Supporting Information

Low-molecular-weight supramolecular hydrogel of riboflavin bolaamphiphile for VEGF-siRNA delivery

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1. General experimental details

All chemicals were purchased from Sigma–Aldrich, Fluka, Lancaster, Proligo, or Glen Research and used without further purification. All reactions were performed in flame-dried glassware under Ar. Flash column chromatography was performed using Merck silica gel 60 (230–400 mesh). The melting points were determined using an electrothermal model IA9100 apparatus. Elemental analyses were performed on a Vario micro cube. High-resolution (HR) mass spectra (FAB) were obtained using a Jeol JMS700 HR mass spectrometer at the Korea Basic Science Center, Daegu, Korea. Purification of siRNAs was performed using an Agilent 1100 high-performance liquid chromatography system (VyDAC C18 column; 10 × 250 mm; 5 μm; pore size: 120 Å). The siRNAs were synthesized using an Expedite 8909 synthesizer; primer oligonucleotides were purchased from Bionics (Korea). ^{1}H, and ^{31}C NMR spectra were recorded using an FT-300 MHz Bruker Aspect 300 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield relative to the internal standard, tetramethylsilane (TMS). Coupling constants are reported in hertz (Hz). Spectral splitting patterns are designed as follows: s, singlet; d, doublet; dd, double doublet; dt, distorted triplet; t, triplet; m, multiplet; br, broad.

Hydrogel formation. The hydrogel was prepared by dissolving **1a** (6.5 mg) in distilled water (400 μL) by gentle heating (up to 80 ^{o}C). After cooling at room temperature, the complete volume of water had become immobilized and could keep its own weight. Hydrogel formation was confirmed by turning the vial upside down. Hydrogel **1a** is stable at least more than four weeks at room temperature.

Fluroscence spectroscopy. Temperature dependent fluorescence studies were carried out in a Varian Cary Eclipse fluorescence spectrophotometer. The samples were taken in a quartz cell of 1 cm path length and were excited at 373 nm. Samples were incubated for one 45 minute at each temperature.

Scanning electron microscopy. The hydrogel found by **1a** was freeze-dried, that xerogel was placed on carbon tape, and SEM images were observed using XL 30S FPG instrument.

Transmission electron microscopy. Solution of [**1a** (3 mg/mL) or mixture of siRNA : **1a** (1 : 500; molar ratio)] was placed on copper formvar/carbon coated grid, air dried for 2 minute, negatively stained with uranyl acetate (1% in water), again air and vacuum dried and then TEM images were observed using a JEM-1011 instrument.

Synthesis of siRNA. VEGF siRNA was synthesized using an automatic RNA synthesizer, according to standard solid phase protocols. The structures of the siRNA samples were confirmed using MALDI-TOF mass spectrometry. MALDI-TOF MS data of RNAs: Calcd for antisense strand of VEGFsiRNA (5'-G AUC UCA UCA GGG UAC UCC UdT): 6608.2; Found: 6608.2; Calcd for sense strand of VEGF siRNA (5'-G GAG UAC CCU GAU GAG AUC UdT): 6711.2; Found: 6710.9; Calcd for fluoroscein tagged antisense strand of VEGF siRNA (5'-G AUC UCA UCA GGG UAC UCC UdT^{Fl}): 7122.2; Found: 7122.4.

Cell culture. Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin, fetal bovine serum (FBS), and Dulbecco's phosphate-buffered saline (DPBS) were purchased from Hyclone-Themo Scientific (Logan, UT). Opti-MEM was purchased from Invitrogen-Gibco (Carlsbad, CA). HeLa cells were cultured in DMEM supplemented with 10% FBS, 100 μg/mL of streptomycin, and 100 U/mL of penicillin at 37 °C in a 5% CO_2 incubator. Cells were split, using trypsin/EDTA medium, when almost confluent. HeLa cells were seeded at a density of 2.5×10^5 cells/well; each well contained 2 mL of 10% FBS-supplemented DMEM and was incubated for 4 h.

Celluar uptake assays. HeLa cells were transfected in the absence of serum with VEGF-siRNA using the RBAs (**1a**, **1b**, or **1c**), lipofectamine™ 2000 (concentration using Invitrogen kit) or without any transfection reagent. The media were removed after the incubation of cell at 37 °C for 6 h in a CO_2 incubator. The cells were washed with DPBS buffer (1×330 μL/well) followed by Trypsin (150 μL/well) addition and incubation for 3 minute. DPBS (150 μL/well) was then added to above trypsin solution, pippeted and transferred it into each well solution of e-tube , centrifuged (at 3000-4000 rpm) and supernate was removed. DPBS (300 μL/e-tube) was added to the pillets of the cells, vortexed each sample for 1 min, centrifuged (at 3000-4000 rpm) and removed the DPPS. The same procedure for washing was repeated for three times. Then added lysis buffer (70 μL/well) and vortexed for 20 min at 25 ^oC, centrifuged (at 3000-4000 rpm) and transferred (50 μL/e-tube) supernate into each well of the 96-well plate (black polystyrene). The absorbance was measured at a wavelength 485/535 nm using VICTOR multi-label plate reader (PerkinElmer).

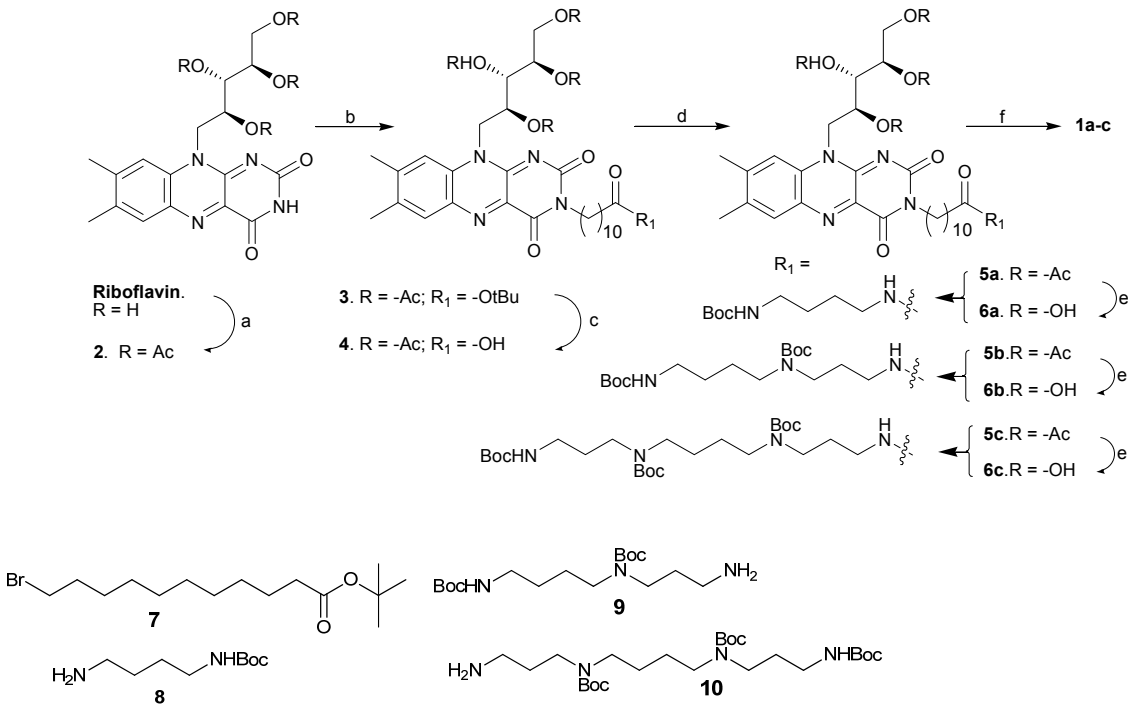
Drug inhibition assay. HeLa cells were preincubated with 0.1% NaN_3/50 mM 2-deoxyglucose, or genistein (200 μM) in serum-free Opti-MEM for 1 h. For the inhibitors, Methyl-β-cyclodextrin (Mβcd) and chlorpromazine, cells were preincubated in serum-free MEM containing either in 5 mM Mβcd or 10 μg/mL chlorpromazine for 15 min at 37°C/5% CO_2. The cells were washed with DPBS, the media were then changed to fresh media containing the si-G1a [siRNA (100 nM):**1a**; 1:500 molar ratio] and further incubated for 3 hour at 37°C/5% CO_2. After exposure to si-G1a and inhibitors for the desired time, the cells were washed with DPBS and then trypsinized and processed for fluorescence intensity as described in cellular uptake experiment. All inhibitors were obtained from Sigma-Aldrich.

WST-1 Assays. RBAs **1a–c** (molar ratios of 100, 200, and 500) were complexed with VEGF-siRNA (100 nM) and added to the cells. Cells were incubated with the complexes at 37 °C under 5% CO_2 for 24 h. After incubation, the media were removed, the cells were washed with DPBS buffer (3×100 μL/well) and then WST-1 reagent (1 mg/mL of WST-1 dissolved in phenol red free medium, Roche; 100 μL) was added to each well. The mixtures were incubated at 37 ^oC for 4 h. After incubation, the absorbance were measured at a wavelength of 450 nm using a micro-plate reader UVM 340 (ASYS) and converted to the percentage of cell viability (relative to control cells).

