Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Dual-Functional Supramolecular Hydrogel Based on Spiropyran-Galactose Conjugate for Target-Mediated and Light-Controlled Delivery of MicroRNA into Cells

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Materials and Methods

1. General materials and methods

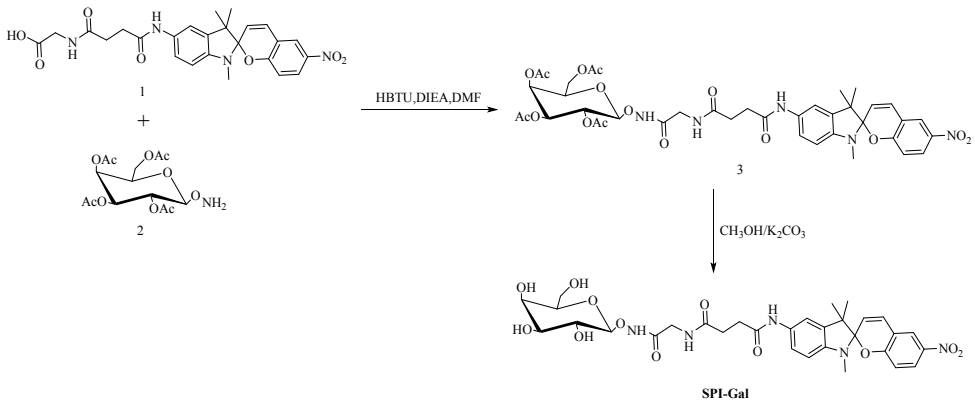
All chemicals and solvents were purchased from J&K chemicals or TCI chemicals. MiR-122 mimics were purchased from RiboBio (Guangzhou, China). High glucose Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serums (FBS), penincilin/streptomycin, Lipofectamine 2000, TRIZOL reagent were purchased from Life Technologies. MTT was purchased from Sigma-Aldrich. Luciferase assay kits were purchased from Promega. A hand-held 12w LED lamp was used as light source. All aqueous solutions were treated by diethy pyrocarbonate (DEPC) before use.

^{1}HNMR and ^{13}CNMR spectra were obtained on a 400 MHz Bruker AVANCE III–400 spectrometer. Chemical shifts are reported in δ (ppm) relative to the solvent residual peak. Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). MS were done on an Agilent 6550 iFunnel Q-TOF LC/MS. Scanning electron micrograph (SEM) pictures were taken on Hitachi S-4800 scanning electron microscope. MiRNA expression was quantified using Taqman miRNA detection assay (Applied Biosystems).

2. Synthesis and characterization of SPI-Gal

The spiropyran (**SPI**) and galactosamine were synthesized according to the literature. [1,2]

Scheme S1. Synthesis of **SPI-Gal**.



SPI-Gal:

Compound 1 (0.1 mmol) and compound 2 (0.2 mmol) were mixed in 5 mL dry DMF, then HBTU (0.15 mmol) and DIEA (0.3 mmol) were added and the mixture was stirred overnight under N_2 atmosphere at room temperature. Ethyl acetate was then added to this mixture, and the well mixed mixture was washed with ddH_2O for 3 times and saturated sodium chloride solution for 1

time. Ethyl acetate was then removed by evaporation.

Then the raw product 3 was dissolved in 10 mL methanol and 70 mg potassium carbonate was added. The mixture was stirred overnight at room temperature. After purification with HPLC, SPI-Gal was collected and lyophilized (28 mg, yield 41.7%).

