pM	10^{-12} to 2×10^{-10} M	6 h	Integration with dye-loaded poly(ethylmethacrylate)-based polymer nanoparticles; cell lysate study.	2
miR-20a	Fluorescence	Catalytic hairpin assembly (CHA) reaction	0.491 pM	5×10^{-13} to 2×10^{-10} M	5 h	Integration with Fe_3O_4 nanoparticles cross-linked carbon (Fe_3O_4@C); study in cell culture samples.	3
miR-141	Fluorescence	Duplex-specific nuclease (DSN) and telomerase amplification	0.28 pM	10^{-12} to 10^{-7} M	5 h	Integration with CdSe/ZnS quantum dots (QDs); serum sample study. However, 55°C heating process is needed.	4
miR-203	Fluorescence	DNA chain displacement recycle (CDR) reaction	10 pM	10^{-11} to 10^{-7} M	1.5-2 h	Integration with gold nanoparticles (AuNPs); study in cell culture samples.	5
miR-141	Fluorescence	Duplex-specific nuclease (DSN) amplification	0.42 pM	5×10^{-12} to 5×10^{-9} M	90 min	Integration with magnetic nanoparticles coated with poly-dopamine (MNPs@PDA); cell lysate study. However, 50°C heating process is needed.	6
miR-21	Fluorescence	λ exonuclease amplification	20 pM	2×10^{-11} to 5×10^{-9} M	70 min	Integration with graphene oxide (GO); spiked biological fluids and cell lysate study.	7
miR-141	Fluorescence	Isothermal strand displacement polymerization/nicking amplification machinery (SDP/NA)	44.9 pM	10^{-10} to 10^{-7} M	90 min	Integration with CdSe/ZnS quantum dots-loaded DNA hydrogel microcapsules; study in spiked serum samples; potential control-released drug carriers.	This study

*It should be noted that the present sensing platform reveals comparable sensitivity to other optical (fluorescence) assays for the detection of miRNA. Several advantages of the present method might include faster detection time-intervals and lack of susceptibility to environmental interferences that might accompany the same of the nanomaterial optical transducers (*e.g.* AgNCs, AuNPs or graphene oxide), such as thiols or dopamine. All sensing platforms, including ours, however, are far below the sensitivity demonstrated by the commercially available methods. Nonetheless, the progress and advances to improve the sensitivity of non-PCR sensing platforms by biocatalytic and DNA-machinery (and adaptation of highly-sensitive DNA machinery[8] and DNA biocatalytic circuitries for miRNA-sensing) are promising pathways to follow. Particularly, faster analytical procedures and the lack of auxiliary instruments demonstrated by these alternative methods could provide effective means for point-of-care detection and field-test analyses of miRNAs.

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