Supporing Information

RGD Anchored C_2-Benzene based PEG-like Hydrogels as Scaffold for Two and Three Dimensional Cell Culture

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1. Material

The gelators M0, M1, M2, M3 were synthesized with high yields through conventional liquid phase reaction in two or three steps according to Scheme S1.[1]

1,4-benzenedicarbonyl dichloride (2.6 g, 13.0 mmol) in dry dichloromethane (DCM) (20 ml) was added dropwise to a solution of $_L$-phenylalanine methyl ester hydrochloride (6.0 g, 26.1 mmol) and Et_3N (8 ml, 58.3 mmol) in dry DCM (100 ml, T=0°C). The solution was stirred at room temperature for 24 h. All the solvents were evaporated under vacuum, and the residue was subsequently dissolved in ethanol (100 ml). After filtration, undissolved substance was collected and dried to give the dimethyl ester of M0 (5.3 g, 10.9 mmol, 84%). For the hydrolysis, aqueous NaOH (10 ml, 2.0 M) was added to a cooled (0°C) suspension of the dimethyl ester of M0 (3.0 g, 6.14 mmol) in MeOH (20 ml). The mixture was slowly brought back to room temperature and stirred for 24 h, and a clear solution was obtained. The solution was then acidified with 3.0 M HCl to pH < 3.0, and a gel-like precipitate formed. The gel phase was filtered, washed with deionized water and finally dried in the vacuum oven to give M0 (2.6 g, 5.6 mmol, 91%). Overall yield of M0: 76%. Melting point (mp): 218-220℃. M0: ^{1}H NMR (400 MHz, DMSO-d_6, δ): δ=3.2(dd, 4H, CH_2), 4.6(dt, 2H, CH), 7.3(m, 10H, Ph-H), 7.8(s, 4H, Ph-H), 8.8(d, 2H, NH), 12.8(s, 2H, OH) ppm. M0: ^{13}C NMR (400 MHz, DMSO-d_6, δ):

1 X. Q. Dou, P. Li, D. Zhang, C. L. Feng, *Soft Matter,* 2012, **8,** 3231.

δ=36.6, 54.6, 126.8, 127.7, 128.7, 129.6, 136.8, 138.5, 166.1, 173.5 ppm. M0: ESI-MS for $C_{26}H_{24}O_6N_2$ calcd. 460.49; found 460.17 [M^+].

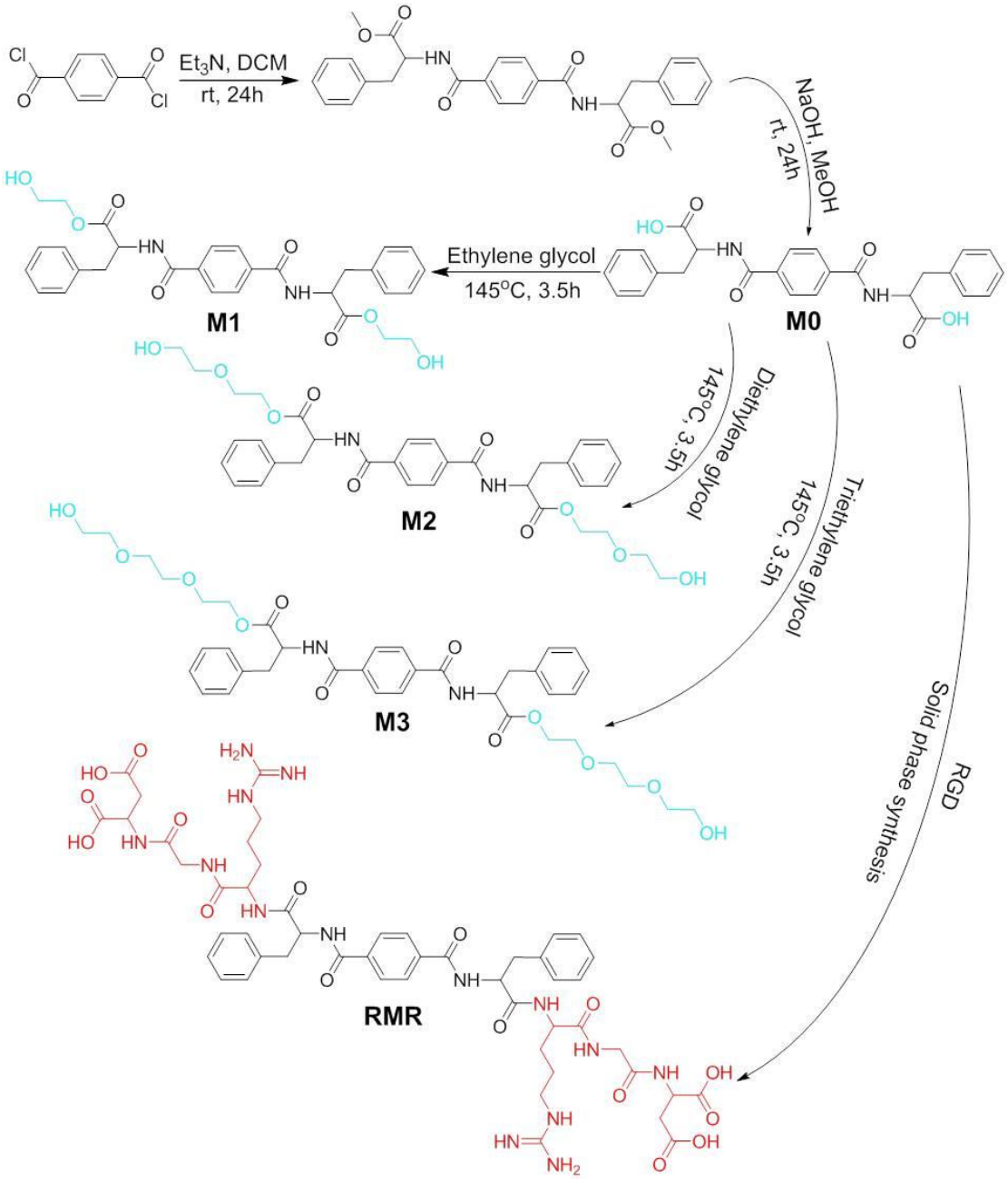
M0 (2.8 g, 6.1 mmol) in ethylene glycol (50 ml) was added to concentrated HCl (0.5ml). The mixture was stirred at 145°C for 3.5 h, then the clear solution was added to a water/ice mixture (350 ml). The gel-like precipitate was collected on a filter, washed with water, and dried in a vacuum oven to give M1 (2.8 g, 5.2 mmol, 85%). Overall yield of M1: 65%. Melting point (mp): 147-148℃. M1: ^{1}H NMR (400 MHz, DMSO-d_6, δ): δ=3.1(dd, 4H, CH_2), 3.6(m, 4H, CH_2), 4.1(t, 4H, CH_2), 4.4(t, 2H, OH), 4.7(dt, 2H, CH), 7.3(m, 10H, Ph-H), 7.8(s, 4H, Ph-H), 8.9(d, 2H, NH) ppm. M1: ^{13}C NMR (400 MHz, DMSO-d_6, δ): δ=36.6, 54.8, 59.3, 66.8, 127.0, 127.9, 128.8, 129.6, 136.6, 138.1, 166.3, 172.2 ppm.M1: ESI-MS for $C_{30}H_{32}O_8N_2$ calcd. 548.22; found 549.22 [M+H] $^+$.

M0 (2.8 g, 6.1 mmol) in diethylene glycol (50 ml) was added to concentrated HCl (0.5ml). The mixture was stirred at 145°C for 3.5 h, then the clear solution was added to a water/ice mixture (350 ml). The gel-like precipitate was collected on a filter, washed with water, and dried in a vacuum oven to give M2 (3.0 g, 4.7 mmol, 77%). Overall yield of M2: 59%. Melting point (mp): 124-126℃. M2: ^{1}H NMR (400 MHz, DMSO-d_6, δ): δ=3.1(dd, 4H, CH_2), 3.4(m, 4H, CH_2), 3.5(m, 4H, CH_2), 3.6(t, 4H, CH_2), 4.2(t, 4H, CH_2), 4.5(t, 2H, OH), 4.7(dt, 2H, CH), 7.2(m, 10H, Ph-H), 7.8(s, 4H, Ph-H), 8.9(d, 2H, NH) ppm. M2: ^{13}C NMR (400 MHz, DMSO-d_6, δ): δ=36.6, 54.9, 60.7, 64.6, 68.6, 72.6, 127.0, 127.7, 128.7, 129.5, 136.7, 137.7, 166.4, 171.0 ppm.M2: ESI-MS for $C_{34}H_{40}O_{10}N_2$ calcd. 636.71; found 637.28 [M+H] $^+$.