SPI-Gal ^{1}H NMR (400 MHz, MeOD) δ 8.11 (d, J=2.7 Hz, 1H), 8.04 (dd, J=9.0, 2.7 Hz, 1H), 7.40 (br s, 1H), 7.33 (d, J=6.4 Hz, 1H), 7.14 (d, J=11.0 Hz, 1H), 6.81 (d, J=9.0 Hz, 1H), 6.66 (br s, 1H), 6.06 (d, J=8.6 Hz, 1H), 4.52 (d, J=11.0 Hz, 1H), 3.88 (br s, 2H), 3.81 – 3.77 (m, 2H), 3.71 (dd, J=11.4, 4.7 Hz, 1H), 3.64 (dd, J=9.5, 8.1 Hz, 1H), 3.57 – 3.56 (m, 1H), 3.52 (dd, J =9.5, 3.2 Hz, 1H), 2.80 (s, 3H), 2.71–2.59 (m, 4H), 1.28 (s, 6H) ^{13}C NMR (100 MHz, DMSO) δ 172.38, 170.19, 168.46, 159.89, 158.98, 158.61, 143.85, 140.91, 136.47, 132.49, 128.79, 126.20, 123.27, 121.72, 119.37, 115.82, 114.62, 107.36, 76.38, 73.29, 69.33, 68.25, 63.23, 60.72, 52.37, 31.91, 30.82, 29.14, 26.06, 20.09

HR MS calcd. for $C_{31}H_{37}N_5O_{12}$ 672.2517 $[M+H]^+$; found 672.2511.

3. Hydrogelation test

The hydrogel was prepared by dissolving SPI-Gal in phosphate buffered saline (PBS) or Dulbecco's Modified Eagle Medium (DMEM) by gentle heating. The gel formed gradually after cooling to room temperature, which was confirmed by inverting the vial.

4. SEM

MCI-Gal gel was prepared by dissolving SPI-Gal in PBS (3.3 mg/mL). SEM samples were prepared by lyophilizing a small amount sample on wafer and sprayed a thin layer of gold for better image resolution.

5. Rheology

Rheology tests were performed on an HAAKE Rheostress 6000 rheometer (Thermo Scientific) and tests were done with a cone and a plate (19.992 mm diameter plate and 1° cone angle). The gap between the cone and the plate was 0.051 mm. The dynamic frequency sweep of the gel was measured at 1% strain.

6. Controlled Release

SPI-Gal was dissolved in PBS with a concentration of 3.3 mg/mL. After heating to 80 °C, 200 pmol miR-122 mimic was added and the mixtures were then cooled to room temperature to form miR-122 embedded MCI-Gal gel. After irradiation by a 12w LED lamp for 3 min, 200 μL PBS were added gently on top of the gel. After incubation for 0 h, 1 h, 3 h, 6 h, or 24 h, the solutions were collected and lyophilized. The miRNA mimics were then dissolved in 20 μL H_2O and analyzed by agarose gel electrophoresis. As the control, gel containing miR-122 but without light irradiation was used.

7. Cell culture

HepG2 and HeLa cells were cultured in high glucose DMEM (Gibco) containing 10% fetal bovine serum and 1% penicillin/streptomycin, and maintained in 5% CO_2 at 37 °C.

For 2D cell culture, cells were added on top of the MCI-Gal gel (3.3 mg/mL in DMEM), cell density is $6 * 10^5$/mL.

8. MTT

HepG2 cells were seeded into a 96 well plate with a concentration of 5, 000 cells per well in 100 μL culture medium. After 24 hours, medium was changed to 100 μL culture medium containing different concentrations of MCI-Gal. MTT assay was then performed at different time points to assess the cytotoxic effects of MCI-Gal. Relative cell viability was calculated by following equation: Relative cell viability = $(A_{sample}-A_{blank})/(A_{control}-A_{blank})$, where A is the absorbance at 490 nm.

To validate whether a LED light irradiation could cause cell death, HepG2 cells were seeded into a 96 well plate with a concentration of 5, 000 cells per well in 100 μL culture medium. After 24 hours, cells were exposed to the irradiation of a hand-held 12W LED lamp for 20 min. MTT assay was then performed, relative cell viability was calculated by following equation: Relative cell viability = $(A_{sample}-A_{blank})/(A_{control}-A_{blank})$, where A is the absorbance at 490 nm.

