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Controllable drug release and effective intracellular accumulation highlighted by anisotropic biodegradable PLGE nanoparticles

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TABLE OF CONTENTS

Experimental Section

1. Materials

L-Lactide (L-LA) and glycolide (GA) (Purac, Netherlands) were twice recrystallized from ethyl acetate. PEG with two terminal hydroxyl groups (Mw = 1000, Beijing Yili Fine Chemical Co., Ltd., China) was dissolved in toluene and dried by an azeotropy method. Ethyl acetate (Beijing Chemical Works, China) was dried by P_2O_5 and then distilled. All other chemicals were used without further purification.

2. Characterization

The molecular weight and molecular weight distribution of PLGE were measured by gel permeation chromatography (GPC) (Waters510 apparatus equipped with Shodex GPC KF-800 columns, Waters, USA) at 35 ^oC. Chloroform served as the eluent with a flow rate of 1.0 mL/min, and polystyrene served as standard. The molecular weight of PLGE were also calculated by 1H-NMR spectrum (Bruker Avance 400, Bruker, Switzerland) using CDCl3 as the solvent and tetramethylsilane as the internal reference. Glass transition temperature (Tg) of PLGE was acquired from differential scanning calorimetry (DSC) (DSC822e, METTLER, Switzerland) and the decomposition temperature (Td) was obtained by Thermal Gravimetric Analysis (TGA).

SEM (JSM-6700F, JEOL, Japan) was used to observe the morphology of the particles. Particle size and distribution were analyzed by SEM images with Image-Pro Plus 6.0 software. Briefly, the sizes of at least 100 particles in each image were measured, and then the size distribution was calculated automatically by the software. A laser confocal scanning microscope (CLSM, Leica TCS-sp2, Germany) was used to observe the distribution of NPs in cells.

3. Synthesis of PLGE copolymer

The PLGE triblock copolymer was synthesized by a ring-opening polymerization of L-LA and GA in the presence of PEG as the initiator. Briefly, L-LA, GA and PEG (molar ratio of LA/GA/PEG was 68.2/29.3/2.5) were added into a polymerization tube followed by adding 0.05 wt% stannous octoate as catalyst. After de-oxygenating with argon three times, the polymerization tube was sealed under vacuum and polymerized at 180 ^oC for 24 h. The obtained crude copolymer was purified with chloroform and precipitated in excess ethanol. Finally, the obtained white precipitation was dried under vacuum at 25 ^oC until constant weight.

4. Synthesis of PLGE NPs with different morphologies

4.1 Synthesis of spherical PLGE NPs

The spherical PLGE NPs were prepared by an emulsification-diffusion method. In detail, 100 mg of PLGE copolymer was dissolved in 10 mL of ethyl acetate, 0.2~1.5 g of PVA was dissolved in 20 mL of deionized water. Then the two solutions were mixed together, and emulsified by a homogenizer at 20000 rpm for 10 min. Subsequently, 90 mL of hot water was added to the emulsion under moderate magnetic stirring. After the organic solvent evaporating in a rotary evaporator, the concentrated suspension of spherical PLGE NPs can be produced.

4.2 Synthesis of anisotropic PLGE NPs

The anisotropic PLGE NPs were prepared by a film stretching/compressing method from the suspension of spherical NPs. Typically, 10% w/v PVA and 2% v/v glycerol were added to the above concentrated suspension of spherical PLGE NPs, then the suspension was poured into a horizontal groove and casted into an even film till homogeneous. After the film was dried at room temperature, it was cut into sections of ca. 1.5 cm × 6 cm and fixed on a homemade apparatus. Then the film was heated under 90 oC for 15 min, and was stretched or compressed at planned ratios. The temperature was kept at 90 oC for another 15 min. Subsequently, the film was quickly cooled by liquid nitrogen. After the films gradually recovered to room temperature, they were immersed in water to redissolve PVA under magnetic stirring. The obtained anisotropic PLGE NPs were separated from the solution, washed with water for three times, and freeze-dried for 48 h.

5. Testing of morphology stability of NPs

To investigate the influence of temperature, nanorod shaped PLGE NPs were redispersed in water, and shook in a water bath for 10 min at 37 ^{o}C and 50 ^{o}C, respectively. The NPs were centrifuged and freeze-dried for SEM imaging. To investigate the influence of solvent and treatment time to morphology stability, the nanorod shaped PLGE NPs were also redispersed in PBS solution for 10 min at 37 ^{o}C, and redispersed in water for 12 h at 37 ^{o}C.