M0 (2.8 g, 6.1 mmol) in triethylene glycol (50 ml) was added to concentrated HCl (0.5ml). The mixture was stirred at 145°C for 3.5 h, then the clear solution was added to a water/ice mixture (350 ml). The gel-like precipitate was collected on a filter, washed with water, and dried in a vacuum oven to give M3 (2.8 g, 3.8 mmol, 62%). Overall yield of M3: 47%. Melting point (mp): 104-106℃. M3: ^{1}H NMR (400 MHz, DMSO-d_6, δ): δ=3.1(dd, 4H, CH_2), 3.4(m, 12H, CH_2), 3.6(m, 4H, CH_2), 4.2(t, 4H, CH_2), 4.6(t, 2H, OH), 4.7(dt, 2H, CH), 7.2(m, 10H, Ph-H), 7.8(s, 4H, Ph-H), 8.9(d, 2H, NH) ppm. M3: ^{13}C NMR (400 MHz, DMSO-d_6, δ): δ=36.5, 55.0, 60.7, 64.5, 68.6, 70.1, 73.0, 127.2, 128.0, 128.7, 129.4, 136.6, 138.1, 166.3, 171.0 ppm. M3: ESI-MS for $C_{38}H_{48}O_{12}N_2$ calcd. 724.32; found 725.33 [M+H] $^+$.

The gelator RMR was purchased from ChinaPeptides and used without further purification. RMR was synthesized through solid phase reaction according to Scheme S1. The purity of the RMR peptide was >95%.

Melting point (mp): 164-167℃. RMR: ^{1}H NMR (400 MHz, DMSO-d_6, δ): δ=1.5(m, 8H, CH_2), 2.0(dt, 4H, CH_2), 2.7(m, 4H, CH_2), 3.1(m, 4H, CH_2), 3.8(m, 4H, CH_2), 4.3(m, 2H, CH), 4.5(m, 2H, CH), 4.7(m, 2H, CH), 5.4(m, 4H, CH_2), 7.1(m, 10H, Ph-H), 7.2(m, 6H, NH), 7.8(s, 4H, Ph-H), 8.2(m, 6H, NH_2, =NH), 8.6(m, 2H, NH), 12.7(s, 4H, OH) ppm. RMR: ESI-MS for $C_{50}H_{64}O_{16}N_{14}$ calcd. 1116.46; found 1117.47 $[M+H]^+$.



Scheme S1. Synthetic route of M0, M1, M2, M3, and RMR.

1.1 ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy

The ^{1}H NMR studies were carried on a Bruker Advance Ⅲ 400 Instrument operating at 400 MHz. All spectra were recorded in DMSO.

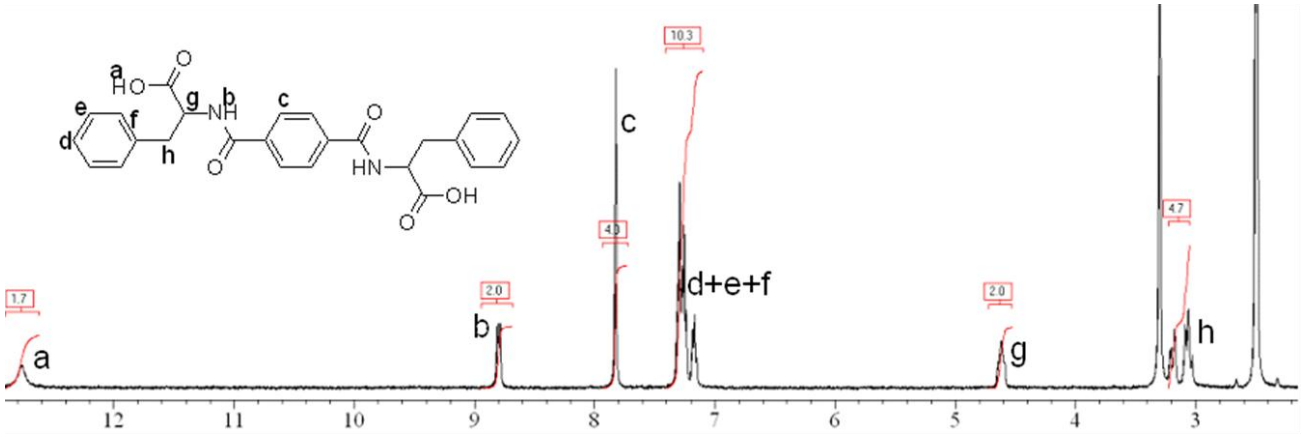


Fig. S1. The ^{1}H NMR spectrum of M0.

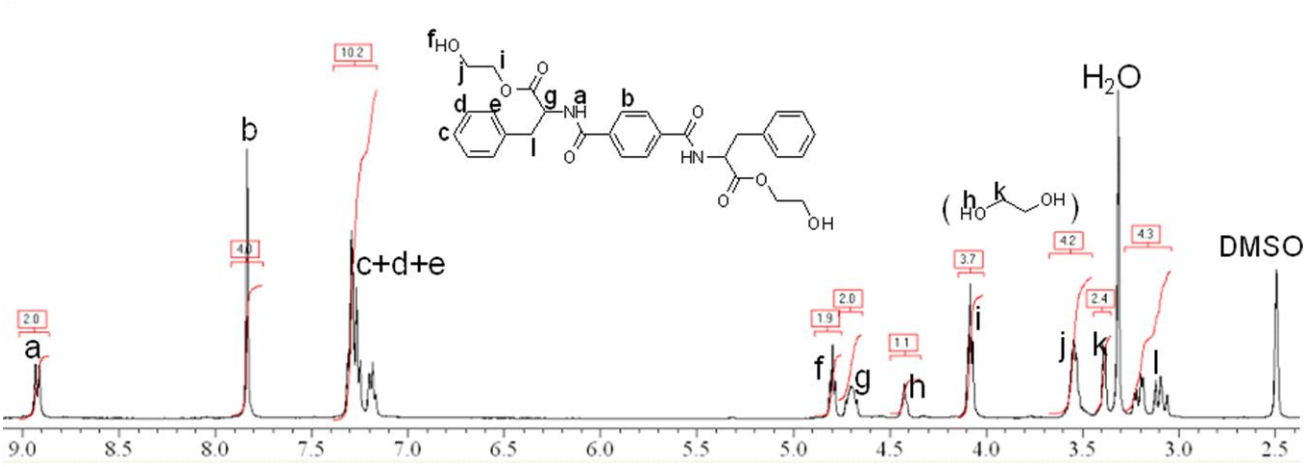


Fig. S2. The ^{1}H NMR spectrum of M1.

