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Supporting Information

Amphiphilic multi-targeting copolymer micelles efficiently deliver pZNF580 to promote endothelial cell proliferation and migration

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Experimental Methods

Preparation and characterization of copolymers

Preparation and characterization of P(CL-co-MMD)(PCLMD).

PCLMD copolymers were obtained by initiating the ring-opening polymerization of ε-caprolactone with 3-methylmorpholine-2,5-dione (MMD) using saturated short-chain diols ($C_nH_{2n+2}O_2$, n=8) as initiator and stannous octanoate ($Sn(Oct)_2$) as catalyst. MMD (2.68 g), ε-caprolactone (8.0 mL), 1,8-octanediol (0.30 g) and freshly prepared $Sn(Oct)_2$ (1 mL) toluene solution were added to the polymerization tube and sealed in a nitrogen atmosphere. After 3 times of vacuum filling with nitrogen, the reaction was carried out at 150 °C for 24 h. The crude product was dissolved with trichloromethane and precipitated with excess cold $CHCl_3$. The precipitate was dried under vacuum for 24 h to obtain the product PCLMD.

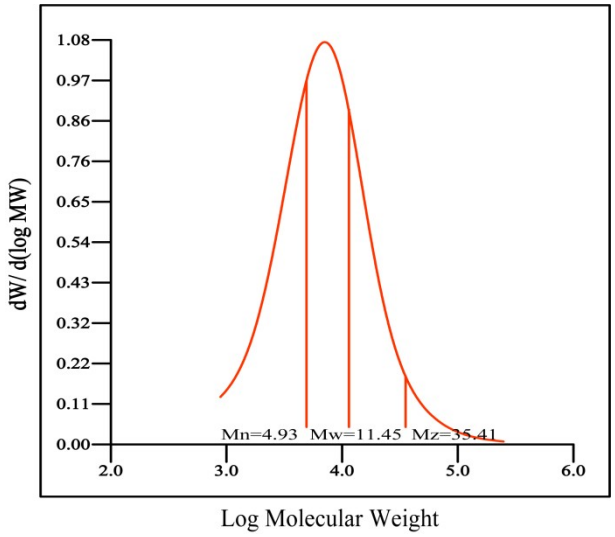

Figure S1. The GPC curve of polymer PCLMD.

Preparation and characterization of P(CL-co-MMD)-g-PPEGMA (PCLMD-PPEGMA) copolymer.

According to the method reported in the literature[33], PCLMD-PPEGMA copolymer was synthesized through ATRP atom transfer radical polymerization using PCLMD-Br as the

macroinitiator and CuBr/bipyridine as the catalyst. Dissolve P(CL-co-MMD)-Br (3.5 g), PEGMA (2.5 g, 5.0 mmol) and bipyridine (0.1 g, 0.60 mmol) in 10 mL of 2-butanone in a dry Schlenk flask. After the mixture was degassed through freeze-vacuum-thaw cycles (3 times), CuBr (0.07 g, 0.40 mmol) was added to the Schlenk flask under nitrogen protection. After three freeze-vacuum-thaw cycles, the reaction was carried out at 50 °C for 24 h. The solution was then passed through a neutral alumina (Al_2O_3) column using chloroform as the eluent to remove the copper catalyst. PCLMD-PPEGMA copolymer was obtained by precipitating in cold n-hexane and drying in vacuum until constant weight. A nanoparticle sizer (Zetasizer 3000 HS, Malvern Instruments, UK) was used to measure the particle size and zeta potential of the copolymer.

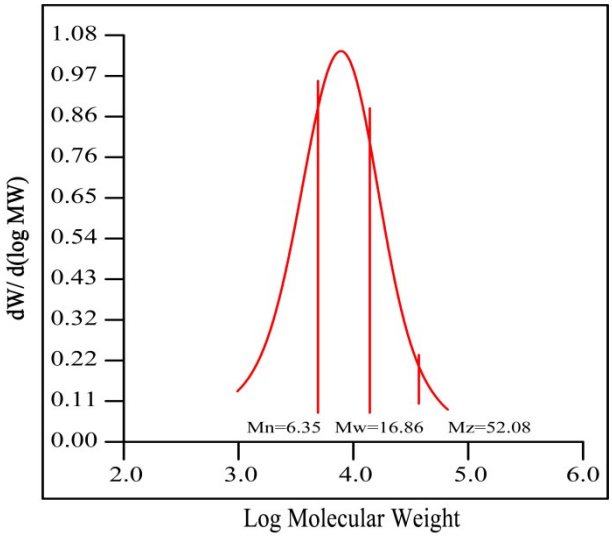

Figure S2. The GPC curve of polymer PCLMD-PPEGMA.

