Supporting Information

Photoactuating Artificial Muscles of Motor Amphiphiles as an Extracellular Matrix Mimetic Scaffold for Mesenchymal Stem Cells

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Materials and Methods

1. Materials. All commercial reagents and solvents were purchased from Aldrich, TCI, Acros, Fluka, or Merck, which were used as received unless otherwise stated. All reactions were performed under an inert atmosphere (Nitrogen). Analytical TLC was performed with Merck silica gel 60 F254 plates and visualization was accomplished by UV light. Flash chromatography was carried out using Merck silica gel 60 (230-400 mesh ASTM). Solvents for spectroscopic studies were of spectrophotometric grade (UVASOL Merck). Compounds 4, i.e., 5-dihyderoxy-9H-thioxanthen-9-one (1) and hydrazone (5) were synthesized according to our previously reported procedures.

2. General. NMR spectra were recorded at 25 °C on Varian AMX400 (1H: 400 MHz, 13C: 100 MHz), Varian Unity Plus (1H: 500 MHz, 13C: 125 MHz), or Bruker Avance 600 (1H: 600 MHz, 13C: 150 MHz) NMR spectrometers. The deuterated solvents (CD2Cl2 and CDCl3) were treated with Na2CO3 and molecular sieves (4Å), followed by degassing with argon prior to use. Chemical shifts (δ) are expressed relative to the resonances of the residual non-deuterated solvent for 1H [CDCl3: 1H(δ) = 7.26 ppm, CD2Cl2: 1H(δ) = 5.32 ppm] and 13C [CDCl3: 13C(δ) = 77.16 ppm]. Absolute values of the coupling constants are given in Hertz (Hz), regardless of their sign. Multiplicities are abbreviated as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), multiplet (m), and broad (br). High-resolution mass spectrometry (HRMS) was performed on an LTQ Orbitrap XL spectrometer with electrospray ionization (ESI) technique. UV-vis measurements were performed on a Hewlett-Packard HP 8543 Diode Array UV-vis Spectrophotometer in a 1 cm path length quartz cuvette. The UV-vis samples were irradiated by using a LED light from Thorlabs (M365FP1, 0.2 A) at 20 °C, positioned at a distance of 1.0 cm from the samples. Photoactuation experiments were carried out with a LED light from Thorlabs (M365FP1, 0.7 A).

3. Synthesis.
Compound 3:

To a suspension of compound 1 (180 mg, 0.74 mmol) and K₂CO₃ (407 mg, 2.95 mmol) in 20 mL DMF, alkyl bromide 2 (516 mg, 1.85 mmol) was added at 25 °C, whereupon the mixture was stirred at 85 °C for 18 h. After cooling down to room temperature, the reaction mixture was poured into water (20 mL) and subsequently washed with water (40 mL) and ethyl acetate (20 mL). The combined organic layers were washed with brine and dried over Na₂SO₄, followed by removing the solvent under reduced pressure. The residue was subjected to column chromatography on SiO₂ (ethyl acetate/ pentane; v/v = 1/5, Rf = 0.5) to allow for the isolation of compound 3 (268 mg, 0.42 mmol, 57% yield) as a pale yellow powder.

¹H NMR (400 MHz, CDCl₃) δ (ppm) 8.18 (d, J = 8.0 Hz, 2H), 7.35 (dd, J = 8.0, 8.0 Hz, 2H), 7.06 (d, J = 8.0 Hz, 2H), 4.12 (t, J = 6.4 Hz, 4H), 4.00 (t, J = 6.8 Hz, 4H), 2.00 (s, 6H), 1.91 – 1.84 (m, 4H), 1.60 – 1.49 (m, 8H), 1.40 – 1.28 (m, 20H).

¹³C NMR (100 MHz, CDCl₃) δ (ppm) 180.3, 171.1, 154.4, 130.0, 128.4, 125.8, 121.2, 113.1, 69.4, 64.6, 29.5, 29.5, 29.3, 29.0, 28.6, 26.0, 26.0, 21.0.

HRMS (ESI): calcd. for C₃₇H₅₂O₇S [M+Na] 663.3326, found 663.3302.