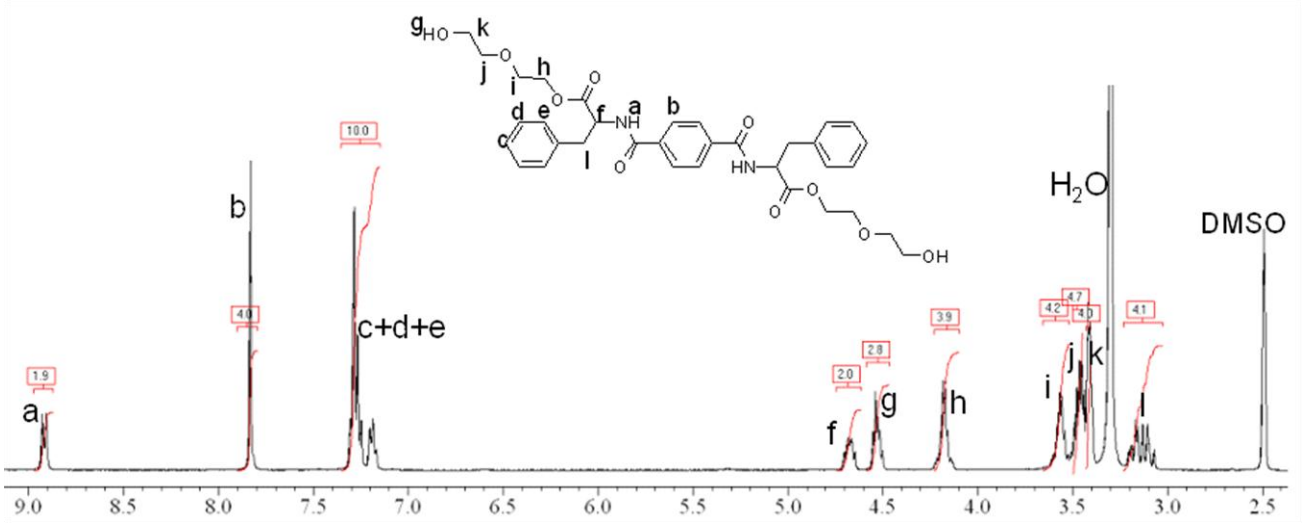


Fig. S3. The ^{1}H NMR spectrum of M2.

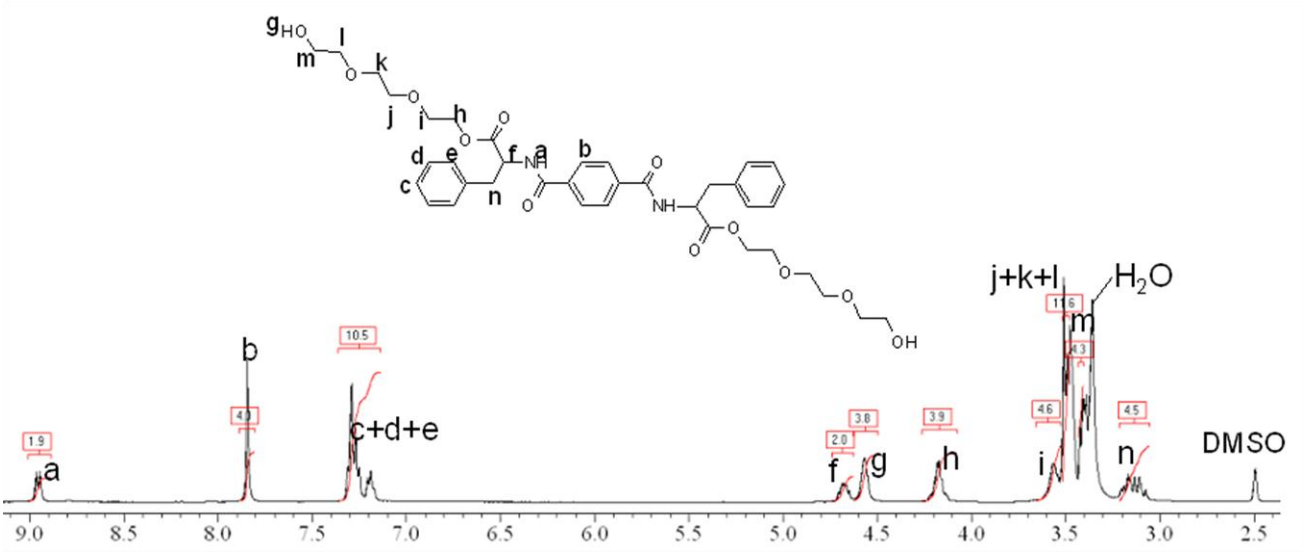


Fig. S4. The ^{1}H NMR spectrum of M3.

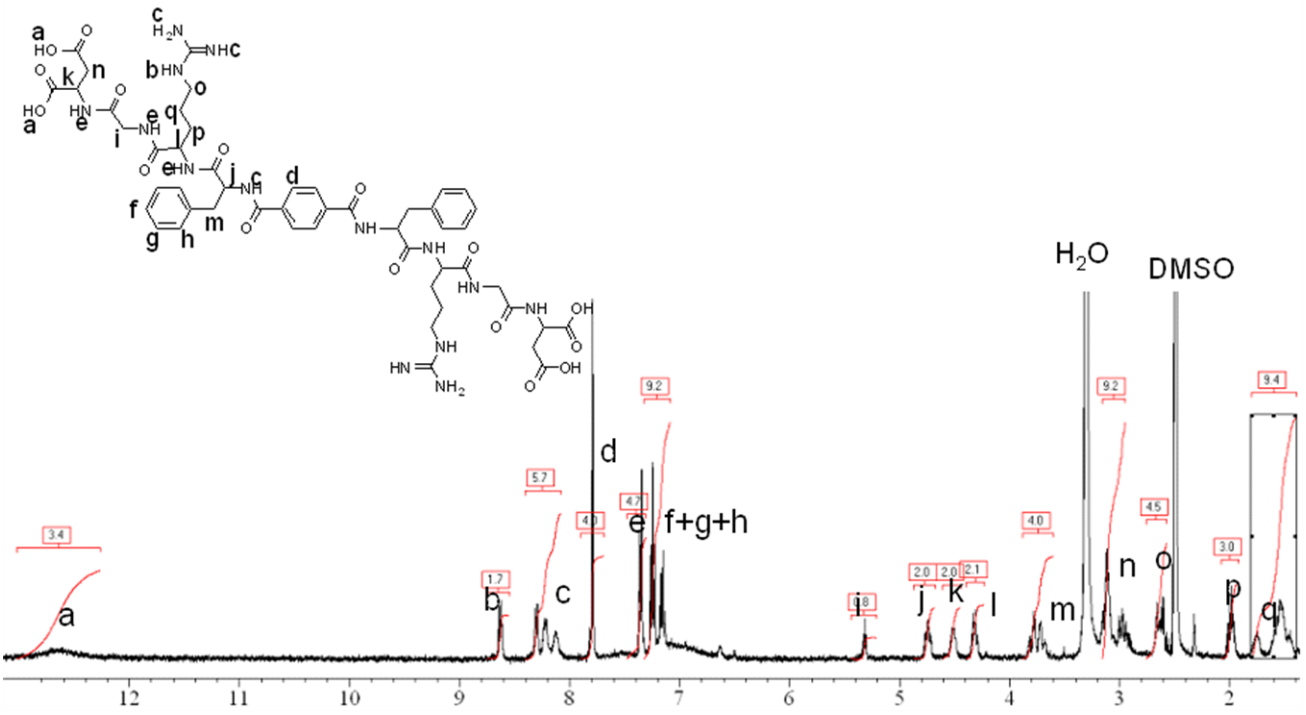


Fig. S5. The ^{1}H NMR spectrum of RMR.

1.2 ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectroscopy

The ^{13}C NMR studies were carried on a Bruker Advance Ⅲ 400 Instrument operating at 400 MHz. All spectra were recorded in DMSO.

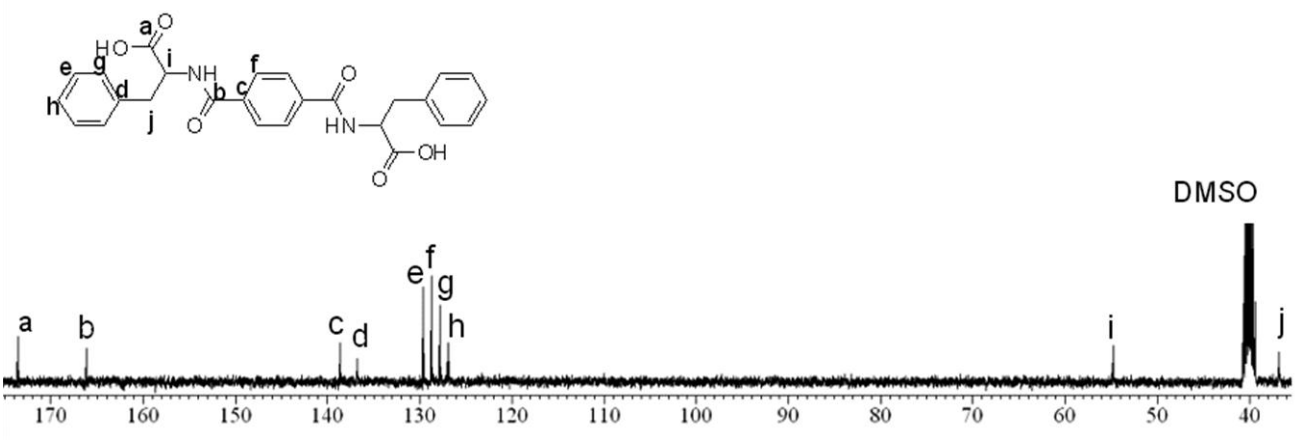


Fig. S6. The ^{13}C NMR spectrum of M0.

