Supporting Information

Hydrogen Sulfide-Releasing Micelles for Promoting Angiogenesis

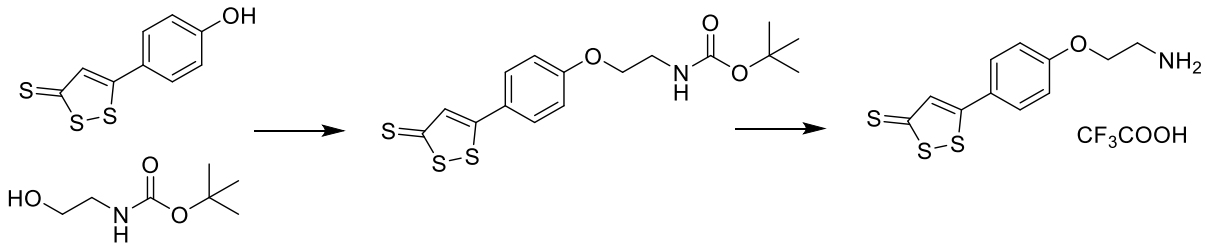
Jerry J. Y. Chen[1], A. J. van der Vlies[2], U. Hasegawa[2,*]

[1] Osaka University, Department of Applied Chemistry, Yamadaoka Suita 565-0825, Osaka, Japan

[2] Kansas State University, Tim Taylor Department of Chemical Engineering, 1005 Durland Hall, 66506, Manhattan Kansas, USA

[*] Corresponding author, Urara Hasegawa, 1013 Durland Hall, 66506, Manhattan Kansas, USA, Telephone: +1-785-532-5486, email: hasegawa@ksu.edu

Scheme S1



(**a**) DIAD, PPh_3, THF, -20°C→25°C, (**b**) TFA, TIS, CH_2Cl_2, 0°C→25°C. For explanation of the used abbreviations see the materials section in the main manuscript.

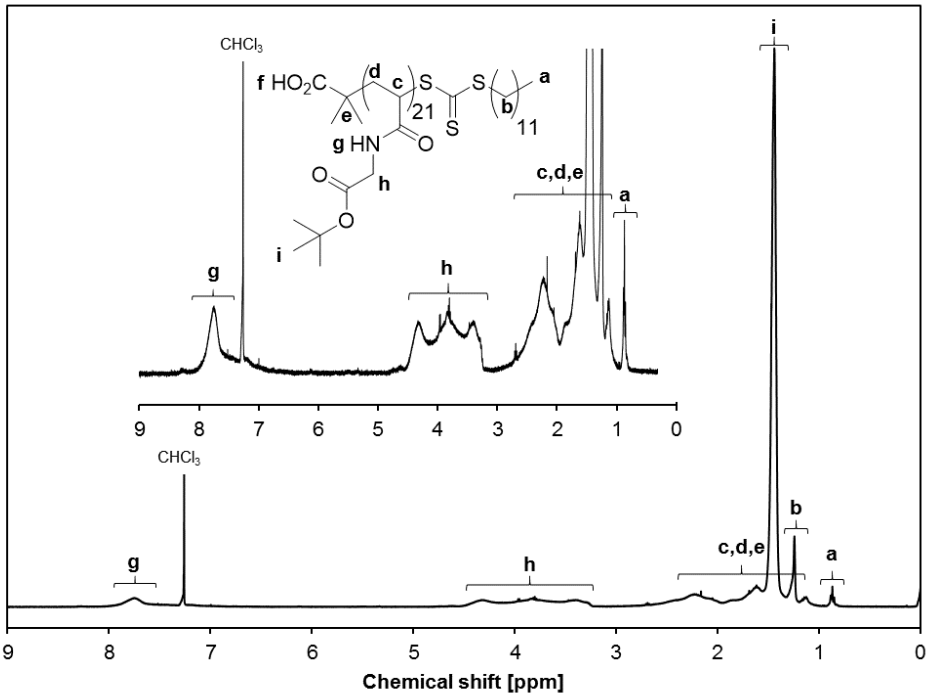


Figure S1. ^1H NMR spectrum of polymer **3** in $CDCl_3$. Signals have been assigned with bold lower-case letters. The CO_2H proton **f**, expected to occur at 10-11 ppm as a broad signal, was not observed (spectral region not shown).