9. RNA isolation and qRT-PCR

0.8 μL miR-122 (100 pmol/μl) were encapsulated in 3.3 mg/mL MCI-Gal gel. 3 * 10^5 HepG2 or HeLa cells were added on top of the gel after light irradiation for 3 min. As controls, 3 * 10^5 HepG2 cells were cultured on MCI-Gal gel without miR-122 mimic or with miR-122 mimic but without light irradiation. After incubation for 24 hours, cells were isolated by degradation of the gel and centrifuging, and then further washed with PBS for three times.

Total RNA was isolated using TRIZOL reagent according to manufacturer's protocol. Mature hsa-miR-122 was quantified using Taqman miRNA detection assay. The reactions were carried out using 1 μg of RNA extracted from cells and U6 was used as internal control.

10. Luciferase assay

HepG2 cells at 80% confluency in 10 cm dish were co-transfected with Luc-miR-122 plasmid (15 μg) and β-galactosidase expressing plasmid (9 μg) by using Lipo-fectamine 2000 according to manufacturer's protocol. β-galactosidase was used as an internal control. After 6 hours, HepG2 cells were collected. Samples were then prepared same as decribed above. HepG2 cells cultured on the gel were further incubated for 48 hours at 37 ^oC and cells were then collected by degradation of the gel and centrifuging. Cells were then further washed with PBS for three times and assayed using luciferase assay kits.

11. Co-localization assay

0.8 μL miR-122-cy3 (100 pmol/μL) was encapsulated in 3.3 mg/mL MCI-Gal gel. 3 * 10^5 HepG2 cells were added on top of the gel after light irradiation for 3min. After incubation for 24 hours, cells were isolated by degradation of the gel and centrifuging and further washed with PBS for three times. Then cells were seeded into a confocal dish and further cultured overnight. After wash with PBS for three times and being fixed by paraformaldehyde, cells were stained with DAPI and Lysotracker, respectively. The imaging acquisitions were carried out immediately using a Leica confocal microscope.

12. Live/Dead cell assay

3 * 10^5 HepG2 cells were seeded on top of the MCI-Gal gel (3.3 mg/mL) after light irradiation for 3min. After incubation for 24 hours, cells were isolated by degradation of the gel and

centrifuging and further washed with PBS for three times. Then cells were seeded into a confocal dish and further cultured overnight. After stain by using LIVE/DEAD® Viability/Cytotoxicity Kit and wash with PBS for three times, the imaging acquisitions were carried out immediately using a Leica confocal microscope.

References

[1] Wei Wang, Jing Hu, Mengmeng Zheng, Li Zheng, Huan Wang and Yan Zhang. *Org. Biomol. Chem.*, 2015, **13**, 11492.

[2] Dennis Schade, Jürke Kotthaus, Nikola Klein, Joscha Kotthaus and Bernd Clement. *Org. Biomol. Chem.*, 2011, **9**, 5249.

Supporting Figures and Tables

Figure S1. (**a**) HPLC analysis and (**b**) UV-Vis characterization of the transformation of **SPI-Gal** to **MCI-Gal** in PBS after heating.

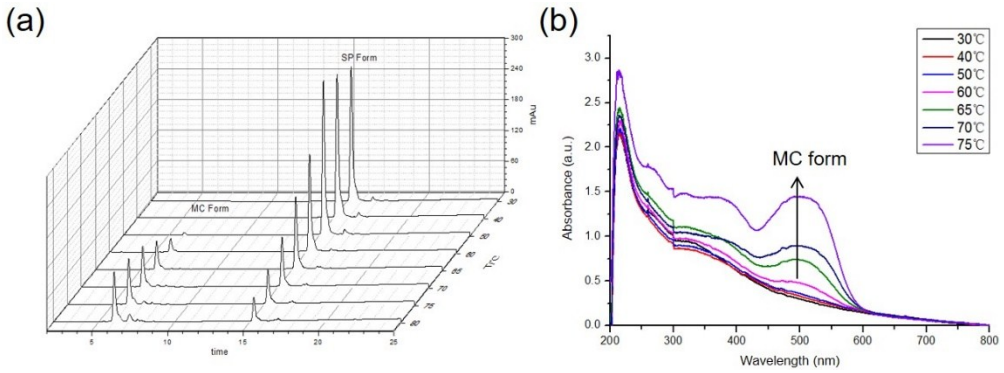


Figure S2. Dynamic frequency sweep of the **MCI-Gal** gel (3.3 mg/mL) at 1% strain.