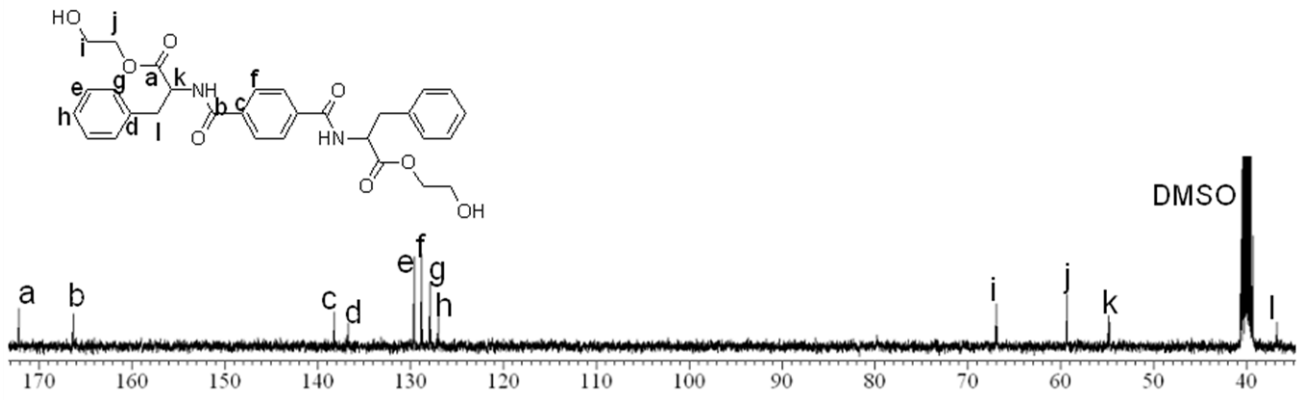


Fig. S7. The ^{13}C NMR spectrum of M1.

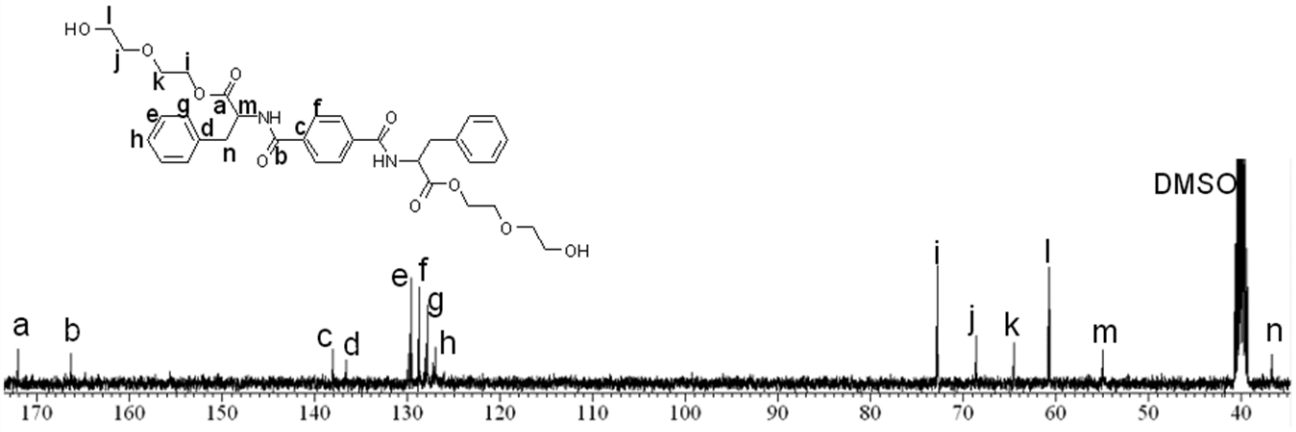


Fig. S8. The ^{13}C NMR spectrum of M2.

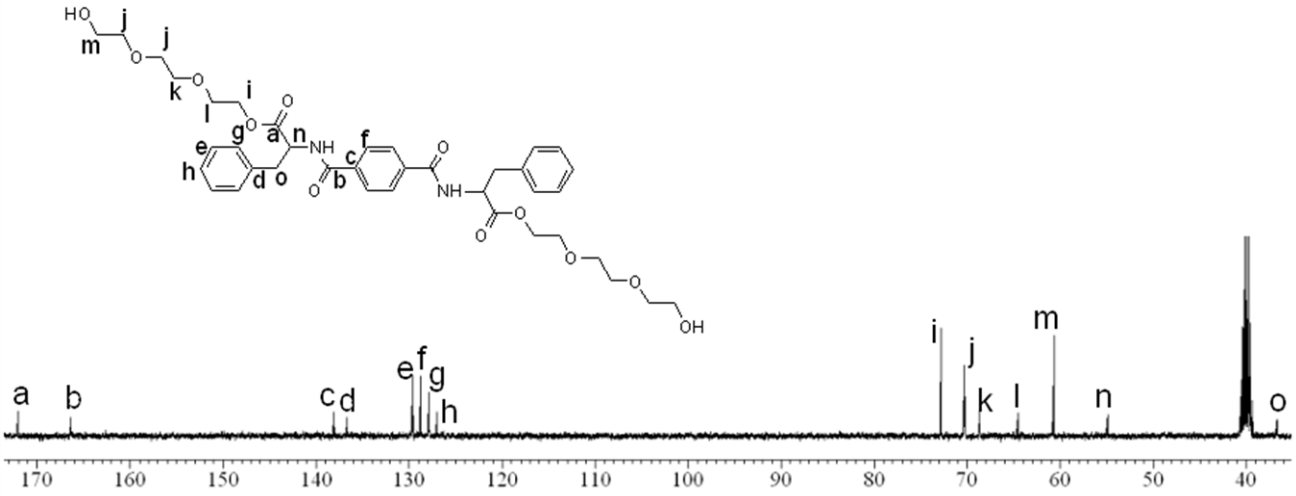


Fig. S9. The ^{13}C NMR spectrum of M3.

1.3 Electrospray Ionization-Mass (ESI-MS) spectroscopy

The ESI-MS spectra were recorded on a Waters Q-Tof Mass Instrument by positive mode electrospray ionization. Methanol was used as the solvent.