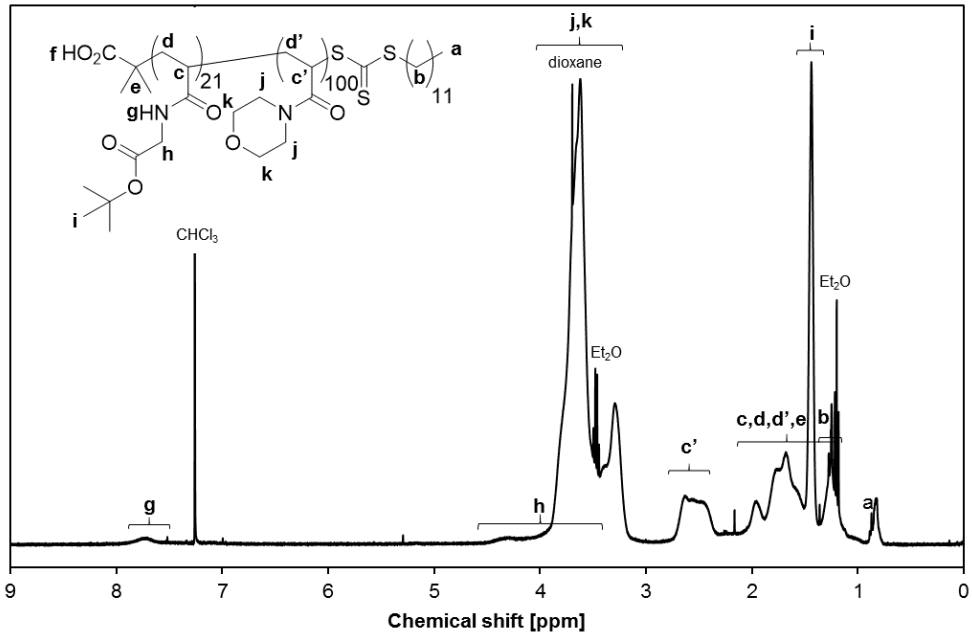


Figure S2. ^{1}H NMR spectrum of polymer **4b** in $CDCl_3$. Signals have been assigned with bold lower-case letters. The CO_2H proton **f**, expected to occur at 10-11 ppm as a broad signal, was not observed (spectral region not shown).

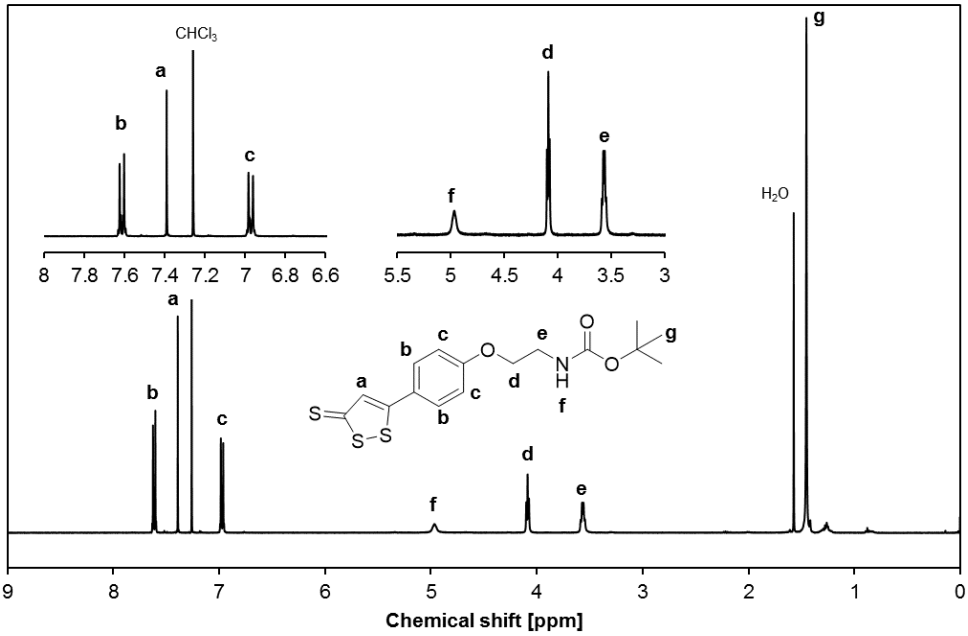


Figure S3. ^{1}H NMR spectrum of Boc-protected **7** in $CDCl_3$. Signals have been assigned with bold lower-case letters.