Compound 6:

A mixture of compound 3 (267 mg, 0.42 mmol) and Lawesson’s reagent (680 mg, 1.7 mmol) in toluene (10 mL) was stirred at 110 °C for 1 h. After solvent removal under reduced pressure, the residue was purified by column chromatography on SiO₂ (ethyl acetate/ pentane; v/v = 1/5, Rf = 0.5) to obtain thioketone (252 mg, 0.38 mmol, 91% yield), i.e., compound 4, as a green solid. Subsequently, a DMF solution (2 mL) of (diacetoxyiodo)benzene (123.7 mg, 0.384 mmol) was added dropwise into a THF solution (3 mL) of compound 5 (164 mg, 0.384 mmol) at −50 °C. After complete addition, the obtained pale pink solution was stirred at −50 °C for 60 s, followed by the addition of a THF (2 mL) solution of compound 4. The reaction mixture was stirred at 25 °C for 2 h. Subsequently, the solvent was removed by using rotary evaporation under reduced pressure and the residue was subjected to column chromatography on SiO₂ (ethyl acetate/pentane; v/v = 1/9, Rf = 0.5) to allow for the isolation of episulfide (191 mg, 0.181 mmol, 50% yield), i.e., compound 6, as a colorless oil.

¹H NMR (400 MHz, CDCl₃) δ (ppm) 8.61 (d, J = 8.4 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.37 – 7.27 (m, 2H), 7.20 (dd, J = 8.0, 8.0 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.70 (s, 1H), 6.31 (m, 2H), 6.12 (dd, J = 8.0, 8.0 Hz, 1H), 4.23 – 4.12 (m, 1H), 4.04 (m, 5H), 3.95 – 3.77 (m, 4H), 2.65 – 2.52 (m, 2H), 2.11 (m, 1H), 2.04 (s, 6H), 1.99 – 1.69 (m, 6H), 1.68 – 1.23 (m, 46H), 1.18 (d, J = 6.0 Hz, 3H), 0.89 (t, J = 6.0 Hz, 3H).

¹³C NMR (100 MHz, CDCl₃) δ (ppm) 193.8, 171.3, 154.5, 153.6, 152.8, 135.0, 132.6, 131.5, 131.2, 130.7, 129.7, 127.0, 126.2, 124.9, 124.5, 124.3, 124.3, 124.1, 123.4, 122.7, 121.9, 110.1, 109.9, 106.1, 69.7, 69.5, 69.5, 65.5, 64.8, 61.9, 40.8, 35.7, 32.1, 29.9, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.1, 28.8, 28.7, 26.2, 26.2, 26.1, 26.1, 22.8, 21.1, 14.3.

HRMS (ESI): calcd. for C₆₃H₈₈O₇S₃ [M+H] 1053.5765, found 1053.5748.
**Compound 7:**

A mixture of episulfide (compound 6, 191 mg, 0.181 mmol) and triphenylphosphine (95 mg, 0.36 mmol) in toluene (10 mL) was stirred at reflux for 16 h. After cooling down to room temperature, the solvent was removed under reduced pressure and the residue was subjected to column chromatography on SiO₂ (ethyl acetate/pentane; v/v = 1/9, Rf = 0.4) to allow for the isolation of compound 7 (158 mg, 0.155 mmol, 85% yield) as a colorless oil.

1H NMR (400 MHz, CDCl₃) δ (ppm) 7.47 (m, 2H), 7.28 (dd, J = 7.6, 7.6 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H), 7.05 (dd, J = 7.6, 7.6 Hz, 1H), 6.94 (s, 1H), 6.87 – 6.82 (m, 2H), 6.36 (dd, J = 7.6, 7.6 Hz, 1H), 6.28 (d, J = 8.0 Hz, 1H), 6.08 (d, J = 7.6 Hz, 1H), 4.21 – 4.13 (m, 3H), 4.08 – 4.05 (m, 6H), 4.00 – 3.86 (m, 2H), 3.75 (dd, J = 11.6, 6.8 Hz, 1H), 3.10 (dd, J = 11.6, 2.8 Hz, 1H), 2.05 (s, 6H), 1.99 – 1.90 (m, 4H), 1.85 – 1.78 (m, 2H), 1.67 – 1.27 (m, 46H), 0.90 (t, J = 6.0 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H).