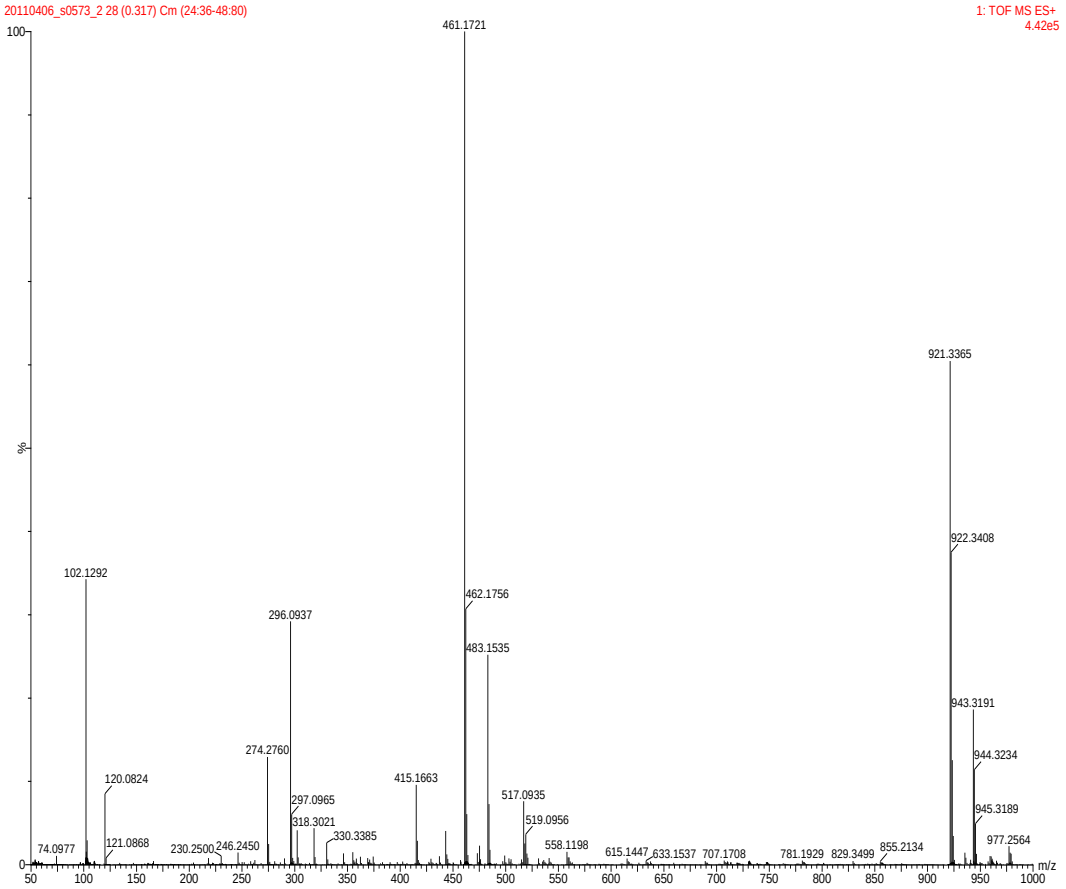


Fig. S10. The ESI-MS spectrum of M0.

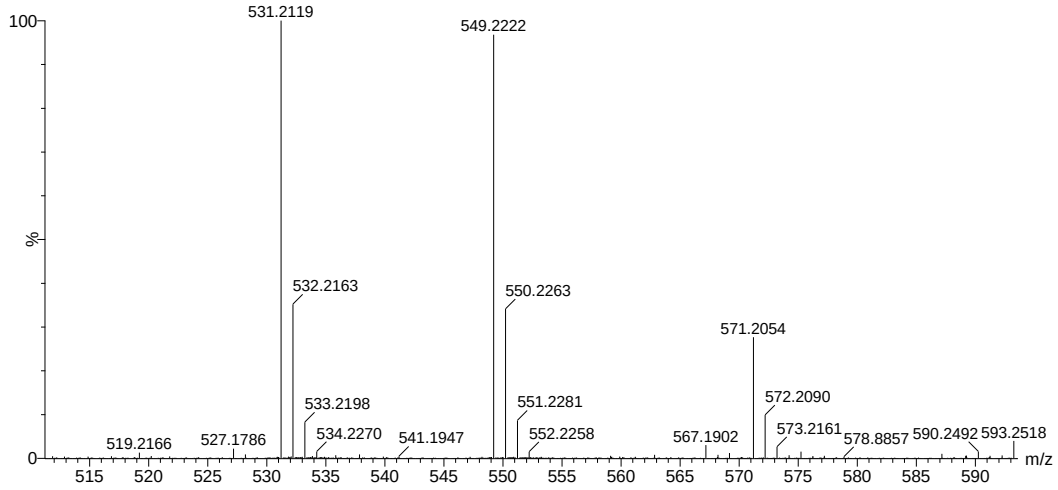


Fig. S11. The ESI-MS spectrum of M1.

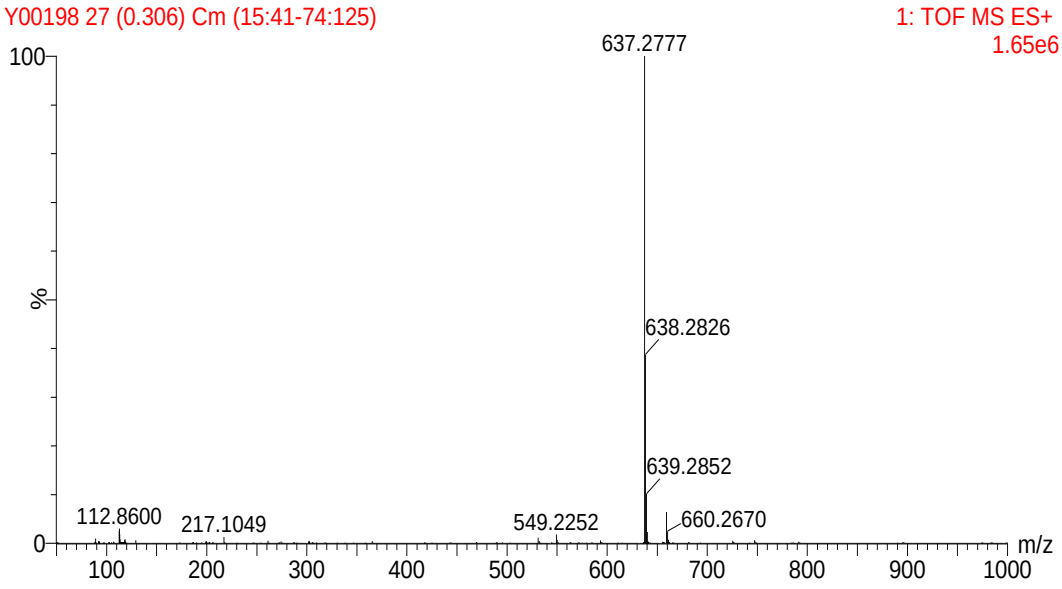


Fig. S12. The ESI-MS spectrum of M2.

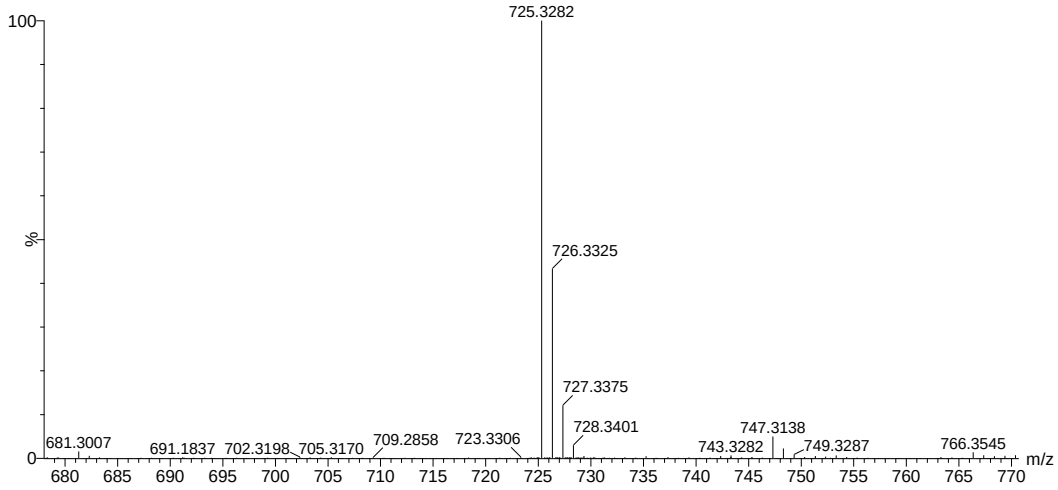


Fig. S13. The ESI-MS spectrum of M3.