VEGF ELISAs. HeLa cells were transfected in the absence of serum with VEGF siRNA using the RBAs (**1a**, **1b**, or **1c**), lipofectamine™ 2000 (Invitrogen), or without any transfection reagent. The cells were left to incubate at 37 °C for 6 h in a CO_2 incubator and washed the cells by DPBS buffer (3 × 330 μL/well), followed by replacement of DMEM containing 10% FBS (330 μL/well). After a further 18 or 42 h of incubation, the cell medium was collected and analyzed for the VEGF expression level using a VEGF ELISA kit (QIA51 VEGF ELISA Kit, Human, Calbiochem).

Confocal microscopy experiments. Cover glasses (1.13 cm^2, Deckglaser) were placed into a six-well plate and then the plate was coated with 0.2% gelatin. HeLa cells were seeded at a density of 2.5×10^5 cells/well; each well contained 2 mL of 10% FBS-supplemented DMEM and incubated for 12 h. HeLa cells were transfected in the absence of serum with 100 nM of fluorescein-tagged VEGF siRNA using lipofectamine™ 2000 (concentration using Invitrogen kit), the RBA **1a**, **1b**, or **1c** (molar ratio, 500:1), or without any reagent. The cells were left to incubate at 37 °C in the presence of Fl-siRNA for 4 h in a CO_2 incubator. After incubation, the media were removed, the cells were washed with DPBS buffer (2 × 2 mL/well), and then 4'-6-diamido-2-phenylindole (DAPI) was added for nucleus staining. After incubation for 20 min, the solution was removed, the cells were washed with DPBS buffer (3 × 2 mL/well), and then the cover glasses were detached from the bottom of the plate. The cover glasses were transferred onto slide glass. Confocal images were obtained from the live cells using a FluoView™ FV1000 confocal microscope (Olympus Optical, Tokyo).

Figure S1. Synthetic route and precursors for the RBAs **1a**–**c**.



Reagent and conditions: (a) Ac_2O, pyridine, 80 °C, 12 h, 71% yield; (b) **7**, NaI, K_2CO_3, DMF, 55 °C, 24 h, 73% yield; (c) 25% TFA, CH_2Cl_2, rt, 8 h, 82% yield; (d) **8**, **9**, or **10**; TBTU, DIPEA, DMF, rt, 12 h, (**5a**–**c**: 60–70% yields); (e) 7 N NH_3 in MeOH, CH_2Cl_2, rt, 5 h, (**6a**–**c**: 70–74% yields); (f) 25% TFA in MeOH, rt, 3 h (**1a**–**c**: quant. yields).

3. Experimental procedures and compounds characterization data

Synthesis of *2',3',4',5'-Tetraacetylriboflavin* (2)[1]

Yield: 71%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (s, 1H), 7.91 (s, 1H), 7.55 (s, 1H), 5.67-5.64 (m, 1H), 5.47-5.37 (m, 1H), 5.12-4.90 (m, 2H), 4.42 (dd, *J* = 2.7, 12.3 Hz, 1H), 4.23 (dd, *J* = 5.4, 12.3 Hz, 1H), 2.55 (s, 3H), 2.43 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.07 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.5, 170.1, 169.9, 159.5, 154.7, 150.9, 148.3, 137.2, 136.3, 134.8, 133.1, 131.4, 115.7, 70.7, 69.6, 69.2, 62.1, 45.2, 21.6, 21.2, 20.9, 20.8, 20.5, 19.6; HRMS-FAB: *m/z* Calcd for $C_{25}H_{29}N_4O_{10}$: 545.1878; Found: 545.1884 $(M+H)^+$.

Synthesis of *2',3',4',5'-Tetraacetyl-3-tert.-butoxycarbonyldecylriboflavin* (3)

A suspension of (1.0 equiv, 5.4 g) of 2',3',4',5'-tetraacetylriboflavin (**2**), potassium carbonate (1.5 equiv), and catalytic amounts of sodium iodide in 30 mL of dry *N,N*-dimethylformamide was stirred at room temperature for 45 min. Then a solution of the alkylating agent **7** (2 equiv, 9.6 g; figure S1) in 10-20 mL of dry *N,N*-dimethyl formamide was added slowly and the stirring was continued until the 2',3',4',5'-tetraacetylriboflavin (**2**) was completely alkylated (24 h). The reaction mixture was diluted with 100 mL of dichloromethane and the organic phase was washed with saturated sodium $NaHCO_3$ solution, water, and brine. Organic solvent was dried over anhydrous Na_2SO_4, evaporated and the remaining residue was purified by column chromatography (EA:HEX; 1:2) to get **3** ((gummy solid; 5.7 g; Yield: 73%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.54 (s, 1H), 5.68-5.66 (m, 1H), 5.47-5.39 (m, 2H), 5.11 (s, 2H), 4.44 (dd, *J* = 2.7, 12.3 Hz, 1H), 4.25 (dd, *J* = 5.7, 12.3 Hz, 1H), 4.06 (t, *J* = 7.5 Hz, 2H), 2.56 (s, 3H), 2.44 (s, 3H), 2.34 (s, 3H), 2.23 (s, 3H), 2.08 (s, 3H), 1.74 (s, 3H), 1.70-1.61 (m, 4H), 1.41 (s, 9H), 1.34-1.27 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 170.8, 170.5, 170.1, 161.7, 159.8, 155.2, 149.3, 147.5, 136.6, 136.3, 136.0, 134.8, 133.1, 131.3, 115.5, 82.1, 70.5, 69.6, 66.2, 62.1, 36.7, 34.5, 31.6, 29.6, 29.56, 29.50, 29.4, 29.3, 28.8, 27.9, 27.2, 25.1, 21.6, 21.2, 21.0, 20.9, 20.5, 19.6; HRMS-FAB: *m/z* Calcd for $C_{40}H_{57}N_4O_{12}$: 785.3967; Found: 785.3973 $(M+H)^+$.

***2',3',4',5'-Tetraacetyl-3-carbonyldecylriboflavin* (4)**

Trifluoroacetic acid (10 mL) was added slowly at 0 ^{o}C to a solution of **3** (4 g, 5.1 mmol) in dichloromethane. The solution was stirred at room temperature for 8 h. After completion of reaction, the reaction mixture was then poured into ice-water, $NaHCO_3$ solution was added to bring the pH of the water phase to approx.5. The product was extracted with dichloromethane and the organic phase was washed with water and brine. Organic solvent was dried over anhydrous Na_2SO_4, evaporated and the remaining residue was purified by column chromatography (EA:EtOH; 20:1) to get **4** (gummy solid; 3 g; Yield: 82%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.54 (s, 1H), 5.69-5.66 (m, 1H), 5.50-5.40 (m, 2H), 5.12-4.90 (bs, 2H), 4.45 (dd, *J* = 2.7, 12.3 Hz, 1H), 4.26 (dd, *J* = 5.7 Hz, *J* = 12 Hz, 1H), 4.07 (t, *J* = 7.5 Hz, 2H), 2.56 (s, 3H), 2.45 (s, 3H), 2.35 (t, *J* = 7.2 Hz, 2H), 2.31 (s, 3H), 2.23 (s, 3H), 2.09 (s, 3H), 1.75 (s, 3H), 1.71-1.58 (m, 4H), 1.35-1.25 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.4, 171.4, 170.9, 170.6, 170.1, 169.9, 159.8, 155.3, 149.3, 147.6, 136.7, 136.0, 134.8, 133.1, 131.4, 115.2, 70.6, 69.2, 62.1, 60.6, 34.1, 29.4,

29.2, 27.9, 27.1, 24.9, 21.6, 21.3, 21.0, 20.5, 19.6; HRMS-FAB: *m/z* Calcd for $C_{36}H_{49}N_4O_{12}$: 729.3331; Found: 729.3337 $(M+H)^+$.

General procedure for coupling reaction by peptide bond (5a-c)

TBTU (1.2 equiv), and DIPEA (1.5 equiv) were added to solution of **4** (1 equiv) in dry DMF (5 mL). The solution was stirred at room temperature for half an hour and then Boc-protected polyamine (1.5 equiv) **7-10** was added. Reaction mixture was then again kept for stirring for 8 h. After the completion of reaction the solvent was evaporated under reduced pressure and extracted in CH_2Cl_2. Organic solvent was dried over anhydrous Na_2SO_4, evaporated and the remaining residue was purified by column chromatography (EA:CH_2Cl_2; 1:4–1:1) to get **5a-c**.