13C NMR (100 MHz, CDCl₃) δ (ppm) 193.9, 171.3, 155.7, 154.7, 153.0, 138.9, 136.5, 135.8, 132.2, 132.0, 131.7, 127.9, 126.5, 126.3, 126.1, 125.5, 125.2, 124.6, 124.6, 123.9, 123.0, 121.8, 119.7, 109.4, 109.4, 105.1, 69.2, 69.1, 68.8, 64.8, 36.8, 32.1, 30.6, 29.8, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 28.8, 26.3, 26.2, 26.1, 26.1, 22.8, 21.1, 18.5, 14.3.

HRMS (ESI): calcd. for C₅₀H₈₆O₇S₂ [M+H] 1021.6044, found 1021.6017.

**Compound 8:**

To a THF/methanol solution (v/v = 1/1, 6 mL) of compound 7 (158 mg, 0.155 mmol), an aqueous NaOH solution (4 M, 0.34 mL, 1.4 mmol) was added and the mixture was stirred at 90 °C for 1 h. The reaction mixture was allowed to cool down to room temperature. The solvent was removed under reduced pressure and the residue was washed successively with water (50 mL) and ethyl acetate (20 mL). The combined organic layers were washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the residue was purified by column chromatography on SiO₂ (methanol/dichloromethane; v/v = 1/9, Rf = 0.5) to obtain compound 8 (142 mg, 0.151 mmol, 98% yield) as a colorless oil.

1H NMR (400 MHz, CDCl₃) δ (ppm) 7.45 (m, 2H), 7.28 (dd, J = 8.0, 8.0 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H), 7.05 (dd, J = 7.2, 7.2 Hz, 1H), 6.94 (s, 1H), 6.87 – 6.82 (m, 2H), 6.36 (dd, J = 8.0, 8.0 Hz, 1H), 6.27 (d, J = 8.0 Hz, 1H), 6.08 (d, J = 7.6 Hz, 1H), 4.21 – 4.12 (m, 3H), 4.10 – 4.04 (m, 2H), 3.99 – 3.86 (m, 2H), 3.75 (dd, J = 11.6, 6.8 Hz, 1H), 3.64 (t, J = 6.8 Hz, 4H), 3.10 (dd, J = 11.2, 2.4 Hz, 1H), 1.99 – 1.89 (m, 4H), 1.85 – 1.78 (m, 2H), 1.65 – 1.26 (m, 48H), 0.89 (t, J = 6.0 Hz, 3H), 0.77 (d, J = 6.8 Hz, 3H).

13C NMR (100 MHz, CDCl₃) δ (ppm) 155.7, 154.7, 152.9, 138.9, 136.5, 135.8, 132.2, 132.0, 131.7, 127.9, 126.5, 126.3, 126.1, 125.5, 125.2, 124.6, 124.6, 123.9, 123.0, 121.8, 119.7, 109.5, 109.4, 105.2, 69.2, 69.1, 68.9, 63.2, 36.8, 32.9, 32.1, 30.6, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 26.3, 26.2, 25.9, 22.8, 18.5, 14.3.

HRMS (ESI): calcd. for C₅₀H₈₆O₇S₂ [M+H] 937.5833, found 937.5812.

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Motor amphiphile (MA$_{P1}$):

