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Bioinspired polynorepinephrine nanoparticles as an efficient vehicle for enhanced drug delivery

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Derivation of polymers from various neurotransmitters

A range of neurotransmitters (epinephrine, serotonin and norepinephrine) were polymerised from 1 mg/mL monomer concentrations under alkaline ethanolic solutions per the conditions used for PNE synthesis unless otherwise indicated (**Table S1)**. TEM micrographs of resulting polymer particles are shown in **Figure S1**.

Table S1. Polymerisation of the Epinephrine, Serotonin and Norepinephrine, under alkaline ethanolic conditions.

Monomer	Reaction solution	Time [hours]	Results
Epinephrine	5 mL Tris buffer solution (10 mM, pH 8.5) + 1mL ethanol	48	No particles
Epinephrine	5 mL Tris buffer solution (10 mM, pH 9.7) + 1mL ethanol	48	Figure S1a
Serotonin	5 mL Potassium Phosphate (500mM, pH 9.5 [1]	48	Figure S1b
Norepinephrine	5 mL Tris buffer solution (10 mM, pH 8.5) + 1mL ethanol	48	Figure S1c

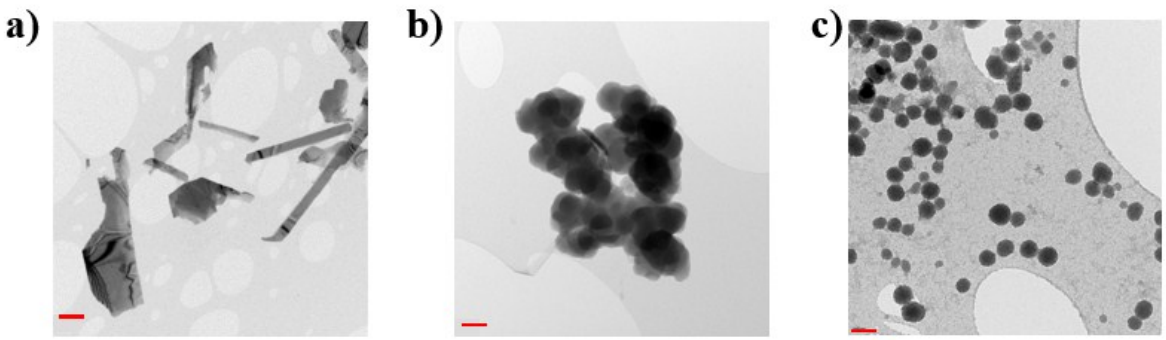

Figure S1.TEM image of a). Polyepinephrine from polymerisation under pH 9.7; b) Polyserotonin; c) Polynorepinephrine, Scale bar 200nm.

Comparison of antifouling properties of PNE vs. PDA

5 mg PNE and PDA nanoparticles were incubated in 5 mL protein solution (1 mg/mL Bovine Serum Albumin (BSA) and lysozyme (LYZ) in (1x PBS pH 7.4) for 4 hours, stirring at room temperature. Size and zeta potential change rate were analysed before and after incubation (**Table S2**). Size change rate= $(S_2-S_1)/S_1$, zeta potential change rate= $(Z_2-Z_1)/Z_1$, where S_1,S_2,Z_1 and Z_2 represent the size before and after incubation, and zeta potential before and after the incubation separately. Experiment was conducted in triplicate, results shown in **Table S2** was the average number of three experiment, standard deviation (S.D.) was presented as well.

Table S2. Antifouling properties of PNE and PDA particles. Size and zeta potential change of PDA and PNE nanoparticles after 4-hour incubation in 1 mg/mL BSA and LYZ.