***2',3',4',5'-Tetraacetyl-3-[(N⁴-(Boc)-1,4-aminobutane-1-amido)undecyl]riboflavin* (5a)**

Yellow gummy solid; 70% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.52 (s, 1H), 5.84 (bs, 1H), 5.66-5.63 (m, 1H), 5.47-5.36 (m, 2H), 4.88 (bs, 1H), 4.67 (bs, 1H), 4.42 (dd, *J* = 2.7, 12.6 Hz, 1H), 4.23 (dd, *J* = 5.7, 12.3 Hz, 1H), 4.03 (t, *J* = 7.2 Hz, 2H), 3.25-3.21 (m, 2H), 3.11-3.09 (m, 2H), 2.53 (s, 3H), 2.42 (s, 3H), 2.28 (s, 3H), 2.20 (s, 3H), 2.14 (t, *J* = 7.2 Hz, 2H), 2.06 (s, 3H), 1.71 (s, 3H), 167-1.57 (m, 4H), 1.51-1.49 (m, 2H), 1.41 (s, 9H), 1.31-1.23 (m, 14H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 170.8, 170.4, 170.0, 169.8, 159.7, 156.3, 155.2, 149.2, 147.6, 136.7, 135.8, 134.8, 132.9, 131.3, 115.5, 79.3, 70.5, 69.5, 69.1, 62.0, 44.6, 42.2, 40.1, 39.1, 36.9, 36.6, 31.6, 29.4, 28.5, 27.7, 27.0, 25.9, 21.5, 21.2, 20.9, 20.4, 19.5; HRMS-FAB: *m/z* Calcd for $C_{45}H_{67}N_6O_{13}$: 899.4761; Found: 899.4767 $(M+H)^+$.

***2',3',4',5'-Tetraacetyl-3-[(N⁴,N⁸-di(Boc)-1,8-amino-4-azaoctane-1-amido)undecyl]riboflavin* (5b)**

Yellow gummy solid; 64% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.52 (s, 1H), 5.67-5.63 (m, 1H), 5.47-5.37 (m, 2H), 4.85 (bs, 1H), 4.62 (bs, 1H), 4.42 (dd, *J* = 3, 12.6 Hz, 1H), 4.24 (dd, *J* = 5.7, 12.3 Hz, 1H), 4.04 (t, *J* = 7.2 Hz, 2H), 3.23-3.10 (m, 10H), 2.54 (s, 3H), 2.42 (s, 3H), 2.29 (s, 3H), 2.21 (s, 3H), 2.19-2.14 (m, 2H), 2.07 (s, 3H), 1.72 (s, 3H), 1.65-1.61 (m, 4H), 1.56-1.48 (m, 4H), 1.46 (m, 2H), 1.46 (s, 9H), 1.43 (bs, 4H), 1.42 (bs, 13H), 1.26 (bs, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 170.8, 170.5, 170.1, 169.8, 159.8, 156.2, 155.2, 149.3, 147.5, 136.6, 136.0, 134.8, 133.1, 131.4, 115.5, 79.9, 70.6, 69.6, 69.2, 62.1, 46.7, 44.6, 42.3, 40.3, 38.8, 37.2, 29.6, 29.5, 28.7, 28.6, 28.2, 27.9, 27.7, 27.2, 26.0, 21.6, 21.3, 21.0, 20.9, 20.5, 19.6; HRMS-FAB: *m/z* Calcd for $C_{53}H_{82}N_7O_{15}$: 1056.5863; Found: 1056.5869 $(M+H)^+$.

***2',3',4',5'-Tetraacetyl-3-[(N⁴,N⁹,N¹²-tri(Boc)-1,12-amino-4,9-azadodecane-1-amido)undecyl]riboflavin* (5c):**

Yellow gummy solid; 60% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.52 (s, 1H), 5.68-5.65 (m, 1H), 5.48-5.38 (m, 1H), 4.89 (bs, 2H), 4.43 (dd, *J* = 2.7, 12.6 Hz, 1H), 4.24 (dd, *J* = 5.7, 12.6 Hz, 1H), 4.05 (t, *J* = 7.5 Hz, 2H), 3.21-3.12 (m, 12H), 2.54 (s, 3H), 2.43 (s, 3H), 2.30 (s, 3H), 2.22 (s, 3H), 2.14 (t, *J* = 7.5 Hz, 2H), 2.07 (s, 3H), 1.73 (s, 3H), 1.65 (bs, 11H), 1.45-1.43 (s, 30H), 1.26 (bs, 4H), 1.25 (bs, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 170.8, 170.5, 170.1, 169.9, 159.8, 156.7, 156.2, 155.2, 149.3, 147.5, 136.0, 134.8, 133.1, 131.3, 115.5, 79.9, 79.4,

70.6, 69.6, 69.2, 62.1, 46.7, 44.6, 42.3, 40.3, 37.2, 29.5, 28.7, 28.6, 27.8, 27.2, 26.0, 21.6, 21.3, 21.0, 20.5, 19.6; HRMS-FAB : *m/z* Calcd for $C_{61}H_{97}N_8O_{17}$: 1213.6966; Found: 1213.6972 $(M+H)^+$.

General procedure for deprotection of acetate group (6a-c)

7 N ammonia in methanol (10 mL) was added to a solution of riboflavin derivatives (**5a-c**; 1mmol) in dichloromethane (1 mL). The solution was stirred at room temperature. After completion of reaction the solvent was removed under high vacuum and dissolved the residue in dichloromethane : methanol (95 : 5), workup with 5% NH_3 in water, removed the solvent under high vacuum and the residue was purified by column chromatography (CH_2Cl_2:MeOH; 10:1–6:1) to get **6a-c**.

***3*-[(*N^4*-(*Boc*)-*1,4-diaminobutane-1-amido*)*undecyl*]*riboflavin* (6a):**

Yellow solid; 74% yield; mp 153-154 °C (decomposed); ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD = 9:1) δ 8.00 (s, 1H), 7.97 (s, 1H), 5.08-5.00 (m, 1H), 4.91-4.86 (m, 1H), 4.45-4.39 (m, 1H), 4.03 (t, *J* = 7.4 Hz, 2H), 3.87-3.77 (m, 3H), 3.74-3.68 (m, 1H), 3.15 (t, *J* = 6.5 Hz, 2H), 3.04 (t, *J* = 6.5 Hz, 2H), 2.56 (s, 3H), 2.46 (s, 3H), 2.15 (t, *J* = 7.2 Hz, 2H), 1.69-1.67 (m, 2H), 1.60-1.55 (m, 2H), 1.49-1.47 (m, 4H), 1.41 (s, 9H), 1.35 (bs, 4H), 1.29-1.25 (bs, 10H); ^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD = 9:1): δ 176.1, 161.5, 158.2, 157.7, 150.3, 149.4, 138.4, 136.5, 136.0, 133.7, 132.4, 118.4, 79.9, 74.9, 73.9, 71.4, 64.8, 42.9, 40.8, 39.9, 37.1, 30.6, 30.3, 30.2, 30.1, 28.9, 28.6, 28.1, 27.8, 27.4, 26.9, 21.7, 19.7; Elemental analysis calculated for $C_{37}H_{58}N_6O_9$: C, 60.80; H, 8.00; N, 11.50; Found: C, 60.74; H, 8.26; N, 11.72; HRMS-FAB: *m/z* Calcd for $C_{37}H_{59}N_6O_9$: 731.4338; Found: 731.4344 $(M+H)^+$.

***3*-[(*N^4*,*N^8*-*di(Boc)-1,8-diamino-4-azaoctane-1-amido*)*undecyl*]*riboflavin* (6b):**

Yellow solid; Yield: 71%). mp 157 -158 °C (decomposed); ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD = 9:1) δ 7.98-7.97 (m, 2H), 5.07-5.00 (m, 1H), 4.90-4.86 (m, 1H), 4.43-4.39 (m, 1H), 4.07 (t, *J* = 7.4 Hz, 2H), 3.86-3.68 (m, 4H), 3.18-3.12 (m, 6H), 3.04 (t, *J* = 6.5 Hz, 2H), 2.56 (s, 3H), 2.45 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 1.69 (m, 4H), 1.58-1.48 (m, 4H), 1.43-1.41 (m, 22H), 1.37-1.35 (m, 4H), 1.29-1.24 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$: CD_3OD = 9:1) δ 175.9, 161.6, 158.1, 157.9, 157.6, 150.3, 149.4, 138.4, 136.5, 136.0, 133.7, 132.4, 118.4, 79.9, 74.9, 73.9, 71.4, 64.8, 46.1, 44.2, 42.9, 40.8, 39.9, 37.1, 30.6, 30.3, 30.2, 30.1, 28.9, 28.6, 28.1, 27.8, 27.4, 27.0, 21.6, 19.6; Elemental analysis calculated for $C_{45}H_{73}N_7O_{11}$: C, 60.86; H, 8.29; N, 11.04; Found: C, 60.88; H, 8.52; N, 11.27; HRMS-FAB: *m/z* Calcd for $C_{45}H_{74}N_7O_{11}$: 888.5441; Found: 888.5447 $(M+H)^+$.