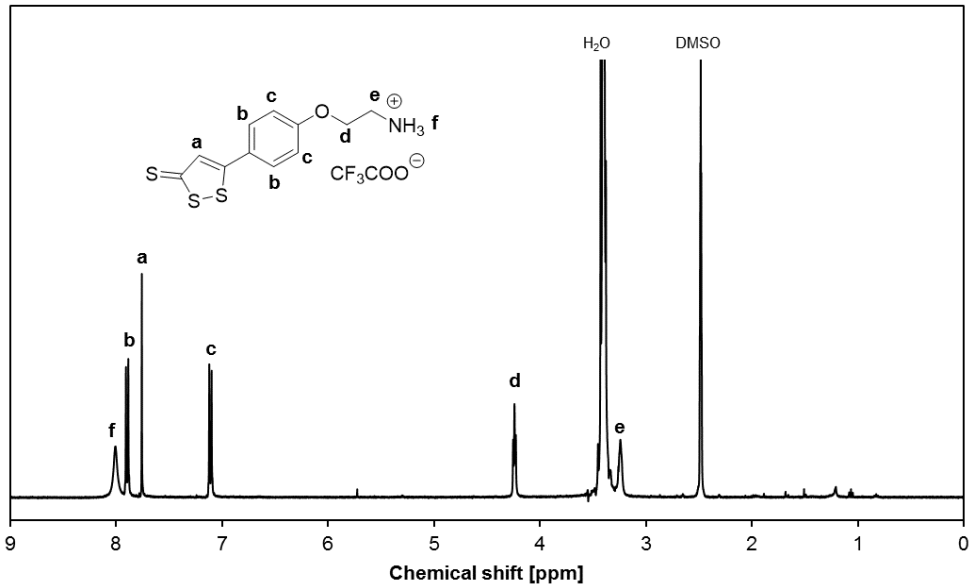


Figure S4. ^{1}H NMR spectrum of **7** in d_6-DMSO. Signals have been assigned with bold lower-case letters.

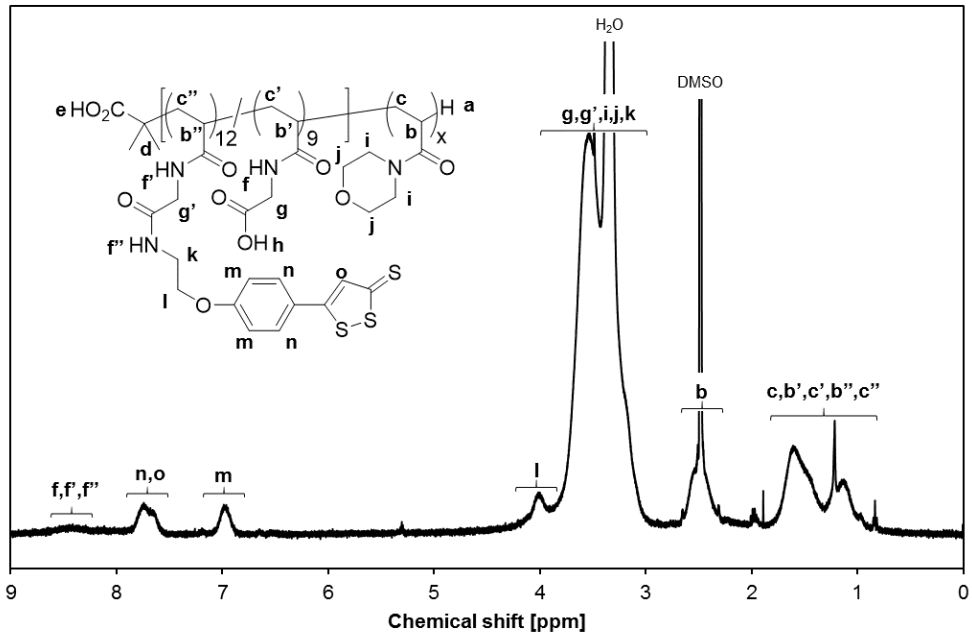


Figure S5. ^{1}H NMR spectrum of polymer **8c** in d_6-DMSO. Signals have been assigned with bold lower-case letters. The CO_2H protons **e** and **h**, expected to occur at 10-11 ppm as broad signals, were not observed (spectral region not shown) and proton **a** could not be assigned due to the overlap with other signals.