To a pyridine solution (3 mL) of compound 8 (110 mg, 0.117 mmol), diphenyl phosphite (192 mg, 0.819 mmol) was added and the mixture was stirred at 25 °C for 16 h. Subsequently, a solution of triethylamine/water (v/v = 1/1, 2 mL) was added and the reaction mixture was continuously stirred at 25 °C for 1 h. The solvent was evaporated at reduced pressure and the residue was subjected to column chromatography on SiO$_2$ (methanol (with 10% triethylamine)/dichloromethane; v/v = 1/9, $R_f$ = 0.5) to allow for the isolation of MA$_{P1}$ (48 mg, 0.0379 mmol, 32% yield) as a pale yellow oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) 7.59 (s, 1H), 7.42 (m, 2H), 7.26 (dd, $J = 5.6, 5.6$ Hz, 1H), 7.15 (d, $J = 7.6$ Hz, 1H), 7.02 (dd, $J = 7.6, 7.6$ Hz, 1H), 6.92 (s, 1H), 6.84 – 6.80 (m, 2H), 6.33 (dd, $J = 8.0, 8.0$ Hz, 1H), 6.25 (d, $J = 8.0$ Hz, 1H), 6.04 (d, $J = 8.0$ Hz, 2H), 4.18 – 4.10 (m, 3H), 4.07 – 4.00 (m, 2H), 3.97 – 3.91 (m, 1H), 3.88 – 3.83 (m, 5H), 3.73 (dd, $J = 11.2, 6.8$ Hz, 1H), 3.09 – 2.99 (m, 13H), 1.97 – 1.86 (m, 4H), 1.82 – 1.75 (m, 2H), 1.66 – 1.26 (m, 66H), 0.87 (t, $J = 6.4$ Hz, 3H), 0.74 (d, $J = 6.8$ Hz, 3H).

$^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ (ppm) 155.6, 154.7, 152.9, 138.8, 136.4, 135.8, 132.1, 131.9, 131.7, 127.8, 126.5, 126.3, 126.1, 125.5, 125.2, 124.6, 124.5, 123.9, 122.9, 121.7, 119.6, 109.4, 109.4, 105.1, 69.2, 69.1, 68.8, 64.2, 64.2, 45.5, 36.7, 32.0, 30.9, 30.9, 30.6, 29.8, 29.8, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 29.3, 26.2, 26.2, 26.1, 26.0, 22.8, 18.4, 14.2, 8.6.

HRMS (ESI): calcd. for C$_{50}$H$_{86}$O$_9$P$_2$S$_2$ [M-H] 1063.5105, found 1063.5129.

Motor amphiphile (MA$_{S1}$):

A mixture of compound 8 (36 mg, 0.038 mmol), sulfur trioxide pyridine complex (37 mg, 0.23 mmol) and triethylamine solution (0.1 mL) in dichloromethane (6 mL) was stirred at 25 °C for 18 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography on SiO$_2$ (methanol (with 10% triethylamine)/dichloromethane; v/v = 1/9, $R_f$ = 0.5) to obtain MA$_{S1}$ (23 mg, 0.018 mmol, 47% yield) as a pale yellow oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) 9.58 (s, 2H), 7.42 (m, 2H), 7.26 (dd, $J = 8.0, 8.0$ Hz, 1H), 7.15 (d, $J = 7.6$ Hz, 1H), 7.03 (dd, $J = 7.6, 7.6$ Hz, 1H), 6.92 (s, 1H), 6.83 – 6.80 (m, 2H), 6.33 (dd, $J = 8.0, 8.0$ Hz, 1H), 6.25 (d, $J = 8.0$ Hz, 1H), 6.04 (d, $J = 7.6$ Hz, 1H), 4.17 – 4.10 (m, 3H), 4.07 – 4.01 (m, 6H), 3.97 – 3.83 (m, 2H), 3.73 (dd, $J = 11.6, 6.4$ Hz, 1H), 3.15– 3.06 (m, 13H), 1.97 – 1.86 (m, 4H), 1.82 – 1.75 (m, 2H), 1.70 – 1.63 (m, 4H), 1.60 – 1.26 (m, 60H), 0.87 (t, $J = 6.0$ Hz, 3H), 0.74 (d, $J = 6.4$ Hz, 3H).

$^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ (ppm) 155.7, 154.7, 152.9, 138.8, 136.4, 135.8, 132.1, 131.9, 131.7, 127.9, 126.5, 126.3, 126.1, 125.5, 125.1, 124.6, 124.5, 123.9, 122.9, 121.7, 119.6, 109.5, 109.4, 105.1, 69.2, 69.1, 68.8, 68.2, 54.9, 46.6, 36.7, 32.0, 30.6, 29.8, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 26.2, 26.2, 26.1, 26.0, 22.8, 18.4, 14.2, 8.8.

HRMS (ESI): calcd. for C$_{50}$H$_{86}$O$_9$P$_2$S$_2$ [M-H] 1095.4812, found 1095.4813.