***3*-[(*N^4*,*N^9*,*N^{12}*-*tri(Boc)-1,12-diamino-4,9-diazadodecane-1-amido*)*undecyl*]*riboflavin* (6c):**

Yellow solid; 70% yield; mp 154-155 °C (decomposed); ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD = 9:1) δ 7.98-7.97 (m, 2H), 5.07-5.00 (m, 1H), 4.90-4.86 (m, 1H), 4.43-4.39 (m, 1H), 4.07 (t, *J* = 7.4 Hz, 2H), 3.86-3.68 (m, 4H), 3.18-3.12 (m, 6H), 3.04 (t, *J* = 6.5 Hz, 2H), 2.56 (s, 3H), 2.45 (s, 3H), 2.16 (t, *J* = 7.2 Hz, 2H), 1.69 (m, 4H), 1.58-1.48 (m, 4H), 1.43-1.41 (m, 22H), 1.37-1.35 (m, 4H), 1.29-1.24 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD = 9:1) δ 175.8, 161.5, 158.4, 158.2, 157.8, 157.7, 150.3, 149.4, 138.4, 136.5, 136.0, 133.7, 132.4, 118.4, 80.1,79.9, 74.9, 73.9, 71.4, 64.8, 46.1, 44.2, 42.9, 40.8, 39.9, 37.1, 30.6, 30.3, 30.2, 30.1, 28.9, 28.4, 28.1, 27.8, 27.2, 27.0, 21.7,

19.6; Elemental analysis calculated for $C_{53}H_{88}N_8O_{13}$: C, 60.90; H, 8.49; N, 10.72; Found: C, 60.84; H, 8.66; N, 10.84; HRMS-FAB: *m/z* Calcd for $C_{53}H_{89}N_8O_{13}$: 1045.6544; Found: 1045.6550 $(M+H)^+$.

Procedure for deprotection of Boc group (1a-c)

Trifluoroacetic acid (25% v/v) was added to the solution of riboflavin derivatives (**6a-c**; 1 mmol) in dry methanol (10 mL). The reaction mixture was stirred for 3 h at room temperature. After completion of starting material, diethyl ether was added to reaction mixture to get a precipitate of corresponding compounds. The precipitate was washed by diethyl ether and dichloromethane, and dried under high vacuum to get **1a-c**.

***3*-[(*1,4-diaminobutane-1-amido*)*undecyl*]*riboflavin* (1a)**

Orange solid; 99% yield; mp 147-148 °C (decomposed); ^{1}H NMR (300 MHz, CD_3OD): δ 8.04 (s, 1H), 7.95 (s, 1H), 5.10-5.02 (m, 1H), 4.91 (bs, 1H), 4.47-4.42 (m, 1H), 4.03 (t, *J* = 7.2 Hz, 2H), 3.88-3.79 (m, 3H), 3.73-3.66 (m, 1H), 3.21 (t, *J* = 6.9 Hz, 2H), 2.97 (t, *J* = 6.9 Hz, 2H), 2.57 (s, 3H), 2.47 (s, 3H), 2.18 (t, *J* = 7.2 Hz, 2H), 1.73-1.65 (m, 4H), 1.63-1.55 (m, 4H), 1.39-1.38 (m, 4H), 1.32-1.29 (m, 10H); ^{13}C NMR (75 MHz, CD_3OD): δ 176.7, 162.2, 158.3, 150.9, 149.5, 138.8, 136.8, 136.7, 134.2, 132.4, 118.9, 75.4, 74.4, 71.4, 65.1, 40.5, 39.6, 37.3, 30.6, 30.5, 30.4, 30.3, 28.9, 28.2, 27.7, 27.1, 25.9, 21.5, 19.5; Elemental analysis calculated for $C_{34}H_{51}F_3N_6O_9 \cdot H_2O$: C, 53.53; H, 7.00; N, 11.02; Found: C, 53.68; H, 7.26; N, 11.18; HRMS-FAB: *m/z* Calcd for $C_{32}H_{51}N_6O_7$: 631.3814; Found: 631.3819 $(M+H)^+$.

***3*-[(*1,8-diamino-4-azaoctane-1-amido*)*undecyl*]*riboflavin* (1b)**

Orange solid; 98% yield; mp 144-145 °C (decomposed); ^{1}H NMR (300 MHz, CD_3OD) δ 8.04 (s, 1H), 7.95 (s, 1H), 5.10-5.02 (m, 1H), 4.69-4.53 (m, 1H), 4.47-4.43 (m, 1H), 4.03 (t, *J* = 7.2 Hz, 2H), 3.89-3.80 (m, 3H), 3.73-3.67 (m, 1H), 3.08-2.99 (m, 6H), 2.57 (s, 3H), 2.47 (s, 3H), 2.23 (t, *J* = 7.2 Hz, 2H), 1.89 (t, *J* = 6.9 Hz, 2H), 1.81-1.79 (m, 4H), 1.70-1.59 (m, 4H), 1.39-1.38 (m, 4H), 1.33-1.29 (bs, 10H); ^{13}C NMR (75 MHz, CD_3OD) δ 177.6, 162.0, 158.2, 150.8, 149.5, 138.7, 136.7, 136.6, 134.0, 132.2, 118.7, 75.3, 74.3, 71.2, 64.9, 46.3, 43.0, 40.0, 36.9, 30.5, 30.4, 30.3, 30.2, 30.1, 28.7, 28.0, 27.8, 26.9, 25.6, 24.3, 21.4, 19.4; Elemental analysis calculated for $C_{39}H_{59}F_6N_7O_{11} \cdot 2H_2O$: C, 49.21; H, 6.67; N, 10.30; Found: C, 49.14; H, 6.89; N, 10.11; HRMS-FAB: *m/z* Calcd for $C_{35}H_{58}N_7O_7$: 687.4392; Found: 688.4398 $(M+H)^+$.

***3*-[(*1,12-diamino-4,9-diazadodecane-1-amido*)*undecyl*]*riboflavin* (1c)**

Orange solid; 97% yield; mp 142-143 °C (decomposed); ^{1}H NMR (300 MHz, CD_3OD) δ 8.05 (s, 1H), 7.97 (s, 1H), 5.11-5.04 (m, 1H), 5.00 (bs, 1H), 4.47-4.43 (m, 1H), 4.04 (t, *J* = 7.2 Hz, 2H), 3.89-3.81 (m, 3H), 3.73-3.67 (m, 1H), 3.04-3.02 (bs, 10H), 2.58 (s, 3H), 2.47 (s, 3H), 2.22 (t, *J* = 7.2 Hz, 2H), 2.04-2.01 (m, 2H), 1.89 (bs, 4H), 1.79 (bs, 4H), 1.61 (bs, 4H), 1.39-1.37 (m, 4H), 1.33-1.29 (m, 14H); ^{13}C NMR (150 MHz, CD_3OD) δ 178.2, 163.2, 158.7, 151.3, 149.9, 139.2, 137.2, 137.1, 134.5, 132.8, 119.2, 75.8, 74.8, 71.8, 65.4, 46.9, 43.5, 40.5, 40.4, 37.4, 37.3, 31.2, 30.9, 30.7, 30.6, 29.2, 28.4, 28.3, 27.4, 26.1, 24.8, 21.8, 19.9; Elemental analysis calculated for

$C_{44}H_{67}F_9N_8O_{13}\cdot 4H_2O$: C, 45.59; H, 6.52; N, 9.67; Found: C, 45.78; H, 6.76; N, 9.89; HRMS-FAB: *m/z* Calcd for $C_{38}H_{65}N_8O_7$: 745.4971; Found: 745.4976 $(M+H)^+$.

References

1. C. Banekovich, I. Ott, T. Koch, B. Matuszczaka, and R. Gustb, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 683.

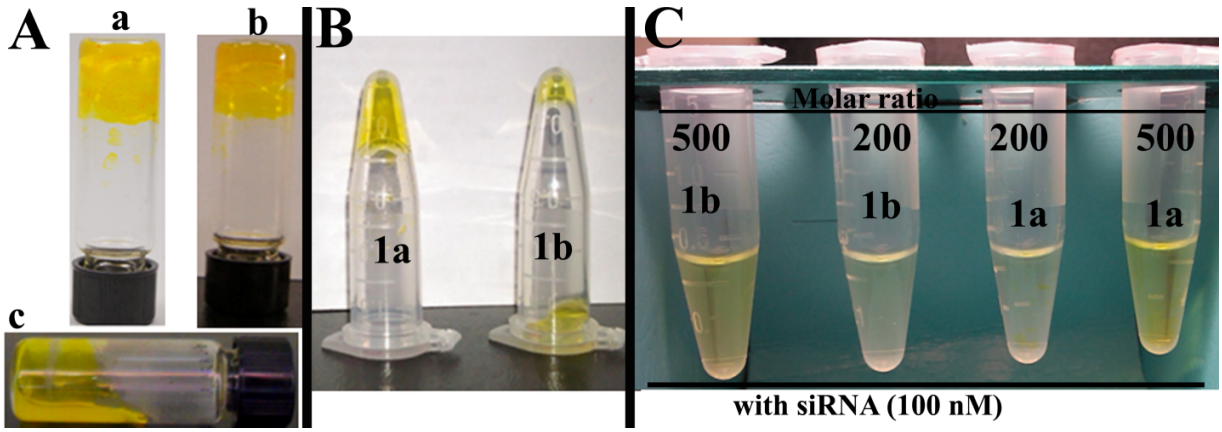


Figure S2. (A) Hydrogelation ability of 1.6 wt% of compound **1a** in different pH buffer solutions (a) pH = 5; (b) pH = 7.4; (c) pH = 9; (B) The gelation behavior of **1a** and **1b** in Opti-MEM and (C) solutions of siRNA (100 nM) with 200 and 500 molar ratio of **1a-b** in Opti-MEM prior to transfection.