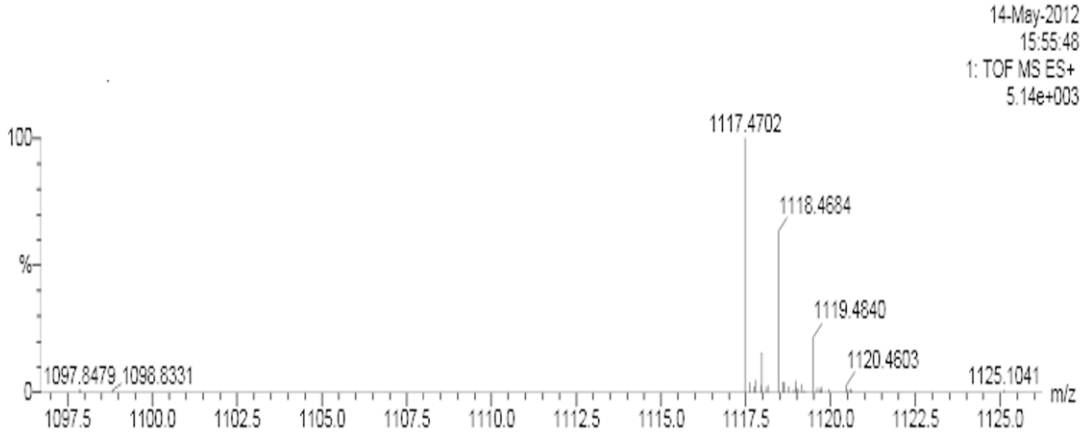


Fig. S14. The ESI-MS spectrum of RMR.

1.4 High Performance Liquid Chromatography (HPLC) spectroscopy

To verify the purity of the RMR peptide, HPLC spectroscopy was performed. The HPLC spectrum was collected utilizing a Waters 2695 pump with a Kromasil 100-5C15 column (length: 250 mm, internal diameter: 4.6 mm, fused silica particles: 5 μm). A 10 μL sample was injected onto the column at a flow rate of 1 mL/minute. The purity of identified peak was determined by the UV detector at 220 nm. The purity of the RMR peptide was >95%.

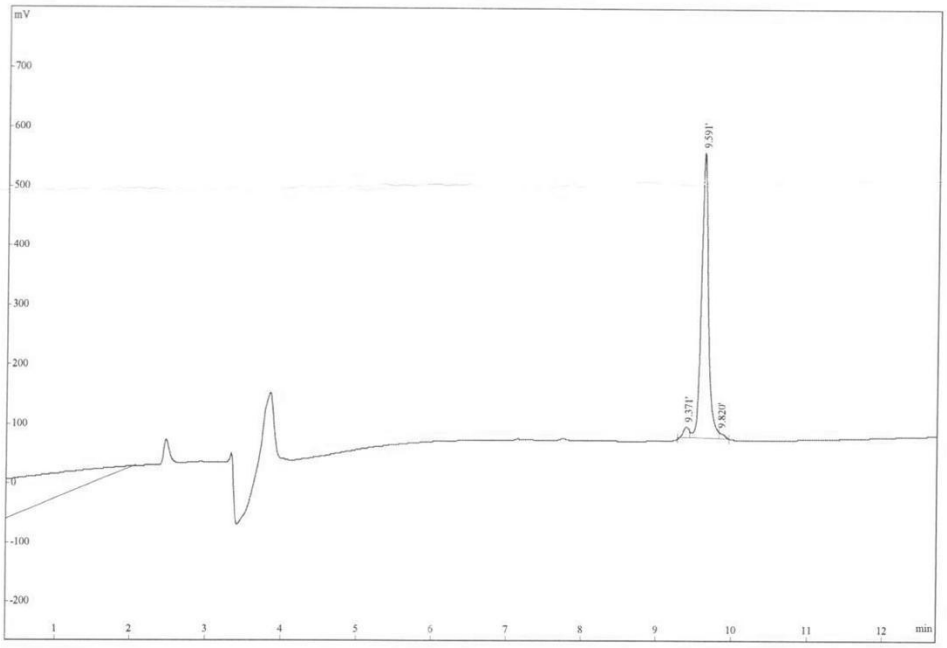


Fig. S15. The HPLC spectrum of RMR.

2. Morphological study of M1 and M3

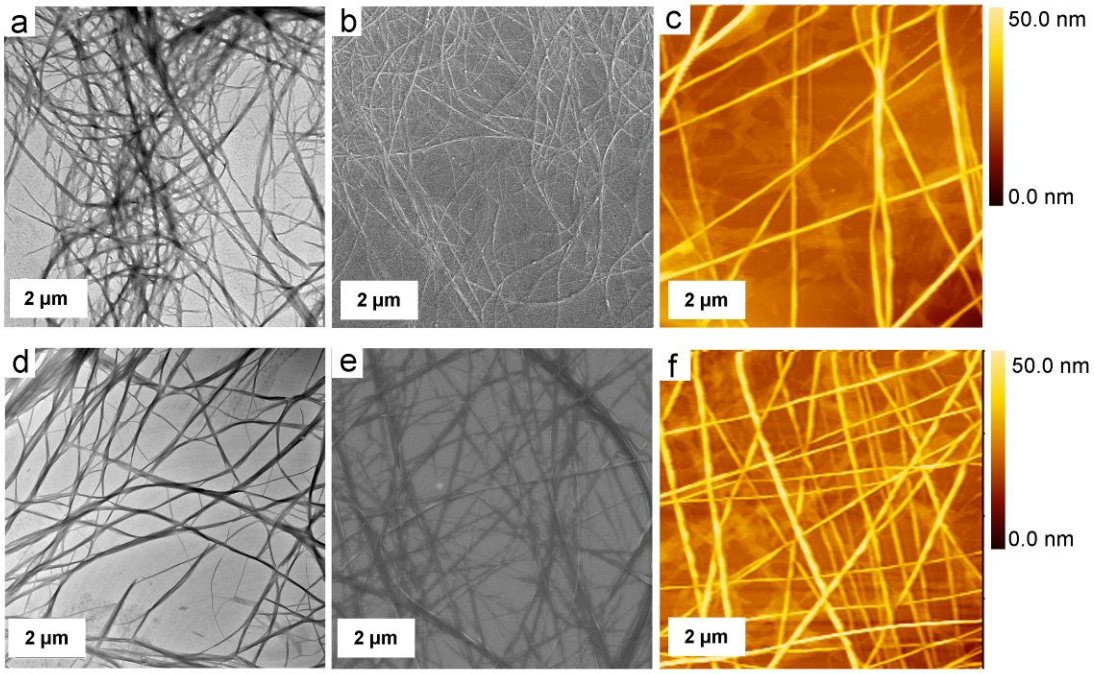


Fig. S16. The TEM images of (a) M1 and (d) M3. The SEM images of (b) M1 and (e) M3. The AFM images of (c) M1 and (f) M3.

3. FT-IR and SAXS study

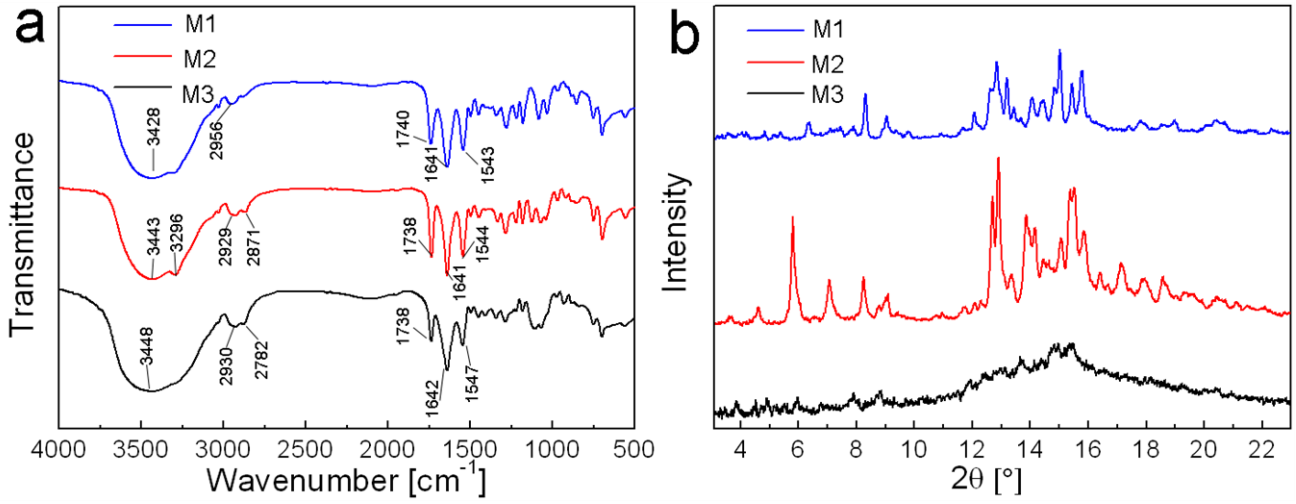


Fig. S17. (a) FT-IR spectra obtained from xerogels M1~ M3. (b) SAXS patterns of M1~ M3.