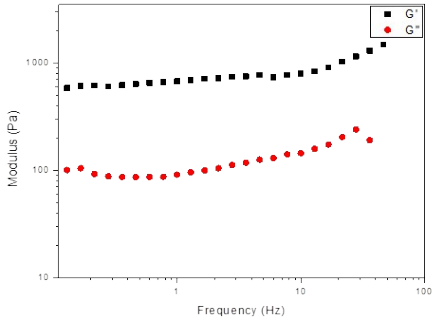


Figure S3. Optical images of the **MCI-Gal** Gel (3.3mg/mL) after irradiation.

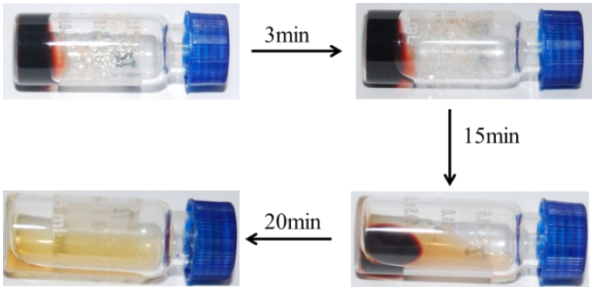


Figure S4. Gel analysis of miRNA release using **MCI-Gal** gel (3.3 mg/mL) encapsulated with 200 pmol miR-122 and subject to irradiation for 3 minutes. Incubation time was from 0 to 24 hours. The

band intensities were measured by Image J and further quantified according to standard.

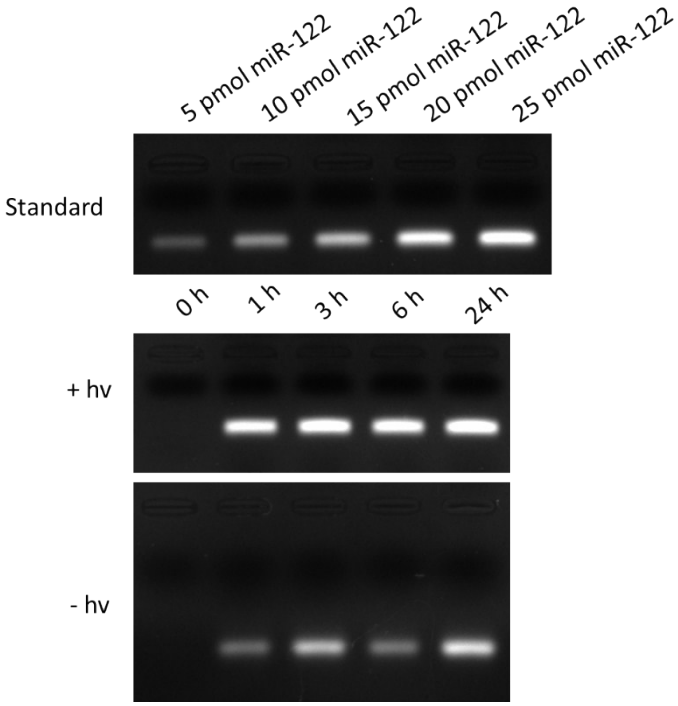


Figure S5. Cell viability of HepG2 cells after treatment with **MCI-Gal** at different concentrations for 1 or 2 days. Data are shown mean ± SEM (n=3).