	Size change (%)	**S.D.**	**Zeta potential change (%)**	**S.D.**
PDA/BSA	41.14	5.6	36.11	2.3
PNE/BSA	2.59	0.7	13.96	1.2
PDA/LYZ	50	17.9	102.77	0.16
PNE/LYZ	15.33	6.5	91.33	4.85

To analyse the stability of PNE nanoparticles, zeta potential of these particles was measured at different pH conditions (**Figure S2a**). Zeta potential of PNE nanoparticles decreases at lower pH levels. This trend is not consistent above ~pH 6.0. To understand the stability of PNE particles, the diameter was measured for 10 days as presented in **Figure** S2b. PNE stability in cell culture media was also observed in **Figure** S2c.

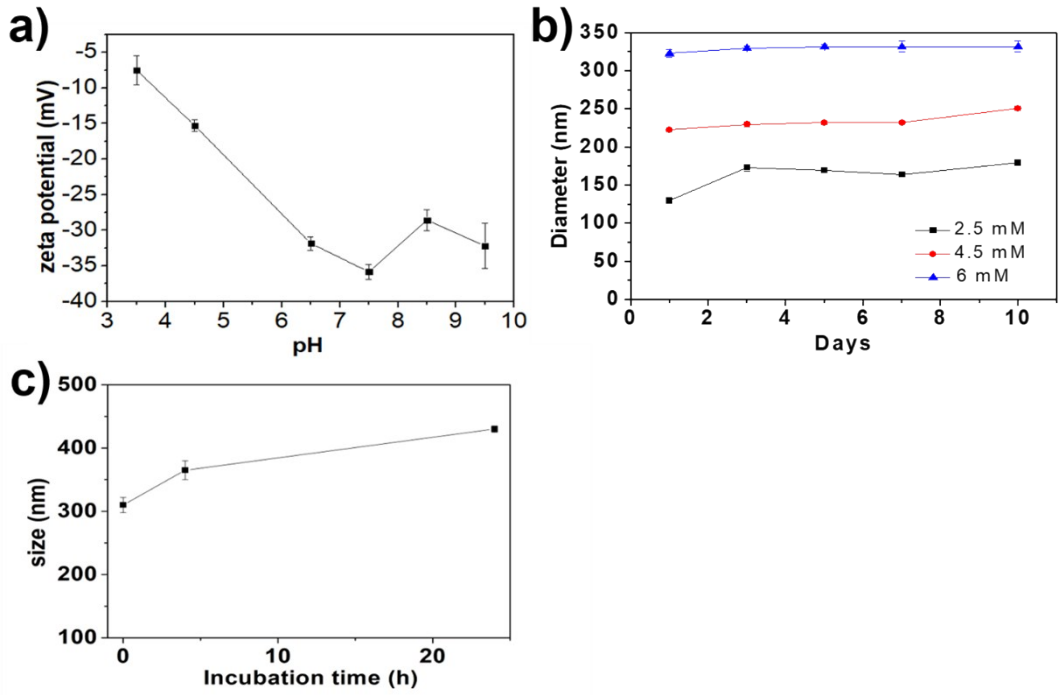

Figure S2. a). PNE zeta potential as a function of pH; b). PNE particles size change over 10 days incubation; c). PNE particles size change in cell culture media DMEM medium supplemented with 10% FBS and 1% P/S, PBS and 0.9% NaCl.

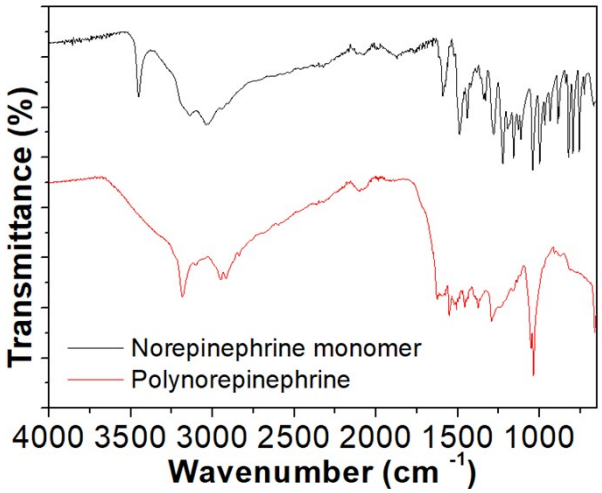

Figure S3. FTIR of norepinephrine and PNE.