4. Morphological study of M2/RMR

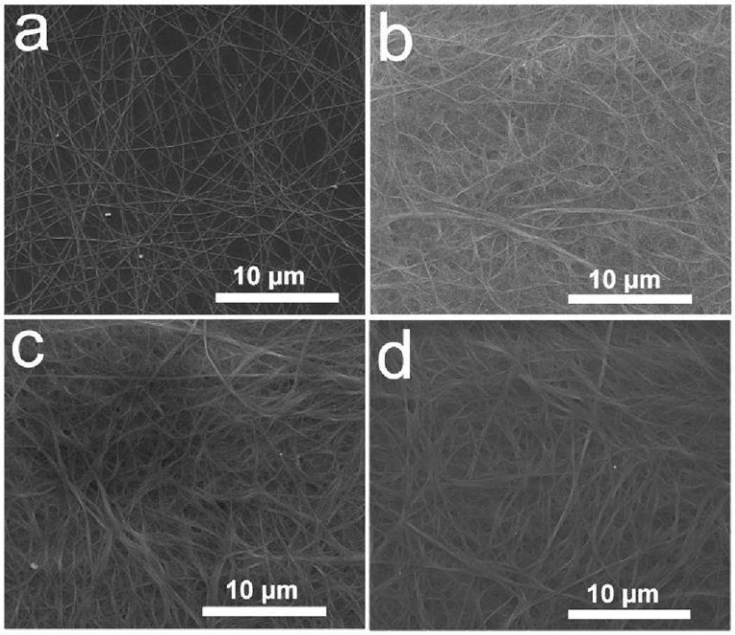


Fig. S18. The SEM images of M2/RMR containing (a) 0 %, (b) 10%, (c) 30%, and (d) 50% RMR.

5. Rheology properties of M2 and M2/RMR

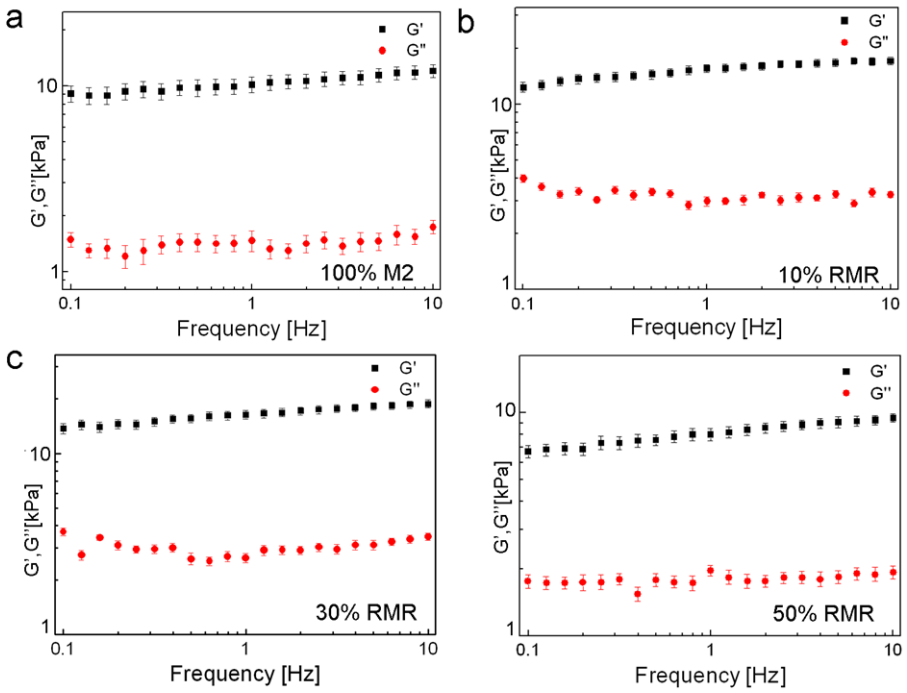


Fig. S19. Rheology properties of M2 and M2/RMR.

6. Protein adsorption study

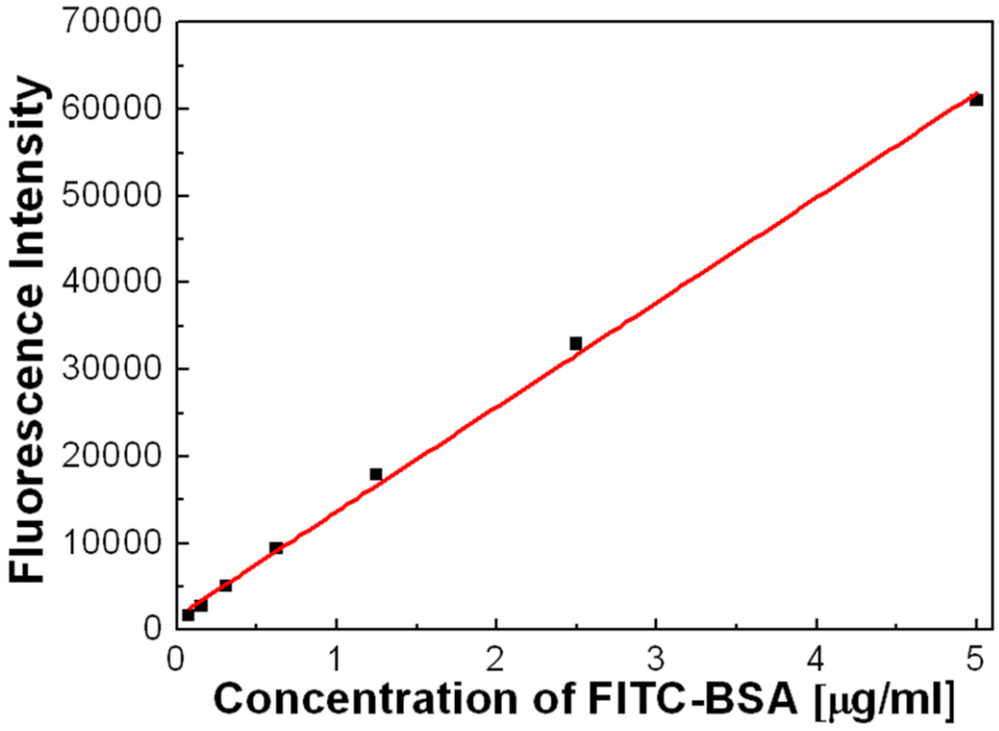


Fig. S20. Calibration curve of FITC-BSA.