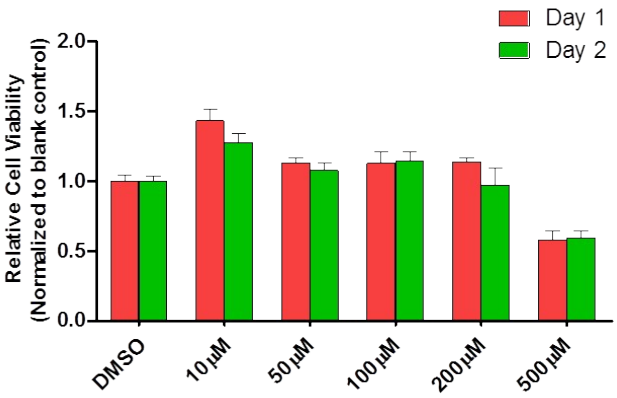


Figure S6. MTT test on HepG2 cells cultured in dish without or with exposure to light irradiation by the 12w hand-held lamp for 20 minutes. Data are shown mean ± SEM (n=3).

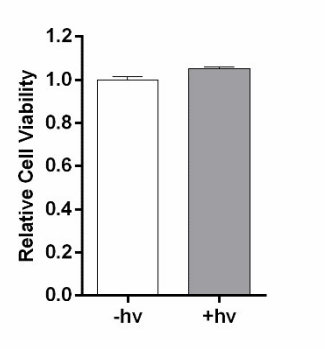


Figure S7. Live/Dead cell assay of HepG2 cells after culture on MCI-Gal gel (3.3 mg/mL) for 24 hours. Cells were collected by photo-degradation of gel by light irradiation and centrifuge, followed by culture in dish and stained with Calcein AM (green) and ethidium homodimer (red) to respectively indicate live or dead cells. Most of the cells remained alive after isolation and collection. Scale bar: 200 μm.

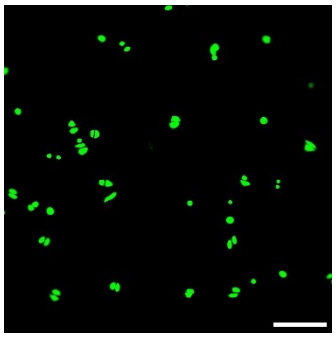


Figure S8. qRT-PCR analysis of miR-122 level of HepG2 cells cultured in dish or on top of 3.3 mg/mL **MCI-Gal** gel for 24 hours. Data are shown mean ± SEM (n=3).

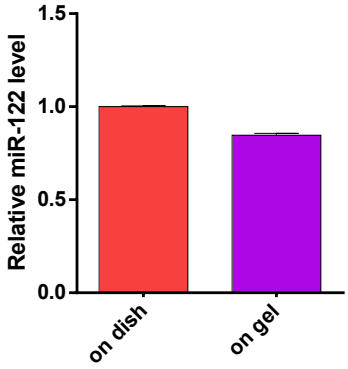


Figure S9. Co-localization of delivered miR-122 in HepG2 cells. Cy3-miR-122 (80 pmol) was

encapsulated in 3.3 mg/mL MCI-Gal gel and miRNA release was induced by light irradiation for 3 minutes. HepG2 cells were then seeded on top of the gel and cultured for 24 hours. After degradation of the gel, HepG2 cells were collected and further seeded in confocal dish. DAPI and LysoTracker were then used to stain nucleus and lysosome, respectively. Scale bar: 20 μm.