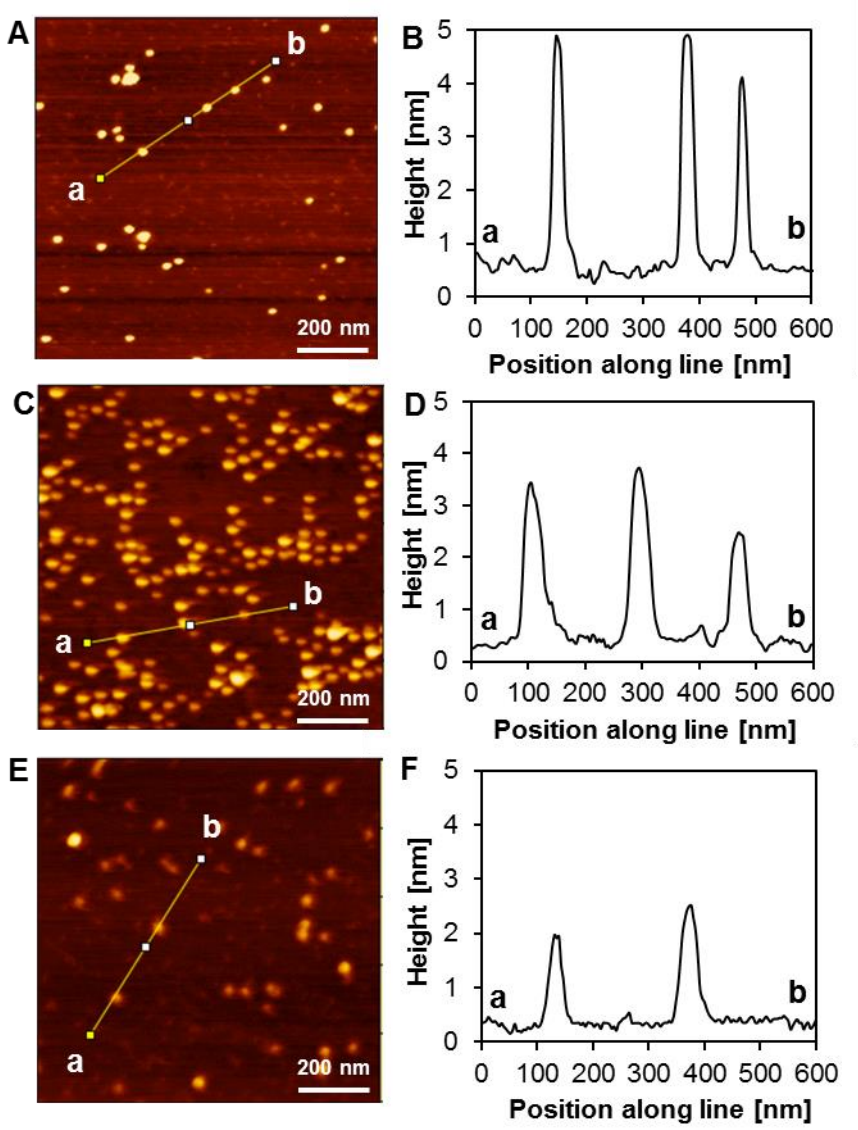


Figure S6. Cross-section curve of the AFM images of the different micelles. The height profiles for micelles (**A**,**B**) **8a** (**C**,**D**) **8b** and (**E**,**F**) **8c** along the yellow lines marked with a and b.

Table S1. Average size distribution of the micelles according AFM

Micelles	D [nm][a]
8a	27.4 ± 10.0
8b	28.9 ± 9.3
8c	28.5 ± 11.4

[a] D: diameter of the micelles.

Synthesis of fluorescently (Cy3)-labeled polymers 8b and 8c.