Preparation and characterization of P(CL-co-MMD)-g-PPEGMA-g-NLS-TAT-REDV (P CLMD-PPEGMA-NLS-TAT-REDV) copolymer.

It was prepared by introducing NLS-TAT-REDV polypeptide onto PCLMD-PPEGMA usi ng NHS-PEG-OPSS as the linking agent. Dissolve 0.25 g PCLMD-PPEGMA in 25 mL D MSO. Take 5 mL of 10 mg/mL PCLMD-PPEGMA copolymer solution in a bottle with m agnetic stirring, and add 5 mL of 10 mg/mL OPSS-PEG-NHS DMSO solution dropwise.

After stirring for 2 h, 5 mL of 10 mg/mL DMSO solution containing NLS-TAT-REDV p olypeptide was added, and then the reaction was stirred at room temperature overnight. Th e product was subjected to dialysis and freeze-drying before use.

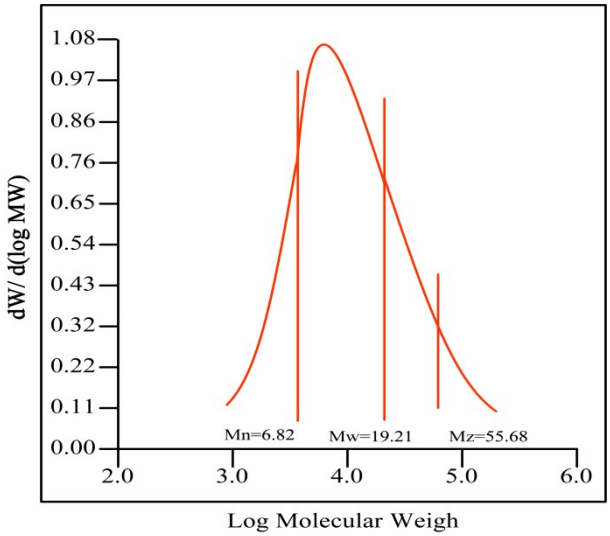

Figure S3. The GPC curve of polymer PCLMD-PPEGMA-NLS-TAT-REDV.

Table 1. The Mn and Mw values for PCLMD, PCLMD-PPEGMA and PCLMD-PPE GMA-NLS-TAT-REDV.

Sample	Mn (kDa)	Mw (kDa)	Mz (kDa)	Mw/Mn
PCL-co-MMD	4.93	11.45	35.41	2.32
(PCL-co-MMD)-g-PPEGMA	6.35	16.86	52.08	2.65
(PCL-co-MMD)-g-PPEGMA-g-NLS-TAT-REDV	6.82	19.21	55.68	2.82

Preparation and characterization of gene complex micelles.

The 0.5 mg/mL pZNF580 (dissolved in PBS) was added dropwise to the PCLMD-PPEG MA-NLS-TAT-REDV solution and mixed, and allowed to stand at room temperature for 3 0 min. pZNF580 concentration was adjusted to obtain different weight ratios of (w/w_{pZNF58_0} = 0.25, 0.5, 1, 2, 3, 4) multi-targeting gene complex micelles (TCMs@pZNF580). NTC Ms@pZNF580 were prepared using the same method. A nanoparticle sizer (Zetasizer 3000 HS, Malvern Instruments, UK) was used to measure the particle size and zeta potential of

the gene complex micelles.

Table 2. The changes of particle size, PDI and zeta-potential for PCLMD-PPEGMA-NLS-TAT-REDV/pZNF580.

$W/W_{pZNF580}$	Size (nm)	PDI	Zeta potential (mV)
0.25	165.68±0.95	0.30±0.06	24.58±1.25
0.5	168.35±2.80	0.41±0.27	24.26±1.32
1	172.42±1.67	0.34±0.15	23.85±0.54
2	181.15±1.74	0.35±0.16	23.58±1.75
3	183.86±1.32	0.31±0.12	23.51±1.45
4	189.31±0.25	0.30±0.08	23.28±0.75

References

[33] Q. Li, X. F. Hao, J. Lv, X. K. Ren, K. Y. Zhang, I. Ullah, Y. K. Feng, C. C. Shi and W. C. Zhang, *J. Mater. Chem. B,* 2017, **5**, 1673-1687.