7. Cell culture

7.1 2D cell culture

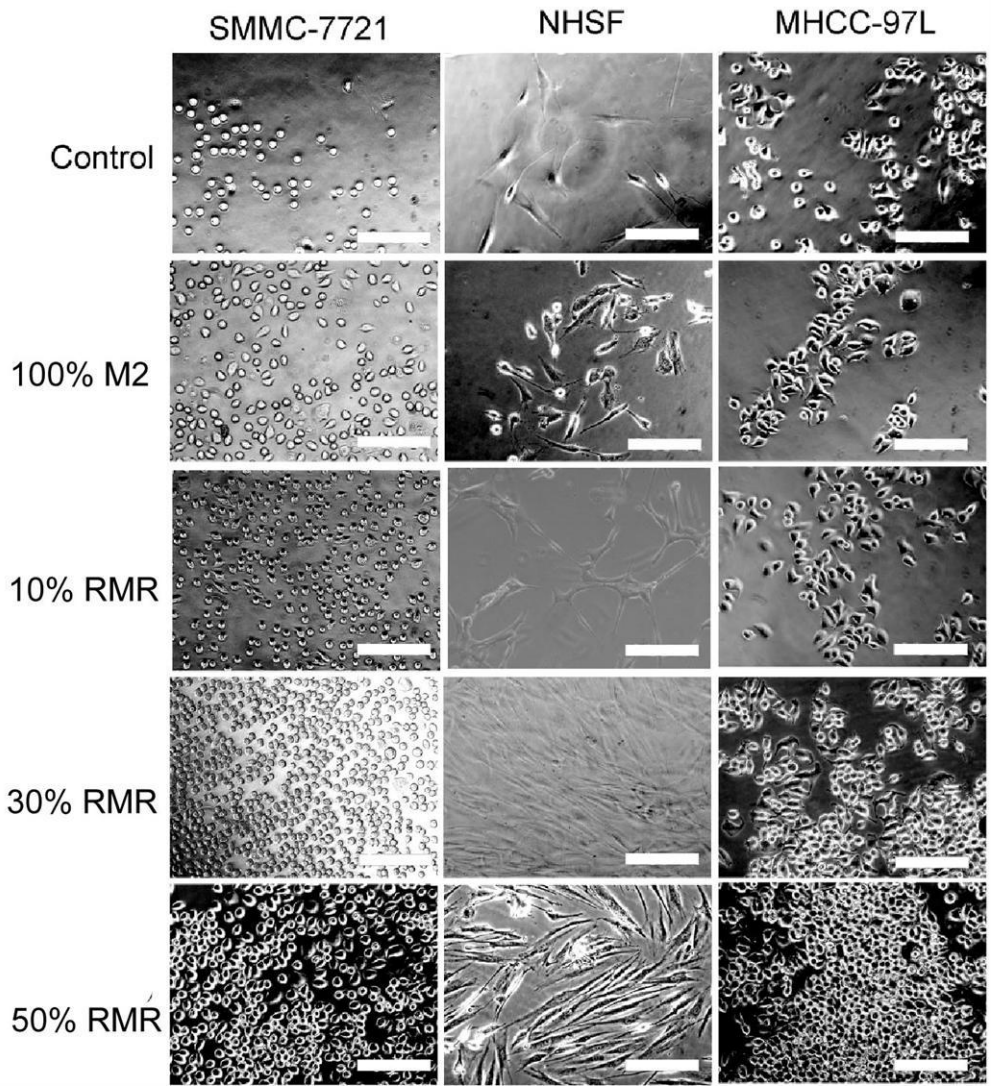


Fig. S21. The phase-contrast images of SMMC-7721, NHSF, and MHCC-97L cultured on 2D surfaces after 24 h. Scale bar represents 400 μm.

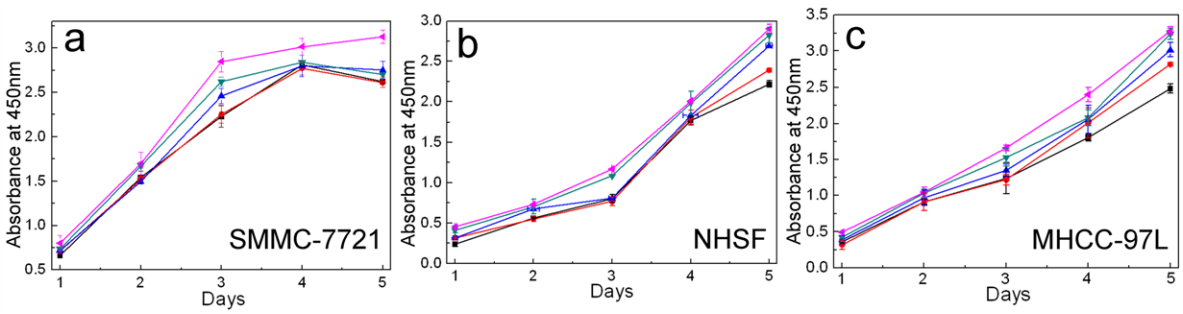


Fig. S22. CCK-8 assay results of (a) SMMC-7721, (b) NHSF, and (c) MHCC-97L cultured on 2D surfaces (—■—: Control; —■—: 100% M2; —▲—: 10% RMR; —▼—:30% RMR; —◀—: 50% RMR).

7.2 3D cell culture

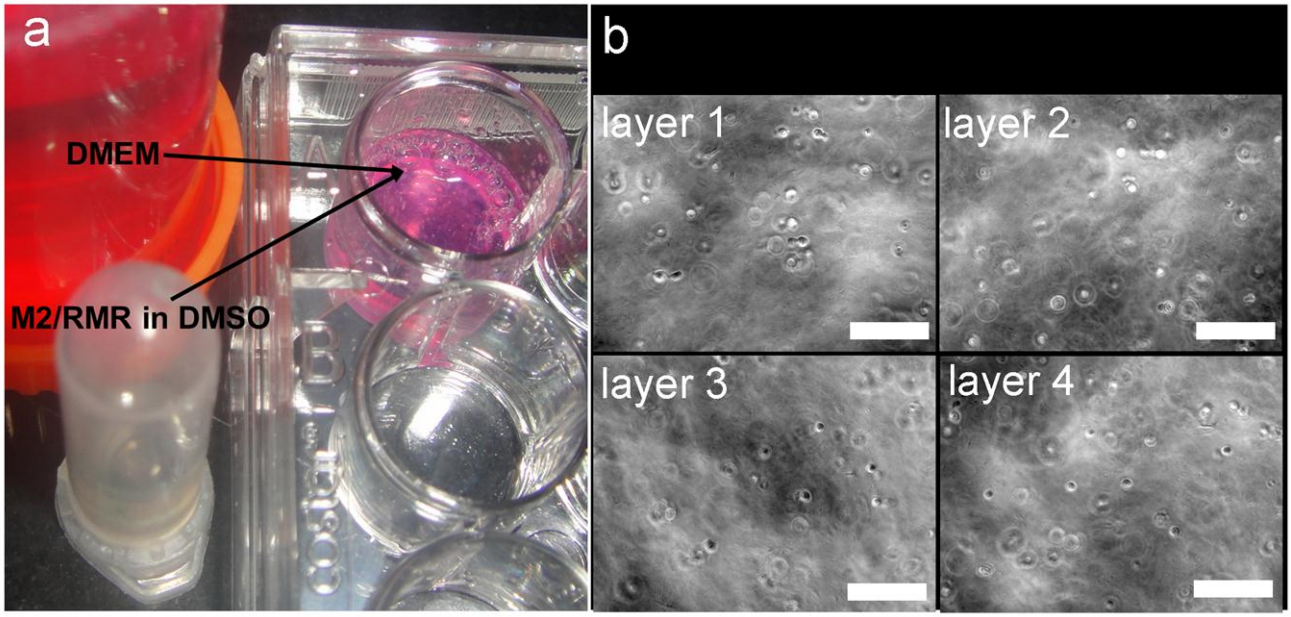


Fig. S23. (a) Photograph of M2/RMR gels prepared in DMEM in 24 well plates. (b) Different layers of cell images in the hydrogel.

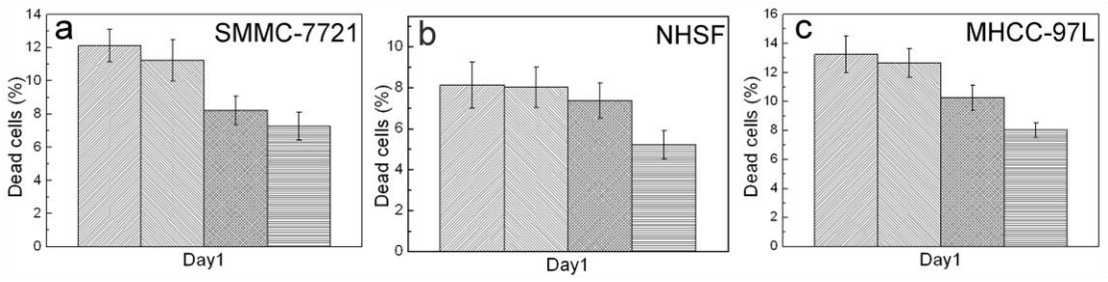


Fig. S24. Dead cells were counted after live-dead staining. The percentage of dead cells of (b) SMMC-7721, (d) NHSF, and (f) MHCC-97L were tested after 24 h in 3D culture. (▨: 100% M2; ▨: 10% RMR; ▨: 30% RMR; ▨: 50% RMR)