6. Synthesis of NR loaded PLGE NPs with different morphologies

The NR loaded PLGE NPs were prepared similar with PLGE NPs. Additional 100 mg of NR was added in the ethyl acetate. All other steps were the same with the synthesis of spherical and anisotropic PLGE NPs. We difine drug loading (DL) and encapsulation efficiency (EE) as follows:

DL (%) = weight of Nile red in PLGE nanoparticles/weight of PLGE nanoparticles,

EE (%) = weight of Nile red in PLGE nanoparticles/weight of Nile red fed initially.

7. Drug release of NR loaded PLGE NPs with different morphologies

15 mg of NR loaded PLGE NPs were dispersed in 5 mL of PBS (pH = 7.4), and the solution was kept in a water bath at 37 ^{o}C with a rotation speed of 60 rpm. 2 mL of the solution were taken out and centrifuged at day 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, and 28, respectively. The precipitation was dispersed in DCM to extract NR completely. After the DCM was removed by evaporation, 2 mL of DMSO was added to test the UV absorption. One value of each sample at predetermined time was analysed in a test cycle, where a test cycle is 28 days. The test was repeated from 0 to 28 day for three cycles, thus three values of each time point were obtained and analysed. Subsequently, the three values were averaged to get each value of drug release of NR loaded PLGE.

8. Endocytosis of NR loaded PLGE NPs with different morphologies

MCF-7 cells was dispersed in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, US) containing 10% of bovine serum and in a six-pore plate with a concentration of 2×10^5 cells/well. The cells were incubated for 24 h at 37 ^{o}C, with a CO_2 concentration of 5% and humidity of 95%. Subsequently，the culture solution was removed and new DMEM solution was added (300 ug/mL and 211 μg/mL, containing equal amount of fluorescent probe). The cells were treated with 4% paraformaldehyde for 30 min at room temperature after an interval of time in the incubator, and then they were fixed on the glass slide. A CLSM was used to observe the distribution and to calculate the fluorescence intensity of NPs in cells, the excitation wavelength and emission wavelength were 490 nm and 590 nm, respectively.