4. Nile Red Fluorescence Assay. 3-5 The self-assembly properties of MA$_{P1}$ and MA$_{S1}$ were analyzed by incorporation of the hydrophobic solvatochromic probe Nile Red (9-diaethlylamino-5-benzo[α]phenoxazinone), which shows a blue shift of the emission wavelength when it is encapsulated in hydrophobic environments. Freshly prepared Nile Red ethanol solution (0.1 mM) was diluted into MA solutions to a final concentration of 0.25 μM, which means that the corresponding samples contain...
only 0.25% ethanol, allowing to eliminate the effects of ethanol on the self-assembly behavior of MAP1 and MAS1. Subsequently, the mixture solutions containing MAP1 (or MAS1) and Nile Red solution (0.25 µM) were excited at 550 nm, and the fluorescence spectra with a wavelength range of 580–750 nm were recorded by using a JASCO FP6200 fluorometer. The blue shifts were calculated by subtracting the emission wavelength of Nile Red in Milli-Q water from the emission wavelength of the sample. Afterward, critical aggregation concentrations (CACs) of MAP1 and MAS1 were determined by plotting the obtained blue shifts values against the concentrations.

5. Cryogenic Transmission Electron Microscopy. The MAP1 solution (or MAS1 solution) was prepared by direct dissolution into double deionized water, followed by heating at 80 °C for 30 min and then cooling to room temperature to obtain a clear solution (3.9 mM). To observe the self-assembly structures by cryo-transmission electron microscopy (cryo-TEM), 2.5 µL of MAP1 solution (or MAS1 solution in identical conditions) was placed on a glow-discharged holy carbon coated grid (Quantifoil 3.5/1, QUANTIFOIL Micro Tools GmbH, Großlößnitz, Germany). After blotting, the corresponding grid was rapidly frozen in liquid ethane (Vitrobot, FEI, Eindhoven, The Netherlands) and kept in liquid nitrogen until measurement. The grids were observed with a Gatan model 626 cryo-stage in a Tecnai T20 (FEI, Eindhoven, The Netherlands) cryo-electron microscope operating at 200 keV. Cryo-TEM images were recorded under low-dose conditions on a slow-scan CCD camera.

6. Standardized Preparation of Artificial Muscle of Motor Amphiphile in Water. Artificial muscles of motor amphiphiles (MAs) were prepared with the standardized conditions as follows: by using a pipette, 3.5 µL of MA solution (55 mM) was manually drawn into an aqueous solution of CaCl2 (150 mM) in a cuvette by the shear-flow method, allowing for the formation of a noodle-like artificial muscle, with a diameter of 300 ± 30 µm and a length of 8.0 ± 2.0 mm (Figure 6). The light source (Thorlabs, M365FP1, 0.7 A) was placed at a distance of 5.0 ± 0.5 mm toward the artificial muscle. The photoactuating speed of the MA artificial muscle was calculated from a saturated flexion angle, i.e., 90°, of bending depending on a particular time. All the photoactuation experiments were performed in triplicate and recorded with a Nikon Coolpix A900 Digital Camera to obtain an averaged value of actuation speed. On the basis of the 1H NMR and UV-Vis absorption studies of MA solutions shown in Figure 2, Figure S2, Figure 3, and Figure S4, a ratio of metastable-MAs : stable-MAs at the photostationary state can be determined as 85:15, which was comparable to our previous report.2,5 Therefore, a similar extent of switching process of MAs in the artificial muscles was expected when the MA artificial muscles reached the saturated flexion angle of 90°.