As an example, the labeling of **8b** is given: 5.3 mg (0.26 µmol, 2.4 µmol COOH groups) **8b** and NHS were dried overnight under vacuum in the presence of P_4O_{10}. The polymer was dissolved in 500 µL DMF and to the clear solution was added 0.27 mg (2.4 µmmol, 1 eq relative to COOH groups) NHS in 115 µL DMF followed by 0.74 mg (3.6 µmol, 1.5 eq relative to COOH groups) DCC. The solution was stirred at room temperature for 16 h and 0.17 mg (0.27 µmol, 1 eq relative to polymer) Cy3-amine (Lumiprobe) in 100 µL DMF, followed by 0.020 mg (0.24 µmol) DMAP in 100 µL DMF and 0.4 µL (0.29 mg, 2.9 µmol) Et_3N in 100 µL DMF. After stirring for 48 h the reaction mixture was acidified by adding 150 µL 1 M $NaHSO_4$ (aq), diluted with 1.5 mL water and dialyzed (MWCO 3400) against 4 L water for 2 d with replacing the water four times. The solution was passed through a plug of glass wool to remove a small precipitate. Lyophilization of the clear solution yielded 5 mg of a red orange solid that was dissolved in 400 µL DMF and loaded on a Sephadex LH20 size exclusion column. Fractions that showed absorbances at 440 nm (ADT) 520 and 560 nm (Cy3) were pooled and concentrated under reduced pressure at room temperature. The residue was dissolved in 500 µL DMF and added to 4.5 mL water and the clear solution dialyzed (MWCO 3400) against 4 L of water for 2 d with replacing water four times. The polymer was recovered by lyophilization to yield 3.8 mg of an orange red solid.

Cellular uptake Cy3-labeled 8b and 8c micelles

The Cy3-labeled polymers **8b** and **8c** were dissolved in NMP at 50 mg/mL and diluted 1:9 with water and dialyzed (MWCO 3400) against 4 L water for 2 d with replacing the water four times. A volume of 50 µL of this solution was mixed with 450 µL medium and sterile filtered. HUVECs were seeded in a glass bottom dish at 1 x 10^3 cells/well. The nuclei were stained by adding 100 µL medium containing 10 nM Hoechst 33342. After 15 min the medium was replaced with the micelle solutions. After incubating overnight, the confocal images were collected.

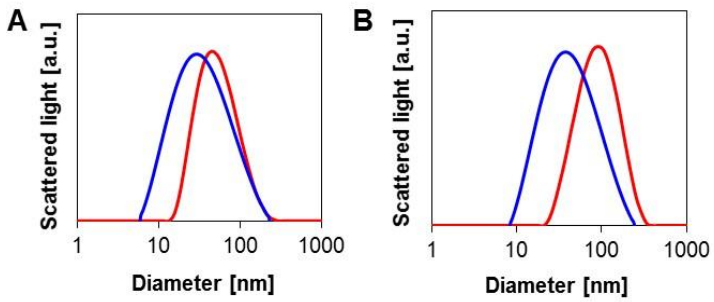


Figure S7. Size distribution of Cy3-labeled (red) and unlabeled micelle (blue) solutions as measured by DLS. (**A**) polymer **8b** and (**B**) polymer **8c**.