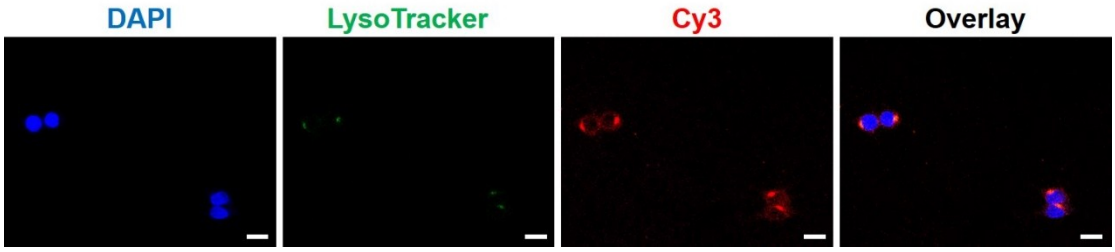
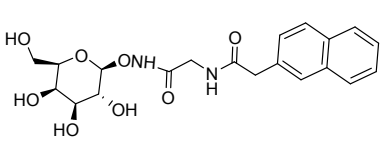
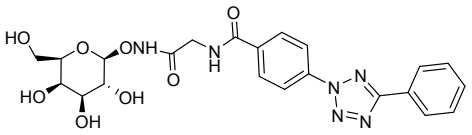


Table S1. Chemical structures and hydrogelation properties of Nap-Gal and Tet-Gal



Nap-Gal



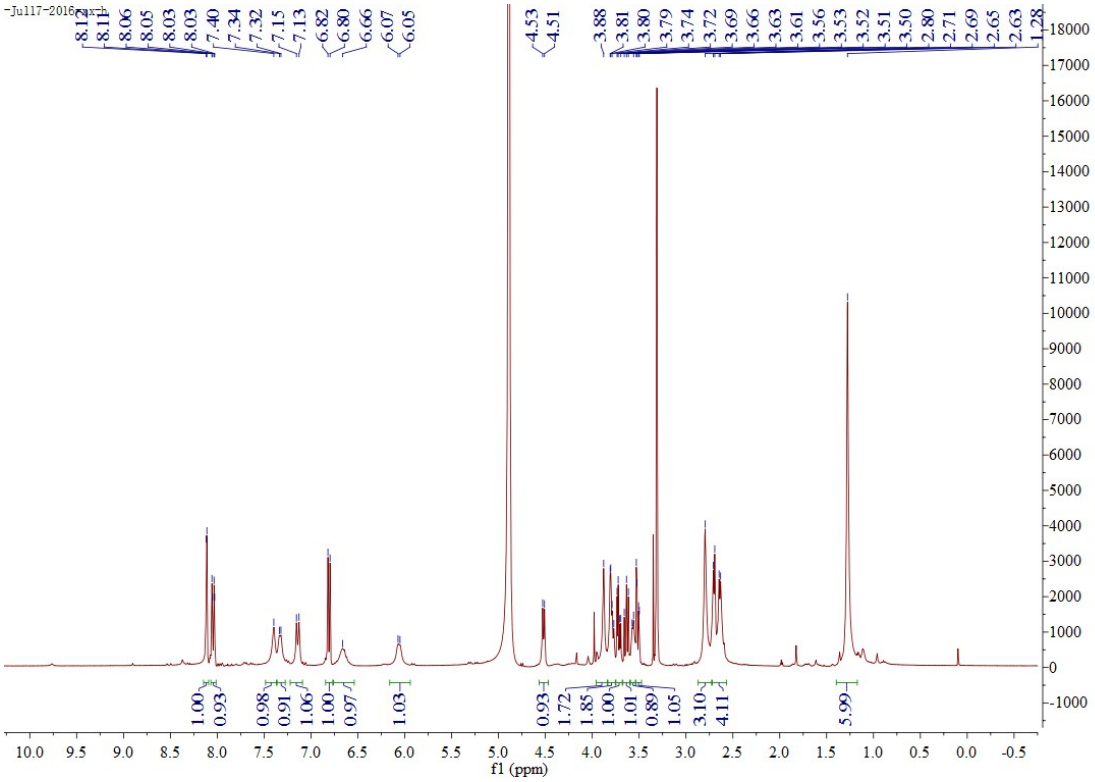
Tet-Gal

Compound	Gelation (Y/N)[a]	State
Nap-Gal	N	P
Tet-Gal	N	S

P: Precipitation; S: Solution; [a]Tested concentration: 3~5 mg/mL.

NMR Spectra

^{1}HNMR of SPI-Gal



^{13}CNMR of SPI-Gal