7. Synchrotron Radiation X-ray Diffraction Analysis.2,5,6 Through view X-ray diffraction (XRD) images of the MA artificial muscles were obtained using the BL45XU beamline at SPring-8 (Hyogo, Japan) equipped with an R-AXIS IV++ (Rigaku) imaging plate area detector or with a Pilatus3X 2M (Dectris) detector. The scattering vector, \( q = 4\pi\sin(\theta)/\lambda \), and the position of incident X-ray beam on the detector were calibrated using several orders of layer reflections from silver behenate (\( d = 58.380 \text{ Å} \)), where \( 2\theta \) and \( \lambda \) refer to the scattering angle and wavelength of the X-ray beam (1.0 Å), respectively. The sample-to-detector distances for through-view XRD measurements were 2.02 m. The obtained diffraction patterns and images were integrated along the Debye-Scherrer ring to afford one-dimensional (1D) intensity data using the FIT2D software. The lattice parameters were refined using the CellCalc ver. 2.10 software.
8. Scanning Electron Microscopy and Polarized Optical Microscopy Analysis. The MA\textsubscript{P\textonehalf} solution (or MA\textsubscript{s1} solution) was prepared by heating at 80 °C for 30 min and then cooling to room temperature to obtain a clear solution (55 mM). By using a pipette, 3.5 µL of the MA solution was manually sheared into an aqueous solution of CaCl\textsubscript{2} (150 mM) on a sapphire substrate to obtain a noodle-like MA artificial muscle with an arbitrary length. After the removal of CaCl\textsubscript{2} solution, the MA artificial muscle was washed with deionized water (three times), and the resulting MA artificial muscle was used directly for polarized optical microscopy (POM, Nikon model Eclipse LV100POL) and XRD experiments. For scanning electron microscopy (SEM), the MA artificial muscle was dried in air for 48 h and subsequently observed under a Hitachi S-5500 Field Emission SEM (FE-SEM).

9. Cell Culture. Human bone marrow-derived mesenchymal stem cells (hBM-MSCs) were obtained from Lonza. A growth medium consisted of Alpha modified Eagle medium (Gibco), 10% (v/v) fetal bovine serum (Gibco), 0.1% (v/v) ascorbic acid 2-phosphate (Sigma), and 2% penicillin/streptomycin (Gibco). hBM-MSCs were incubated in T75 culture flasks with the conditions of 37 °C and 5% CO\textsubscript{2}. The growth medium was changed every 3 d and the hBM-MSCs were detached with trypsin for 3 – 5 min at 37 °C and harvested at about 80 – 90% confluence. The corresponding hBM-MSCs were used for cell culture experiments.

Freshly prepared artificial muscles of MA\textsubscript{C1} and MA\textsubscript{P\textonehalf} were placed in 24-well plates, which were washed with PBS (0.5 mL) 3 times and subsequently washed by growth medium (0.5 mL) 3 times. Afterward, the hBM-MSCs with particular densities were evenly seeded into the growth medium containing MA artificial muscles. The samples were incubated in a 5% CO\textsubscript{2} humidified atmosphere at 37 °C. The growth medium was changed every 3 d.

10. Cytotoxicity and Cell Viability Analysis. The vitro cytotoxicity of MA artificial muscles was tested by using a direct contact method between hBM-MSCs and MA artificial muscles via a live/dead staining assay. The hBM-MSCs with a density of 20,000 cells/well were evenly seeded into 24-well plates containing growth medium and MA artificial muscles, and incubated in a 5% CO\textsubscript{2} humidified atmosphere at 37 °C. After incubation for 24 h, the cytotoxicity of hBM-MSCs was analyzed by the live/dead assay by using calcein-AM (2 µL, Molecular Probes, Invitrogen Detection Technologies) and ethidium homodimer-1 (4 µL, Molecular Probes, Invitrogen Detection Technologies) in PBS to stain the hBM-MSCs for 30 min. After staining, the MA artificial muscles were moved out from the 24-well plates to a well plate (diameter: 60 mm, containing PBS) for being observed under a Leica fluorescence microscopy equipped with a 10× NA 0.30 objective and a Leica confocal laser scanning microscopy (CLSM) equipped with a 40× NA 0.80 objective.