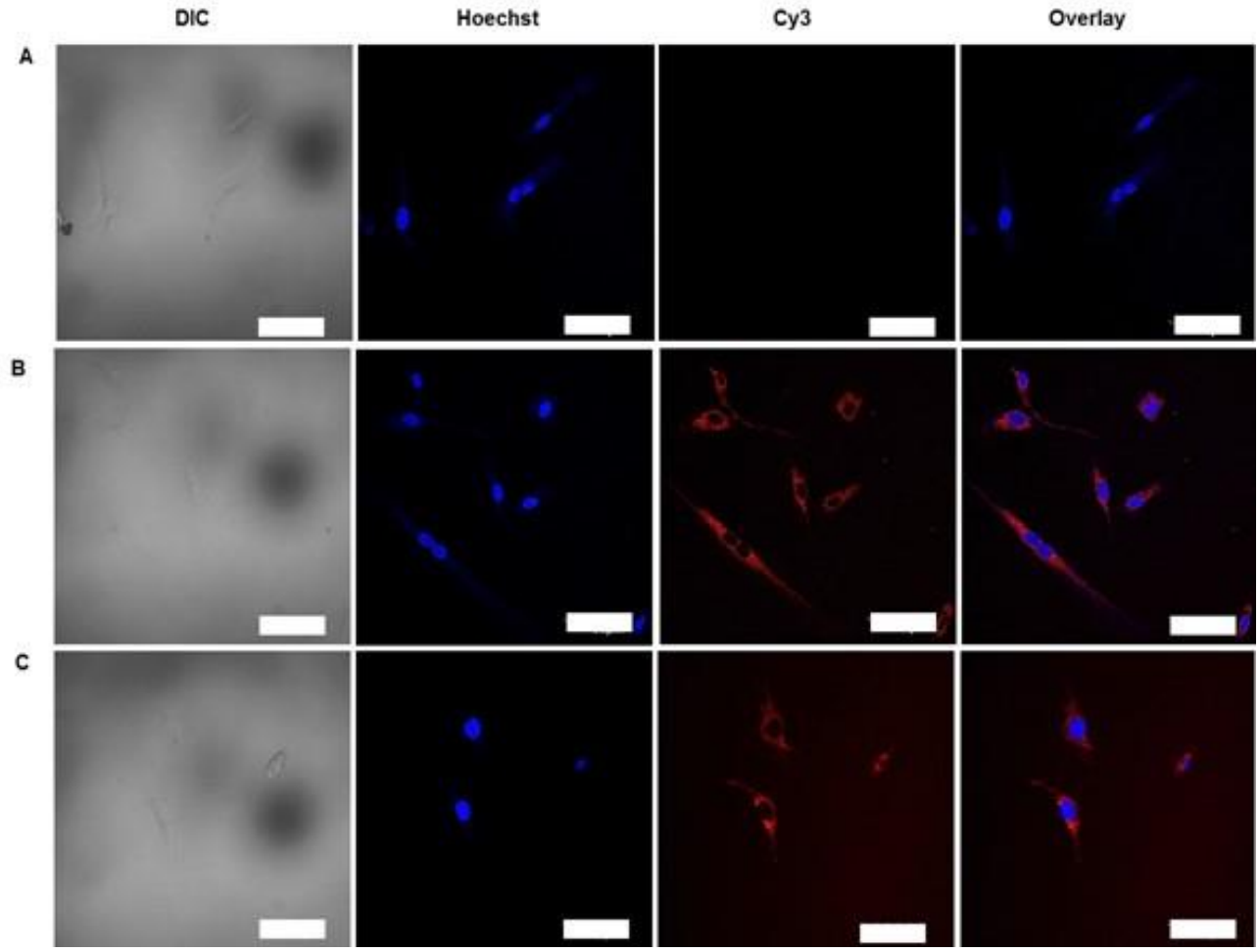


Figure S8. Cellular uptake micelles in HUVECs. The cells were treated with (**A**) medium alone, Cy3-labeled micelles (**B**) **8b** and (**C**) **8c** overnight. Cell nuclei were stained with Hoechst. Scale bars: 60 µm.