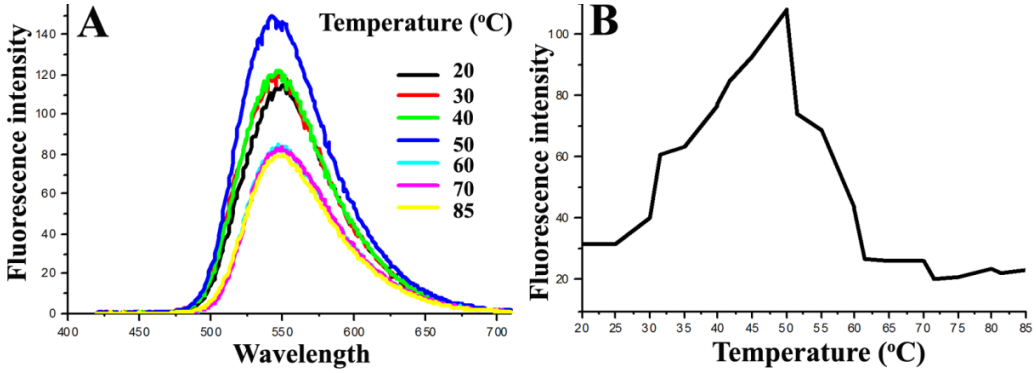


Figure S3. Fluorescent emission intensity vs. (A) wavelength at indicated temperatures, (B) temperature diagram (1.6 wt% hydrogel of **1a;** excited at 373 nm; emission wavelength: 546 nm).

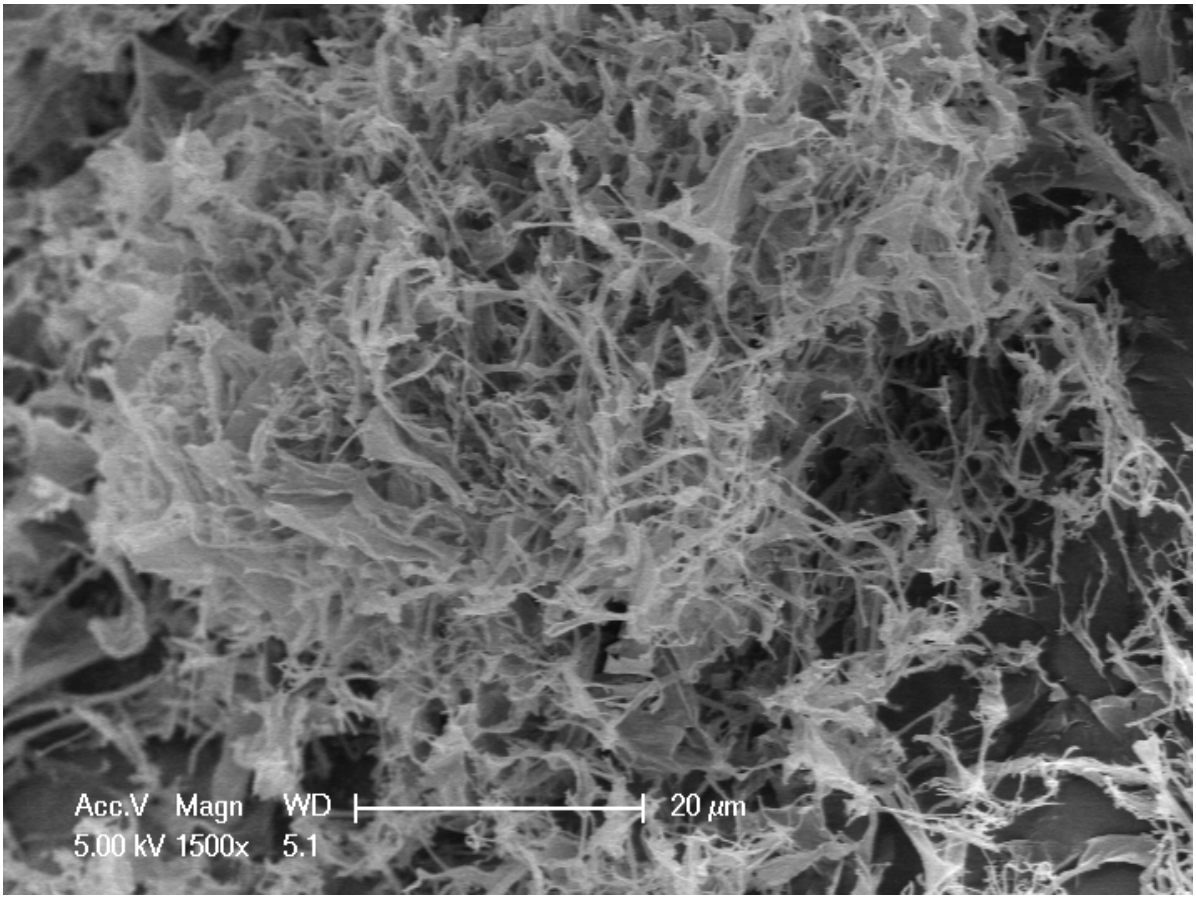


Figure S4. SEM image of xerogel of **1a** (Scale bar = 20 μm).

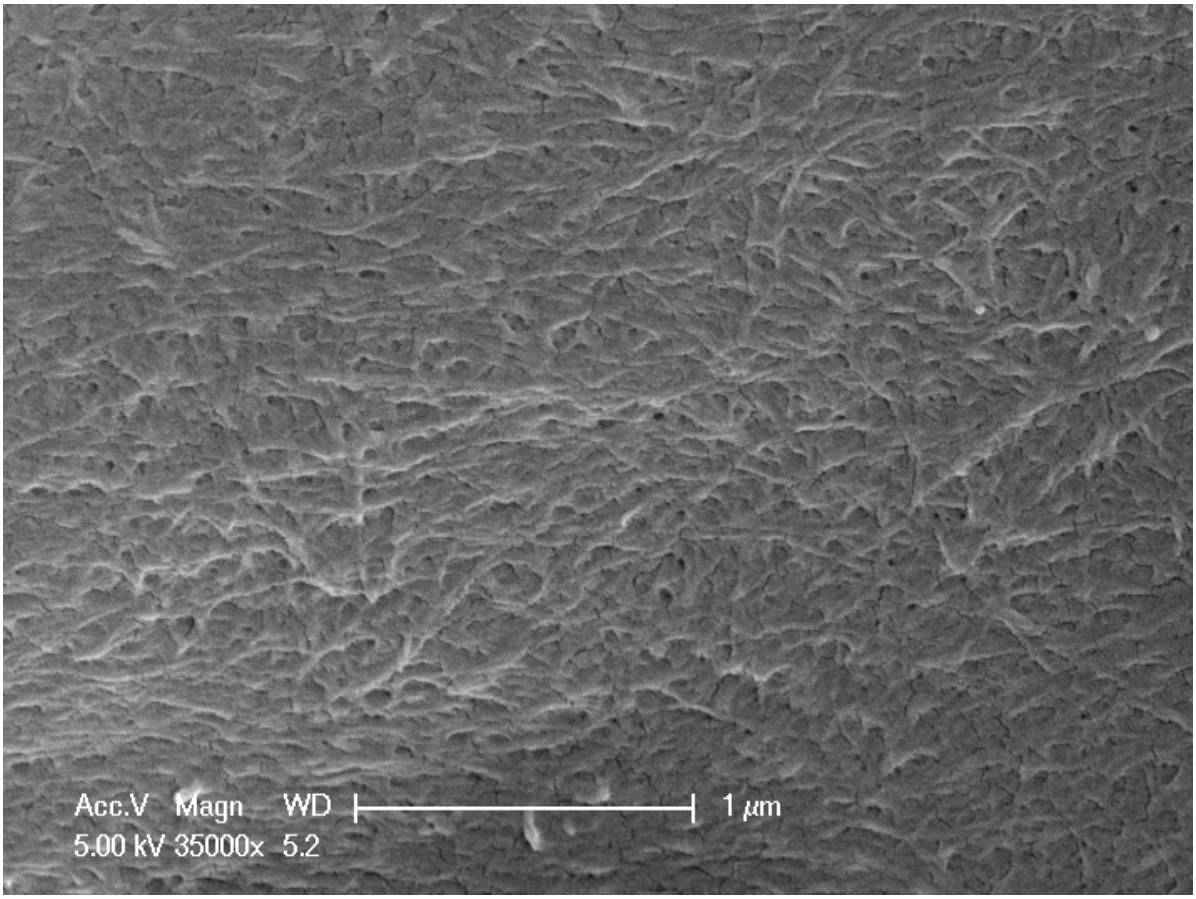


Figure S5. SEM image of xerogel of **1a** (Scale bar = 1μm).