Additionally, a deeper insight into the condition of hBM-MSCs cultured on the surface of the MA artificial muscles with prolonged incubation time is performed by F-actin and nuclei staining. The hBM-MSCs with a density of 2,500 cells/well were evenly seeded into 24-well plates containing growth medium and MA artificial muscles and incubated in a 5% CO\textsubscript{2} humidified atmosphere at 37 °C for 1, 3, and 5 d. After incubation, the hBM-MSCs were fixed with 3.7% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min at room temperature and then washed by PBS for three times. Subsequently, the cell membrane was permeabilized with a 0.5% TritonX-100 solution for 3 min, followed by washing three times with PBS. The obtained hBM-MSCs were stained with 4′,6-diamidino-2-phenylindole (DAPI) and tetramethylrhodamine isothiocyanate(TRITC)–phalloidin for the cell nuclei and F-actin, respectively, which were observed under the Leica confocal laser scanning...
microscopy equipped with a 40× NA 0.80 objective. The cell nuclei stained by DAPI were not shown because of the strong background fluorescence of the MA artificial muscles. The area of cell F-actin and the surface area of the MA artificial muscles were determined by the software of ImageJ. A spread cell shape with well-defined actin stress fibers of hBM-MSCs is commonly quantified as being in a viable state. Due to the overlapping fluorescence between the strong background of MA artificial muscles and the cell nuclei stained by DAPI, the change of cell F-actin area with prolonged incubation time, instead of the number of cell nuclei, was used to indicate the cell proliferation.

11. Post-Photoactuation Experiment of Artificial Muscles with Adhered hBM-MSCs. The hBM-MSCs with a density of 20,000 cells/well were evenly seeded into 24-well plates containing growth medium and MA artificial muscles, and incubated in a 5% CO₂ humidified atmosphere at 37 °C for 6 h. After incubation, hBM-MSCs were stained by using calcein-AM (2 µL) and ethidium homodimer-1 (4 µL) in PBS for 30 min. Subsequently, the MA artificial muscles were moved out from the 24-well plates to a well plate (diameter: 60 mm, containing PBS) for observation under the Leica fluorescence microscopy equipped with a 10× NA 0.30 objective. The MA artificial muscles were irradiated by using a Thorlabs LED light (M365L2-C1, 120 mW). The photoactuation process upon irradiation was simultaneously recorded by fluorescence microscopy with cell visualization at a micro-length scale and by an iPhone camera at a macroscopic scale, as shown in Figure S1.

![Figure S1](image.png)

**Figure S1.** Photographs of instruments for photoactuation experiments of artificial muscles with adhered hBM-MSCs.

12. Description of Supplementary Movies.

**Movie 1:** Three-dimensional visualization of MA artificial muscles with adhered hBM-MSCs by using confocal laser scanning microscopy.

**Movie 2:** Post-Photoactuation of MAC1 artificial muscle with adhered hBM-MSCs.

**Movie 3:** Post-Photoactuation of MAP1 artificial muscle with adhered hBM-MSCs.
**1H NMR Spectra of Photoisomerization of MAs**

Figure S2. (a) Schematic representation of photoisomerization of MAs. 1H NMR spectra (CD2Cl2, 25 °C, 500 MHz) of (b) MAp1 and (c) MAs1 in a stable state (black) and a photostationary state mixture (red) isomers after irradiation.

Table S1. Integration values of the selective protons in the 1H NMR spectra (CD2Cl2, 25 °C, 500 MHz) of MAs at photostationary state after irradiation.

| Samples | Hc | CH3(ax) (Stable isomers) | CH3(eq) (Metastable isomers) |
|---------|----|--------------------------|-------------------------------|
| MAp1    | 1  | 0.47                     | 2.61                          |
| MAs1    | 1  | 0.46                     | 2.54                          |
Kinetic Studies of Thermal Helix Inversion Step of $\text{MAP}_1$ and $\text{MAS}_1$ in Acetonitrile

Figure S3. Kinetic studies of the thermal helix inversion step from metastable $\text{MA}$ to stable $\text{MA}$ of (a) $\text{MAP}_1$ and (b) $\text{MAS}_1$ in CH$_3$CN solutions at five temperatures (55 °C, 60 °C, 65 °C, 70 °C, and 75 °C) by UV-vis absorption spectral changes at 342 nm. Purple bands indicate 95% confidence interval.

The rate constants $k$ of the first-order decay at different temperatures were obtained using the equation $A/A_0 = e^{-kt}$. Activation parameters were obtained by fitting to the linearized form of the Eyring equation using origin software. Analysis of these data using equation $(\Delta^f G = RT \ln (k_B/h) - \ln (k/T))$, provides the Gibbs energy of activation ($\Delta^f G$). The half-lives of metastable $\text{MAP}_1$ and $\text{MAS}_1$ at 25 °C (298.15 K) were obtained by a linear fitting of $\ln k/T$ and $1/T$. The activation parameters and half-life of metastable $\text{MAP}_1$ and $\text{MAS}_1$ in acetonitrile are presented in Table S2.

