Electronic Supplementary Information

Anti-phagocytosis and tumor cell targeting micelles prepared from multifunctional cell membrane mimetic random copolymers

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\textit{p}-Nitrophenyloxycarbonylpoly(ethylene glycol) methacrylate (NPEM) was prepared according to the method reported by Konno et al.[T. Konno, J. Watanabe, and K. Ishihara, Biomacromolecules, 2004, 5, 342-347] Briefly, 17.05 g poly(ethylene glycol) (360) monomethacrylate and 4.80 g TEA were added and dissolved by 50 mL chloroform in a 250 mL three necked flask equipped with a dropping funnel, a thermometer, and a mechanical stirrer. After the solution was cooled at $-30^\circ$C, \textit{p}-nitrobenzoyl chloride (9.62 g) dissolved in 60 mL chloroform was added dropwise to the stirred solution over a period of 1 h. After the reaction mixture was maintained at $-30^\circ$C for 2 h, the temperature was risen to 25 $^\circ$C slowly. The precipitate formed in the reaction mixture was filtered off and the solvent in the filtrate was evaporated under reduced pressure. The residue solution was further precipitated by small amount of dry diethyl ether and removed the TEA chloride by filtration. By evaporation of the filtrate under reduced pressure, NPEM was obtained as a yellow oily liquid.

The structure of NPEM was confirmed by $^1$H NMR spectrum measured in CDCl$_3$ (Fig. S1). $\delta$(ppm) = 8.30 (m, 2H), 7.42 (m, 2H), 6.16 (m, 1H), 5.60 (m, 1H), 4.45 (m, 2H), 4.28 (m, 2H), 3.68 (m, 20H), 1.94 (m, 3H). The newly appeared peak at 4.45 ppm suggests the success of esterification between the hydroxyl group and the \textit{p}-nitrobenzoyl chloride. All the proton signals appeared and were clearly attributed. Furthermore, the peaks a at 4.28
ppm and d at 8.30 ppm from the two components showed almost the same areas, suggesting the successful synthesis of PMEN active ester and high purity of the compound (Biomacromolecules, 2004, 5, 342-347).

**Fig. S1.** $^1$H-NMR spectra of NPEM in CDCl$_3$.

2. **Synthesis of Chol-NH$_2$**

5.02 g (0.0112 mol) cholesteryl chloroformate was dissolved with 60 mL of anhydrous DCM in a dropping funnel and dropped to a cooled (0 °C) three-necked flask (250 mL) containing 37.5 mL anhydrous ethylenediamine (EDA) and 50 mL of anhydrous dichloromethane (DCM). After dropping, the solution was stirred and reacted in the dark at room temperature overnight. The reacted solution was extracted three times with equal volume of distilled water to remove ethylenediamine hydrochloride and remainder EDA. The organic phase was then dried over MgSO$_4$ for overnight. After filtration, the filtrate was evaporated under reduced pressure at 30 °C and white powder Chol-NH$_2$ was obtained.

The structure of Chol-NH$_2$ was confirmed by $^1$H NMR as shown in **Fig.S2**. $\delta$(ppm) = 5.37 (m, 1H), 5.02 (s, 1H), 4.49 (m, 1H), 3.24 (m, 2H), 2.80 (m, 2H). The two methylene proton signals of the ethylene diamine segment appeared at 2.80 ppm and 3.24 ppm respectively, and the almost equal areas of the two peaks suggested less impurity. The signal peak at
5.02 ppm attributed to the proton of newly formed amide group (PNAS, 2012, 109, 11294–11299) and the peak area ratio 2:1 of the a:b (at 2.80 ppm and 5.38 ppm respectively) further support the successful preparation of the compound.

Fig. S2. $^1$H-NMR spectrum of Chol-NH$_2$ in CDCl$_3$.

3. **Synthesis of FA-NH$_2$**

Synthetic route of FA-NH$_2$ was shown in Fig. S3. Firstly, 4.40 g (0.0732 mol) of anhydrous ethylenediamine (EDA) was dissolved in 110 mL mixed solvents of anhydrous methanol (99 mL) and anhydrous TEA (11 ml), and reacted with 3.63 mg (0.0172 mol) of di-tert-butyl dicarbonate (Diboc) overnight at room temperature. The mixture solvent was evaporated by a rotary evaporator to remove methanol. The oily liquid was redissolved in 100 mL of DCM, extracted three times with equal volume of distilled water supplemented with 10% Na$_2$CO$_3$, and then dried with MgSO$_4$ overnight. After filtration and evaporation, mono-protected EDA (BOC-NH$_2$) was obtained.

Secondly, 1.00 g (0.0023 mol) of folic acid (FA) dissolved in 40 mL of anhydrous dimethyl sulfoxide (DMSO) containing 0.5 mL of anhydrous TEA was activated by 0.50 g (0.0023 mol) N,N-dicyclohexylcarbodiimide (DCC) and 0.52 g (0.0046 mol) N-hydroxysuccinimide (NHS) stirred at room temperature for 24 h. The result solution was
filtrated to remove the by-product 1,3-dicyclohexylurea. 0.39 g (0.0025 mol) of BOC-NH₂ was then added into the above filtrate in the dark and stirred at room temperature overnight. After filtration, the filtrate was dropped into a stirred 400 mL mixed solvent of ether/acetone (1:1, v/v) cooled at 0 °C. After the yellow precipitate was separated and washed three times with ether by centrifugation, the mono-amino protected EDA coupled folic acid (FA-NHBOC) was obtained.

Finally, FA-NHBOC (0.46 g, 0.0008 mol) dissolved in 4 mL trifluoroacetic acid was stirred at room temperature for 2 h. The solution was evaporated under reduced pressure and the yellow powder was redissolved in 5 mL N,N-Dimethylformamide, and then precipitated in 25 mL ether. After centrifuged at 4000rpm/min and washed with ether for three times, the sample was dried under vacuum at room temperature for 24 h. FA-NH₂ was obtained as a yellow powder and the structure was confirmed by ¹H NMR as shown in Fig. S4. After the substitution reaction and purification, two methylene hydrogen of the ethylene diamine segment peaks appeared at 2.90 ppm and 3.10 ppm (Polymer, 2011, 52, 987-995; Chem. Commun., 2010, 46, 2632–2634), and both the peak areas are equal (a:b = 1:1). These evidences indicated that FA-NH₂ was prepared by the proposed synthesis procedures.
Fig. S3. Structure and synthetic route of FA-NH$_2$.

Fig. S4. $^1$H-NMR spectrum of FA-NH$_2$ in DMSO-d$_6$. 
Fig. S5 Number average size of PMNCF-31 micelles affected by storage, pH, dilution, PBS, and redispersion after freeze-drying.

Fig. S6. Fluorescence microscopic images of MADB-106 cell uptake efficiency for PMNCF micelles bearing different molar ratios of folic acid incubated for different periods.
Fig. S7. MADB-106 cytotoxicity of PMNCF micelles analyzed by MTT assay. Phenol was used as the positive control. Data represent mean ± SD, **P < 0.01, n = 6.
Fig. S8. XPS high resolution spectra of N1s and P2p in differently dried PMNCF-23 micelle films. The concentration of nitrogen in the air dried micelle film (5.17%) is significantly higher than that in the freeze dried micelle film (4.21%). Meanwhile, the concentrations of phosphorus in the two samples are almost same, suggesting more of the folic acid groups located near the surface in the air dried micelle than the freeze dried micelle.