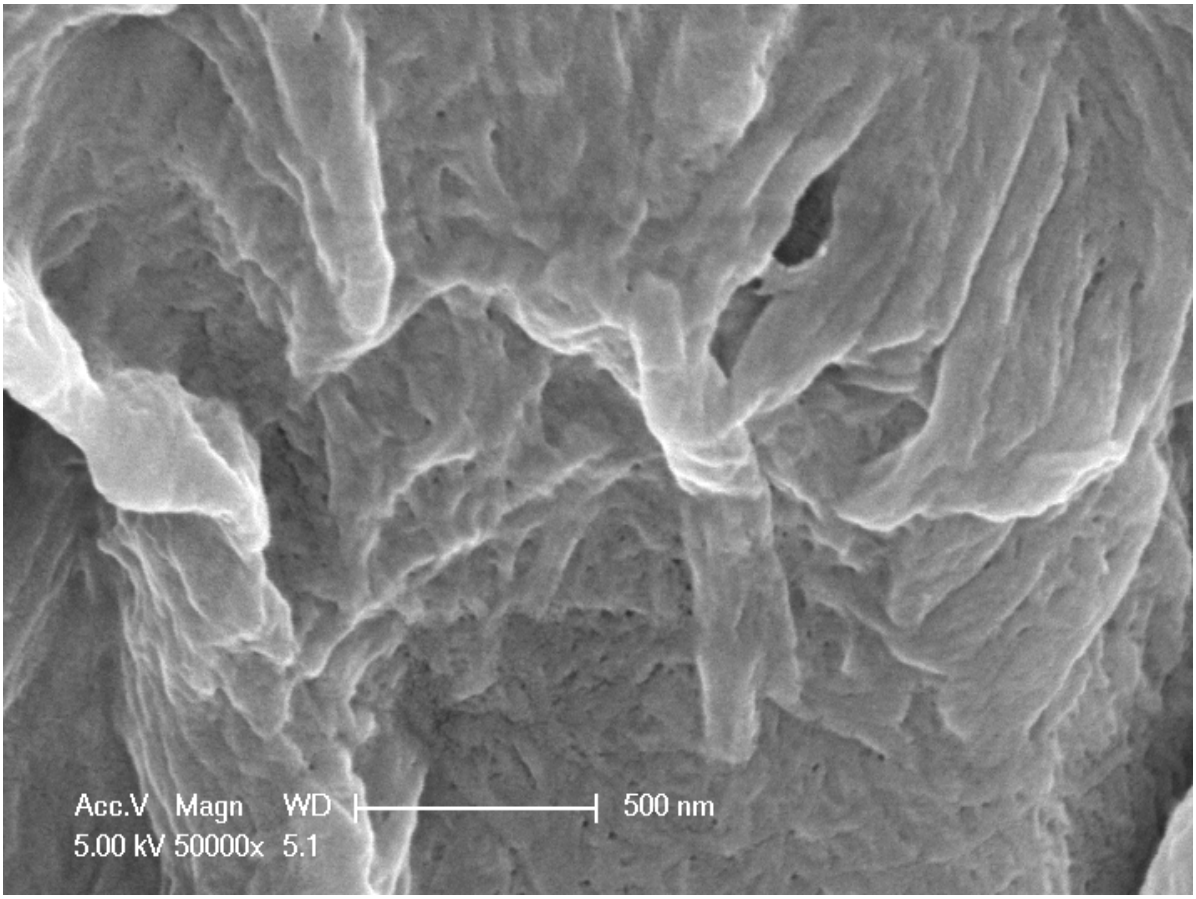


Figure S6. SEM image of xerogel of **1a** (Scale bar = 500 nm).

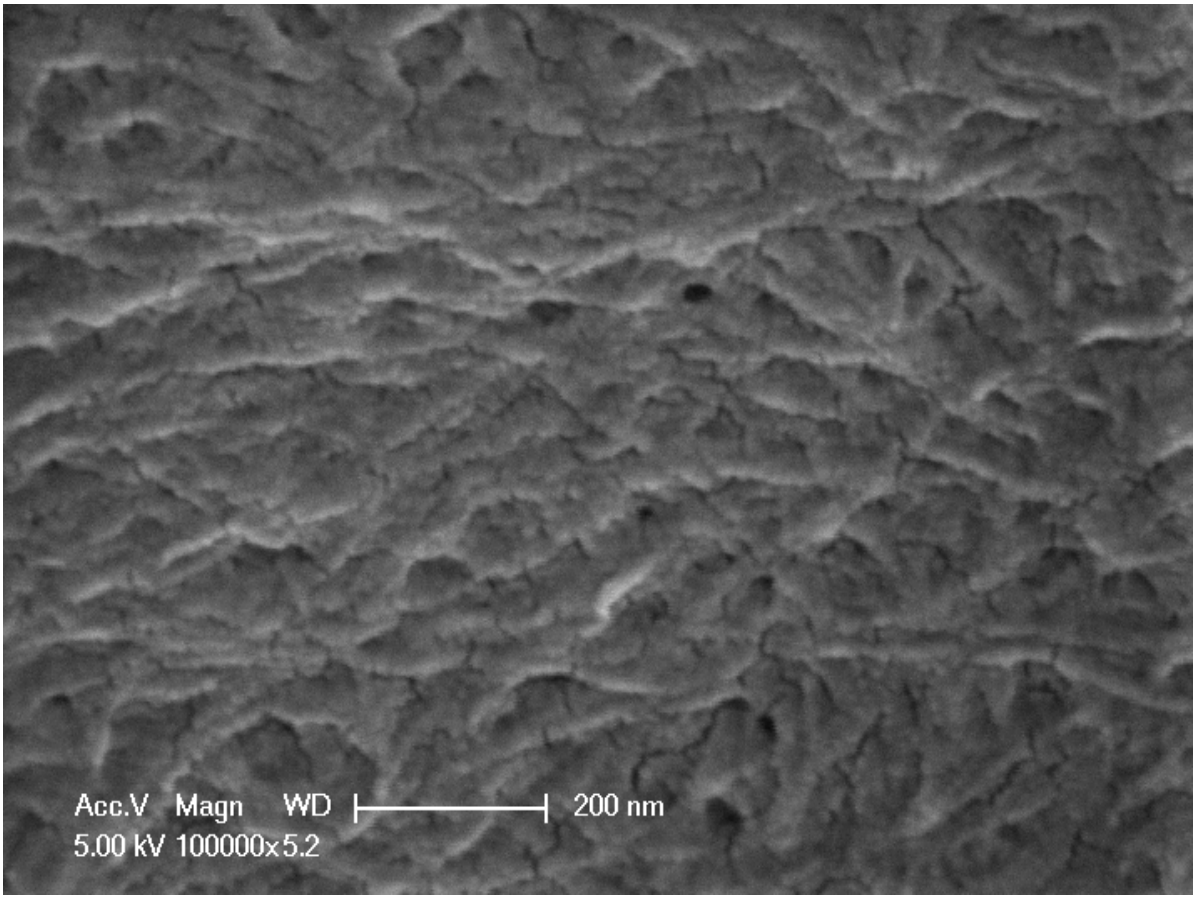


Figure S7. SEM image of xerogel of **1a** (Scale bar = 200 nm).