Table S2. Activation parameters and half-lives of metastable $\text{MAP}_1$ and metastable $\text{MAS}_1$.

| Samples | $t_{1/2}$ at 298.15 K (h) | $\Delta^f G$ at 298.15 K (kJ/mol) | $\Delta^f H$ (kJ/mol) | $\Delta^f S$ (J/K/mol) |
|---------|--------------------------|---------------------------------|----------------------|-----------------------|
| $\text{MAP}_1$ | ~27.9 | 102.5±0.9 | 48.4±0.7 | -181.5±2.1 |
| $\text{MAS}_1$ | ~46.7 | 103.8±3.8 | 59.9±2.8 | -147.2±8.4 |
UV-vis Absorption Spectra of Photoisomerization of MAs in Water

Figure S4. UV-vis absorption spectra of (a) MA<sub>C1</sub>, (b) MA<sub>P1</sub>, and (c) MA<sub>S1</sub> in water before 365 nm light irradiation (black), upon irradiation from 1 min to 3 min (pink), and after irradiation to PSS (red).
Nile Red Fluorescence Assay for Determining Critical Aggregation Concentration

Figure S5. Nile Red fluorescence assay for the determination of critical aggregation concentration (CAC) of MAP1 and (b) MAS1.
Figure S6. The OM (top) and POM (bottom) images of a noodle-like artificial muscle of MAP1 under crossed polarizers. The POM images of MAP1 artificial muscle were tilted at 0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315° relative to the transmission axis of the analyzer. Scale bar applied for all panels.
Fluorescence Microscopy Images for Cytotoxicity

**Figure S7.** Cytotoxicity of artificial muscles of (a) \( \text{MA}_{\text{C1}} \) and (b) \( \text{MA}_{\text{P1}} \) for \( \text{hBM-MSCs} \) after 24 h incubation, determined by a live/dead staining assay in triplicate. The images were taken by fluorescence microscopy, which shows the fluorescence images (top) and the merge images combined with the bright field image (bottom). Scale bar: 100 \( \mu \text{m} \), applied for all panels.
Figure S8. Snapshots showing (a) macroscopic photoactuation (scale bar: 5 mm) of an MA$_{P_1}$ artificial muscle with adhered hBM-MSCs on the surface and (b) in situ observation of actuation movement accompanied with cell visualization under fluorescence microscopy upon 365 nm light irradiation for 200 s (scale bar: 100 μm).
Analytical Data

Figure S9. $^1$H NMR spectrum (400 MHz) of compound 3 in CDCl$_3$ at 25 °C.

Figure S10. $^{13}$C NMR spectrum (100 MHz) of compound 3 in CDCl$_3$ at 25 °C.
Figure S11. $^1$H NMR spectrum (400 MHz) of compound 6 in CDCl$_3$ at 25 °C.

Figure S12. $^{13}$C NMR spectrum (100 MHz) of compound 6 in CDCl$_3$ at 25 °C.
Figure S13. $^1$H NMR spectrum (400 MHz) of compound 7 in CDCl$_3$ at 25 °C.

Figure S14. $^{13}$C NMR spectrum (100 MHz) of compound 7 in CDCl$_3$ at 25 °C.
Figure S15. $^1$H NMR spectrum (400 MHz) of compound 8 in CDCl$_3$ at 25 °C.

Figure S16. $^{13}$C NMR spectrum (100 MHz) of compound 8 in CDCl$_3$ at 25 °C.
Figure S17. $^1$H NMR spectrum (400 MHz) of MAP1 in CDCl₃ at 25 °C.

Figure S18. $^1$C NMR spectrum (150 MHz) of MAP1 in CDCl₃ at 25 °C.
Figure S19. $^1$H NMR spectrum (400 MHz) of MA$_{S1}$ in CDCl$_3$ at 25 °C.

Figure S20. $^1$C NMR spectrum (150 MHz) of MA$_{S1}$ in CDCl$_3$ at 25 °C.
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