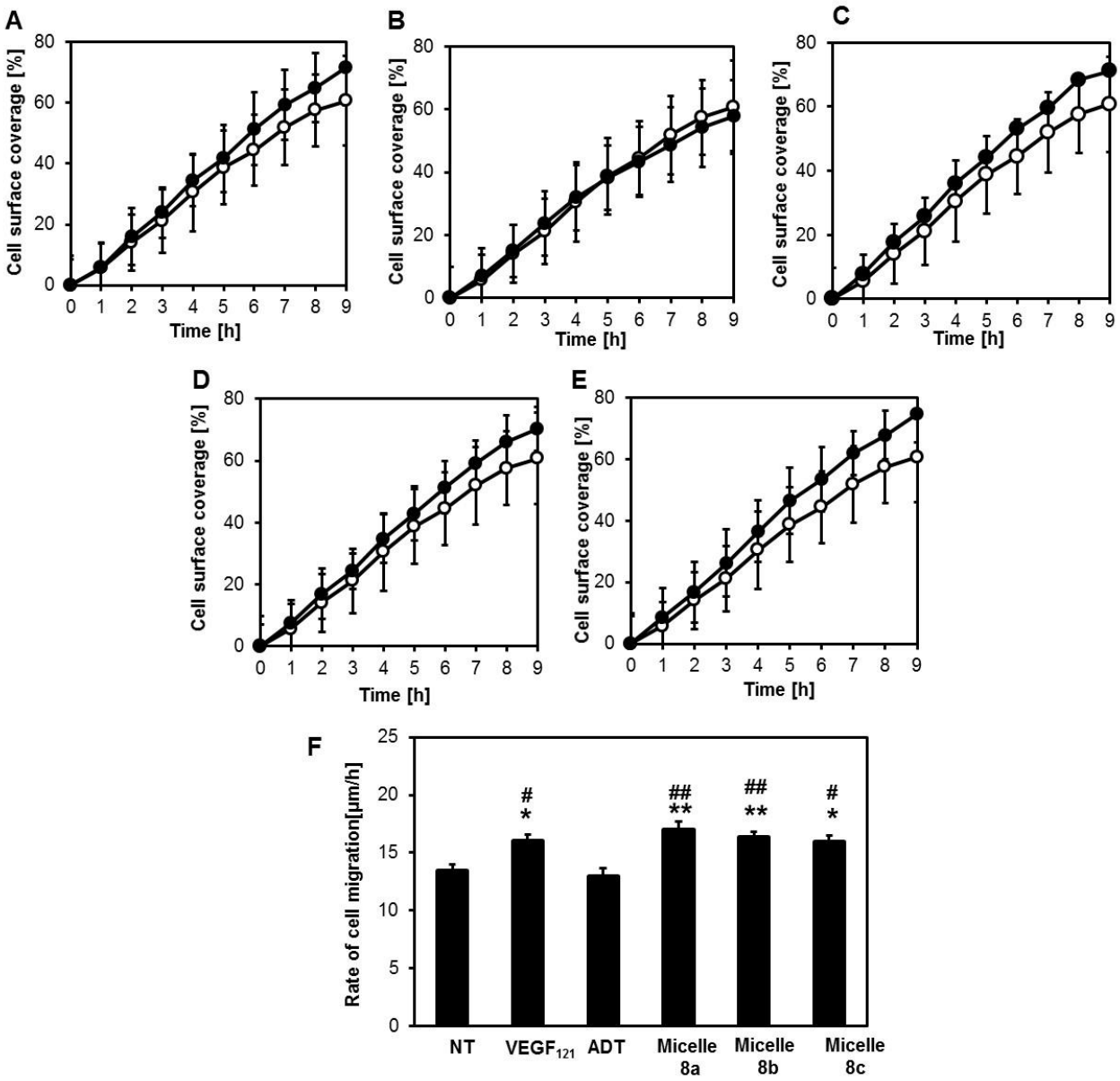


Figure S9. Cell surface coverage and rate of cell migration as determined by the gap closure migration assay. Cell surface coverage as function of time for (**A**) $VEGF_{121}$, (**B**) ADT (**C**) micelles **8a**, (**D**) **8b**, (**E**) **8c** (black circles) relative to no-treatment (NT) (white circles). (**F**) Rate of HUVECs migration. * $p < 0.05$, ** $p < 0.01$ versus NT, $^{\#}$ $p < 0.01$, $^{\#\#}$ $p < 0.001$ versus ADT, $n = 12$.

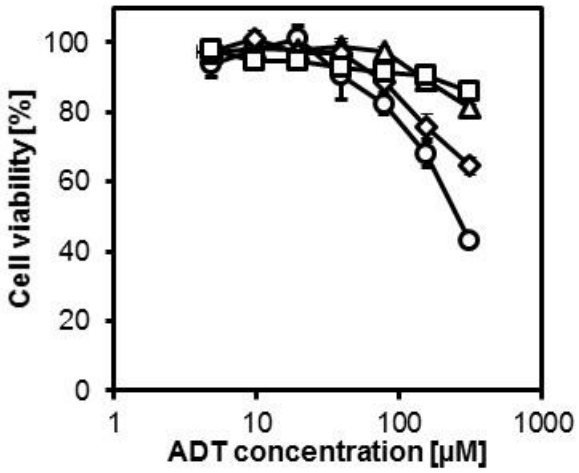


Figure S10. Cytotoxicity of the micelles **8a-c**, and ADT in HUVECs. Metabolic activity of HUVECs treated with the micelles **8a** (square), **8b** (triangle), **8c** (rhombus), and ADT (circle) for 1 d was determined by the MTT assay. *n*=3.

Formulation	No. tubes
8a	1.3 ± 2.3
8b	8.3 ± 4.0
8c	12 ± 3.0
VEGF	17 ± 5.2

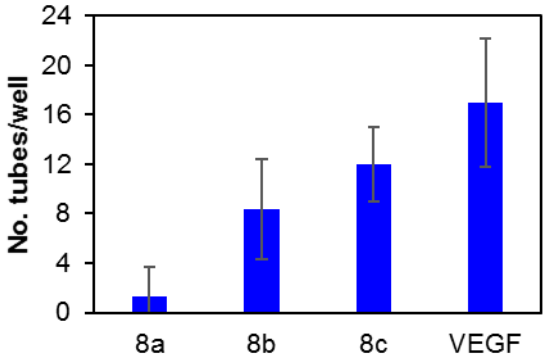


Figure S11. Number of capillary-like tubes counted inside the µ-slide angiogenesis well (surface area: 0.125 cm^2). n= 3.