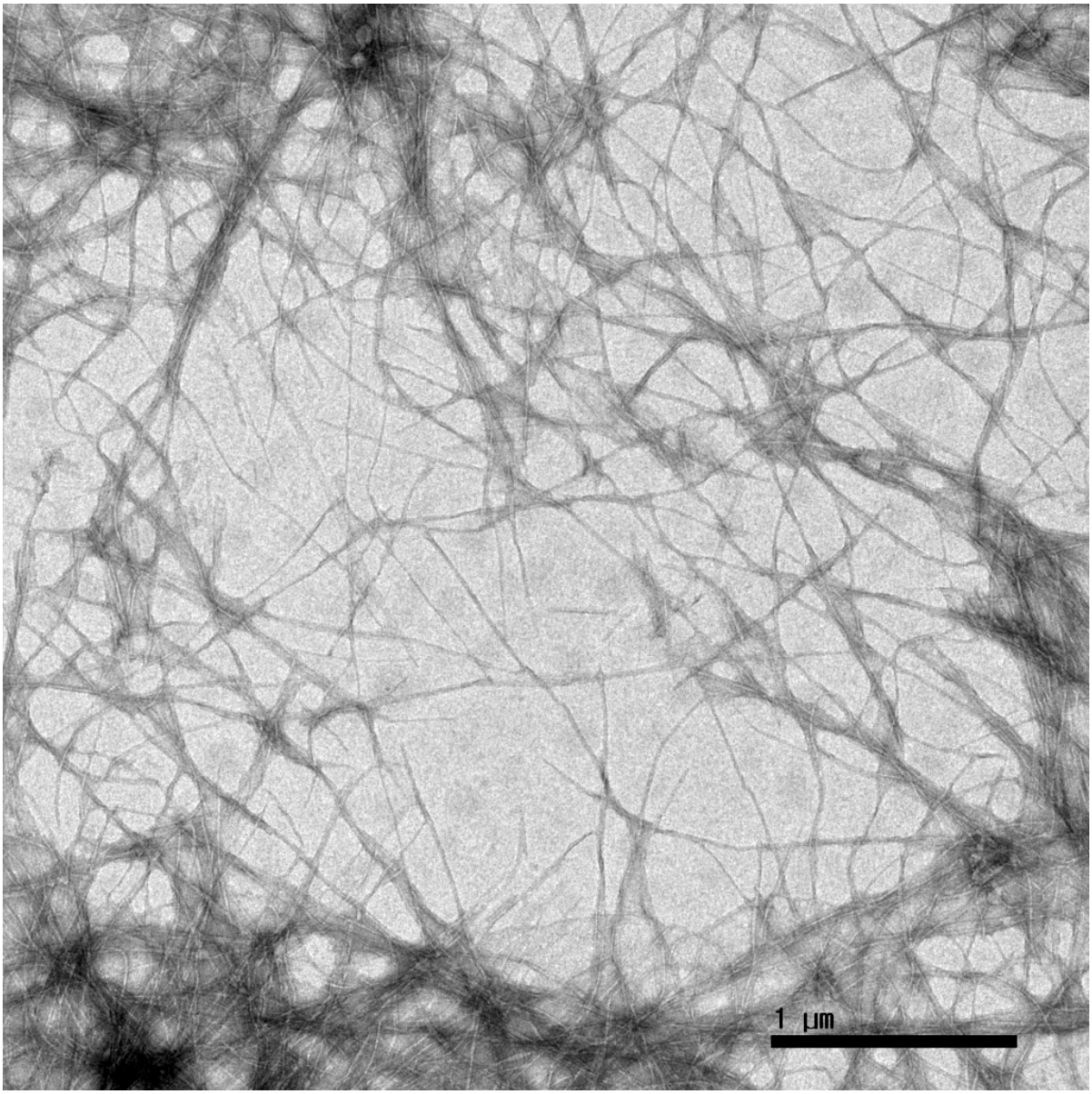


Figure S8. TEM image of xerogel of **1a** (Scale bar = 1 μm).

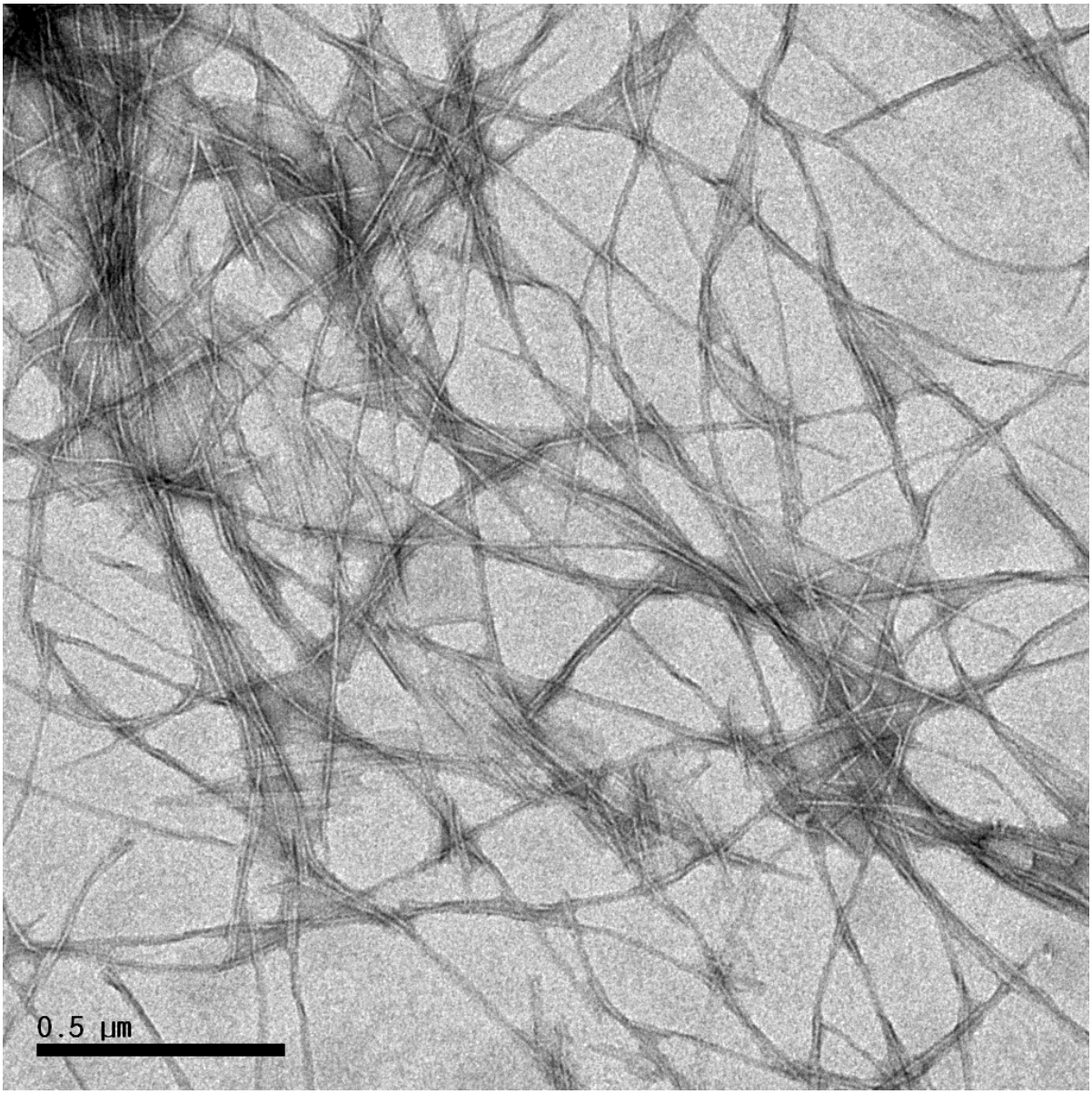


Figure S9. TEM image of xerogel of **1a** (Scale bar = 0.5 μm).

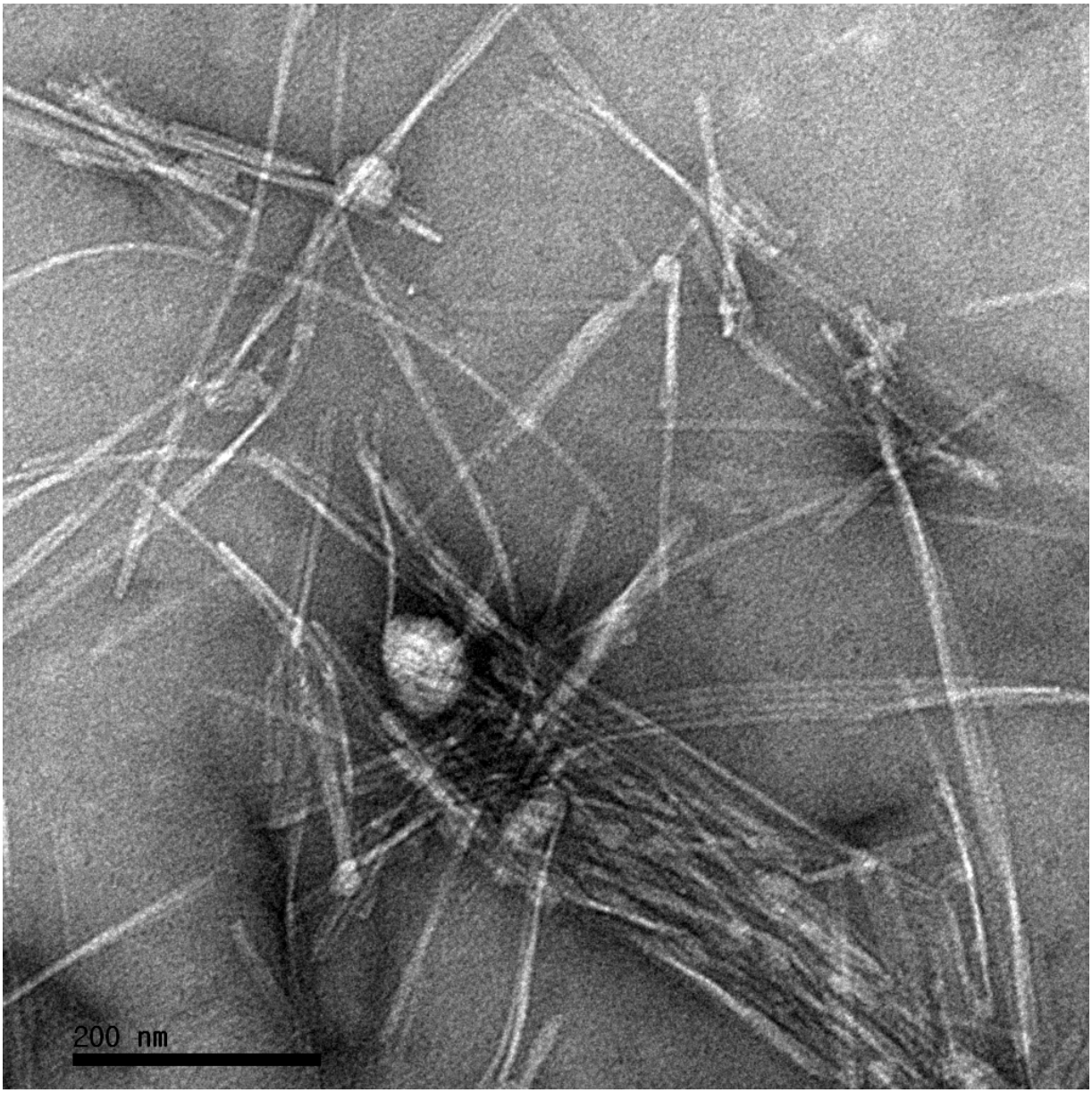


Figure S10. TEM image of xerogel of **1a** with siRNA at 5 μM concentration (Scale bar = 200 nm).

