Supporting Information Available

Pegylated polyaspartamide–polylactide based nanoparticles penetrating cystic fibrosis artificial mucus

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Synthesis and characterization of PHEA-RhB copolymer

Derivatization of PHEA with RhB, to obtain PHEA-RhB copolymer, was carried out by using a recently described procedure[20].

Briefly, CDI (0.0128 g, 0.079 mmol) was dissolved in a-DMF (0.5 mL) under argon atmosphere, and added drop-wise to a RhB (0.030 g, 0.063 mmol) solution in a-DMF (3.5 mL), and stirred at 40°C for 4 h under argon atmosphere. After activation time a solution of PHEA (1 g, 6.3 mmol) in a-DMF (30mL) containing DEA (0.033 mL, 0.32 mmol) as catalyst, was added drop-wise to the CDI-activated RhB solution. The reaction mixture was left under argon atmosphere and continuous stirring at 40°C for 48 h, then precipitated in diethyl ether and centrifuged at 4°C for 15 min, at 9800 rpm by using an Allegra X-22 R centrifuge (Beckman Coulter), equipped with a F0850 rotor and temperature control. The obtained solid product was recovered, washed four times with ethanol. Then, the product, dried under vacuum, was obtained with a yield of 85 wt% based on the starting PHEA weight.

^{1}H-NMR spectra were obtained by a Bruker Avance II-300 spectrometer, working at 300 MHz.

^{1}H-NMR (300 MHz, D_2O, 25°C, TMS): δ 1.15 (m, $12H_{RhB}$ **CH_3**-CH_2-); δ 2.71 (m, $2H_{PHEA}$ -CO-CH-**CH_2**-CO-NH-); δ 3.29 (t, $2H_{PHEA}$ -NH-**CH_2**-CH_2-O-); δ 3.58 (t, $2H_{PHEA}$ -NH-CH_2-**CH_2**-O-); δ 4.65 (m, $1H_{PHEA}$ -NH-**CH**$(CO)CH_2$-); δ 8.00-8.50 (m, $10H_{RhB}$ **H**-Ar).

$\overline{M}_w$ of PHEA-RhB graft copolymer, determined by SEC, was equal to 34.8 kDa ($\overline{M}_w/\overline{M}_n$ = 1.41).

Molar Extinction Coefficient (ε) of RhB and PHEA-RhB were evaluated as reported elsewhere[20]. The absorption at maximum wavelength was measured and the ε was calculated from the curve obtained by plotting absorbance versus sample solution concentrations in concentration ranging between 10^{-7}-10^{-4} M, by recording UV spectra with a by a RF-5301PC spectrofluorometer (Shimadzu, Italy). Each experiment was repeated in triplicate. For RhB, the maximum wavelength was 554 nm (y = 117044x, R^2 = 0.9996), while for

PHEA-RhB was 561 nm ($y = 53200x$, $R^2 = 0.99$).

Synthesis and characterization of PHEA-RhB-PLA copolymer

Derivatization of PHEA-RhB with PLA, to obtain PHEA-RhB-PLA copolymer, was carried out by using a recently described procedure, reported elsewhere[20].

Briefly, CDI (0.114 g, 0.71 mmol) dissolved in a-DMF (0.1 mL) under argon atmosphere, was added drop-wise to a PLA (5 g, 0.35 mmol) solution in a-DMF(28.5 mL), and left to continuous stirring at 40°C for an activation time of 4 h under argon atmosphere. After activation time a solution of PHEA-RhB (0.7 g, 4.43 mmol) in a-DMF (8.5 mL) containing DEA (0.185 mL, 3.54 mmol) as catalyst, was added drop-wise to CDI-activated PLA (aPLA) solution. The reaction mixture was left under argon atmosphere and continuous stirring at 40°C for 70 h, and then precipitated in diethyl ether:dichloromethane mixture (15:1 vol/vol). The obtained suspension was centrifuged at 4°C for 15 min, at 9800 rpm, and the obtained solid product was recovered, washed four times with the same mixture, separating the product by centrifugation. Then, the solid product, dried under vacuum, was obtained with a yield of 280 wt% based on the starting PHEA-RhB weight.

^{1}H-NMR (300 MHz, d_7-DMF, 25°C, TMS): δ 1.15 (m,12H_{RhB} **CH_3**-CH_2-); δ 1.3 and δ 1.7 (2d, 582 H_{PLA} -O-CO-CH(**CH_3**)-O-); δ 2.8 (m, 2H_{PHEA} -CO-CH-**CH_2**-CO-NH-); δ 3.3 (t, 2H_{PHEA} -NH-**CH_2**-CH_2-O-); δ 3.59 (t, 2H_{PHEA} -NH-CH_2-**CH_2**-O-); δ 4.2-4.5 and δ 5.1-5.5 (m, 194 H_{PLA} -O-CO-**CH**(CH_3)-), and δ 4.8 (m, 1H_{PHEA} -NH-**CH**(CO)CH_2-); δ 7.0-8.0 (m, 10H_{RhB} **H**-Ar).

$\overline{M}_w$ of PHEA-RhB-PLA graft copolymer, determined by SEC analysis, was found to be 205.0 kDa ($\overline{M}_w/\overline{M}_n$ = 1.45).

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