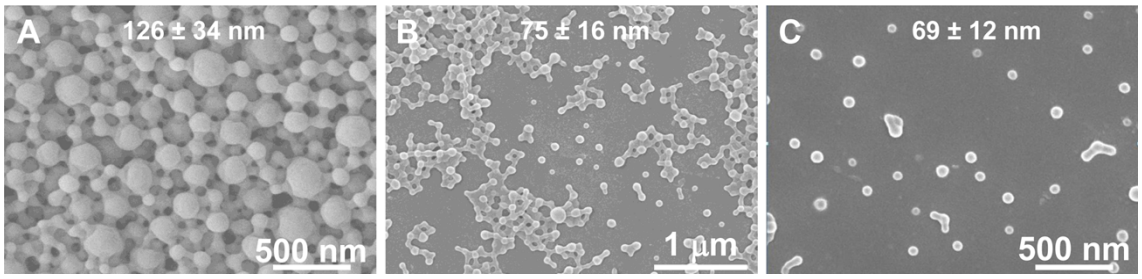
Supplementary information

Supplementary Table S1 Detailed characterization of PLGE copolymer

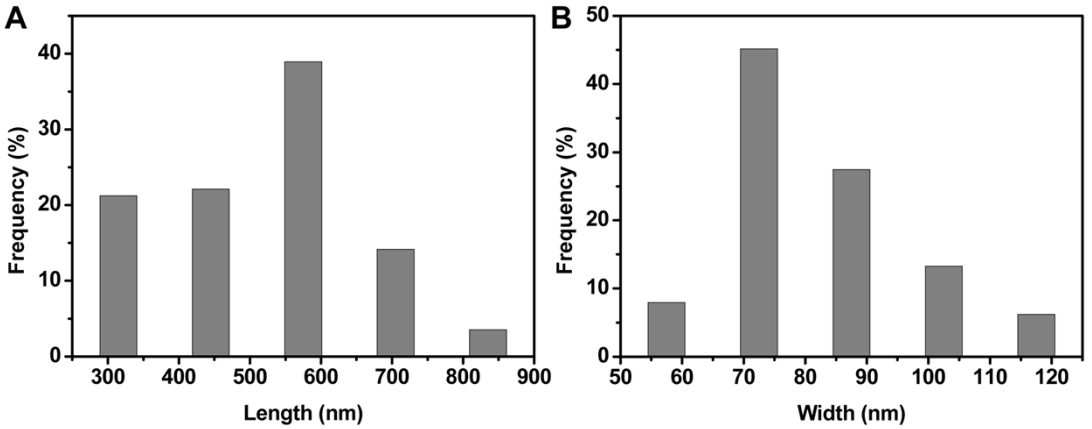
Polymer	M_{PEG}	LA/GA/PEG (Molar Ratio)[b]	LA/GA/PEG (Molar Ratio)[a]	Mn[c]	Mn[d]	Mn[e]	P.D.	Tg (°C)	Td (°C)
PLGE	1000	67.9/29.7/2.4	68.2/29.3/2.5	60000	63600	50049	1.38	46.6	240.5

[a] Molar ratio of LA/GA/PEG fed initially; [b] Molar ratio of LA/GA/PEG calculated by ^{1}H NMR; [c] Molecular weight of PLGE designed initially; [d] Molecular weight of PLGE calculated by ^{1}H NMR ; [e] Molecular weight of PLGE calculated by GPC.

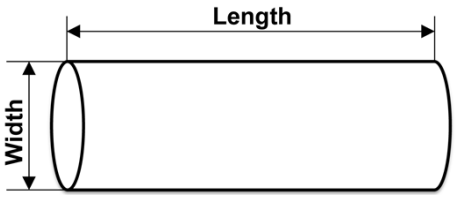
Supplementary Fig. S1 Spherical PLGE NPs with different sizes. (A) Sphere1, 1% PVA, 30 mL; (B) Sphere2, 2% PVA, 30 mL; (C) Sphere3, 5% PVA, 15 mL.



Supplementary Fig. S2 Size density plot of rods with average length of 523 ± 141 nm and average width of 82 ± 15 nm. (A) Length; (B) Width. The sizes are counted from 112 nanoparticles.



Supplementary Fig. S3 A schematic diagram illustrating the parameters of the rod.



Supplementary Cal. S1 Calculation of the volume and surface area of the NPs. We calculated the volume and surface area of the spheres, rod1 (stretched by 60%), and rod2 (stretched by 120%). The following calculation (Cal. S1) is based on the assumption that they are uniform particles and that the top and bottom of the rod are circular.

1. Average volume of the sphere:

$$V_S = \frac{4}{3}\pi r^3 = \frac{4}{3}\times 3.14\times(\frac{128}{2})^3 = 1.10\times 10^6\, nm^3 / particle$$

2. Average volume of rod1:

$$V_{R1} = \pi r^2 L = \frac{1}{4}\pi W^2 L = \frac{1}{4}\times 3.14\times 103^2\times 366 = 3.05\times 10^6\, nm^3 / particle$$

3. Average volume of rod2:

$$V_{R2} = \pi r^2 L = \tfrac{1}{4}\pi W^2 L = \tfrac{1}{4}\times 3.14\times 82^2 \times 523 = 2.76\times 10^6\, nm^3 \,/\, particle$$

4. Average surface area of the sphere:

$$S_S = 4\pi r^2 = 4\times 3.14\times (\tfrac{128}{2})^2 = 5.14\times 10^4\, nm^2 \,/\, particle$$

5. Average surface area of rod1:

$$S_{R1} = 2\pi r^2 + 2\pi rL = \tfrac{1}{2}\pi W^2 + \pi WL = \tfrac{1}{2}\times 3.14\times 103^2 + 3.14\times 103\times 366 = 1.35\times 10^5\, nm^2 \,/\, particle$$

6. Average surface area of rod2:

$$S_{R2} = 2\pi r^2 + 2\pi rL = \tfrac{1}{2}\pi W^2 + \pi WL = \tfrac{1}{2}\times 3.14\times 82^2 + 3.14\times 82\times 523 = 1.45\times 10^5\, nm^2 \,/\, particle$$

Supplementary Fig. S4 The morphological stability of rods at 37 °C (A) for 12 h in deionized water; (B) in PBS solution.