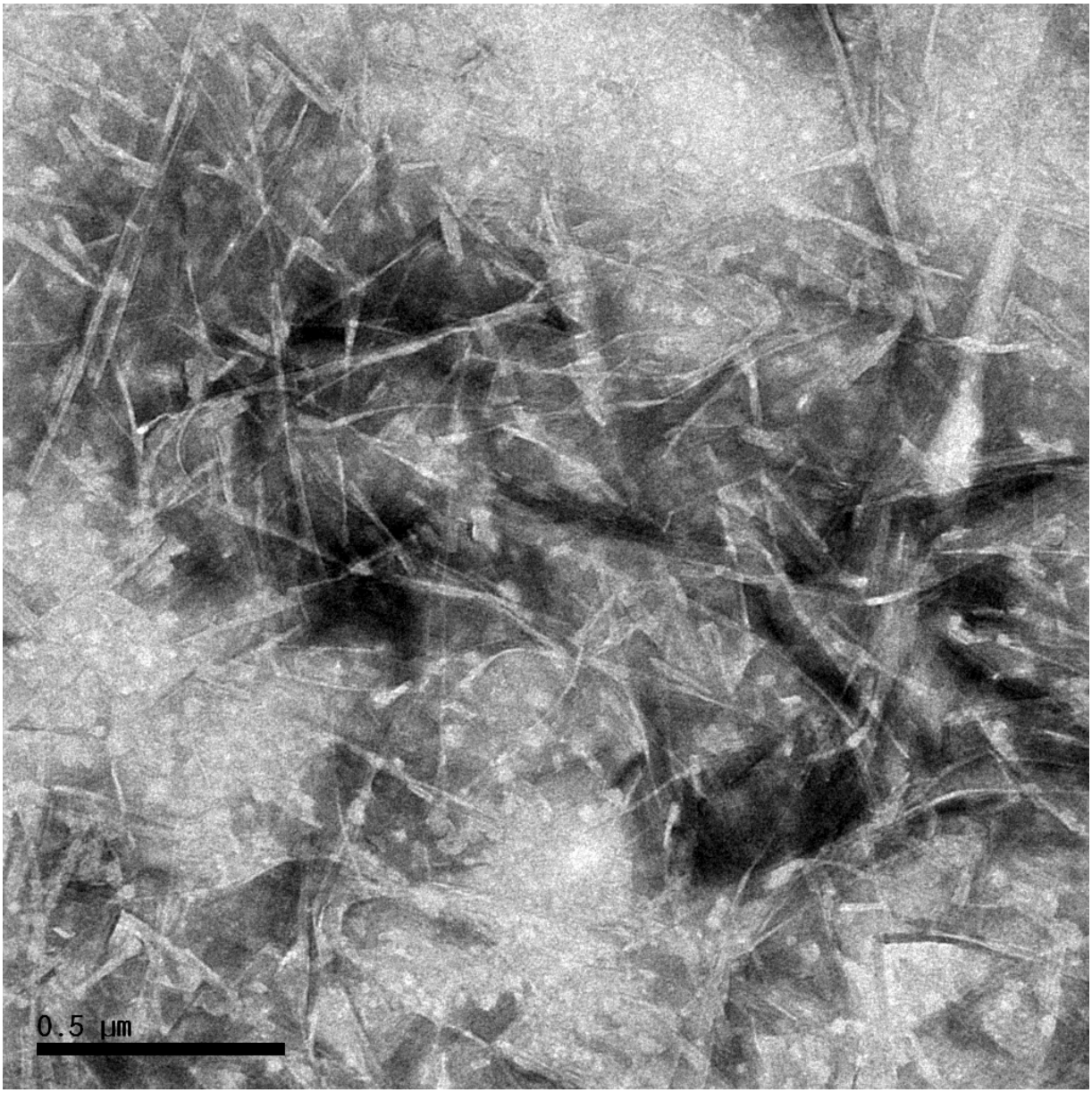


Figure S11. TEM image of xerogel of **1a** with siRNA at 10 µM concentration ((Scale bar = 0.5 µm).

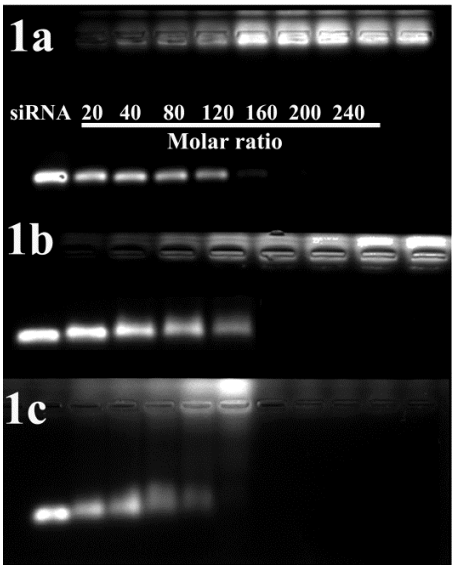


Figure S12. Gel retardation assays of the RBAs **1a**–**c**.

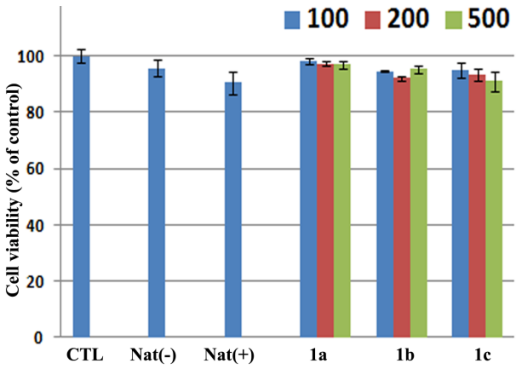


Figure S13. Cell viability assays of 100 nM VEGF-siRNA with **1a**–**c** at molar ratios of 100, 200, and 500. CTL: untreated cell; Nat(–): only siRNA; Nat(+): siRNA with lipofectamine2000; ($N = 3$).

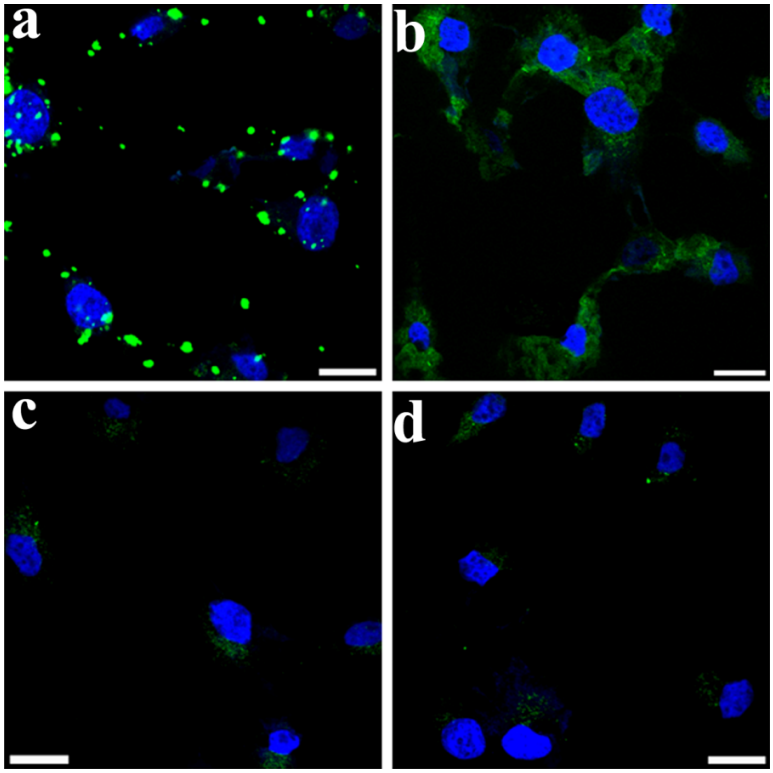


Figure S14. Live cell images using confocal microscopy (low magnification); Fl-siRNA (100 nM) incubated with (a) lipofectamin2000; (b) Hydrogel **1a**; (c) **1b**; (d) **1c**. (Scale bar = 20 μm).