The fluorescence spectra of PNE/DOX water solution and free DOX solution was shown in **Figure S4a.** a linear standard calibration curve of 0.99 was obtained at DOX concentration range from 0.1 to 6 μg/mL (**Figure S4 b**). Based on the standard curve here, we conclude the mass of loaded DOX, the loading efficacy= loaded DOX divided by mass of PNE/DOX (w/w %). When DOX/PNE mass ratio raise from 20% to 300%, DOX loading efficacy increases from 1% to 18% as shown in **Figure** S4c and shows the tendency of higher loading efficacy when mass ratio keeps raising.

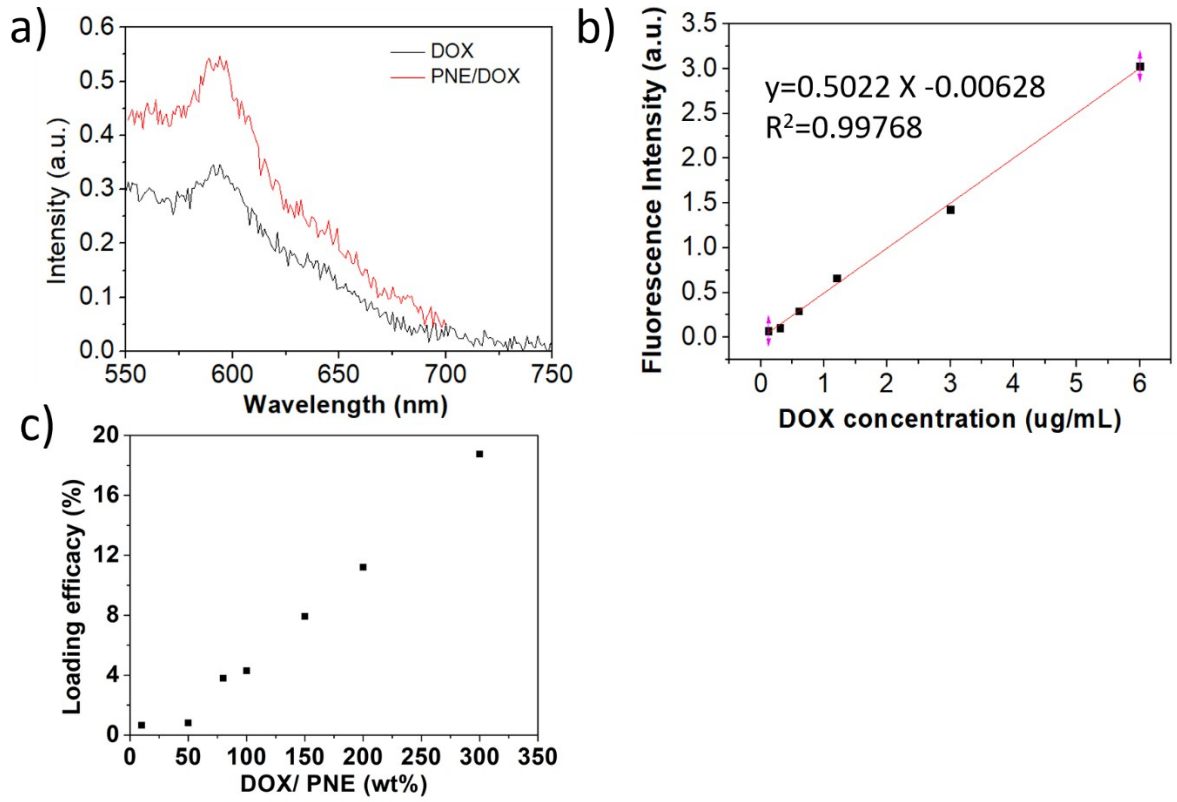

Figure S4 a). Fluorescence intensity of DOX and PNE/DOX; b). DOX standard curve; c). DOX loading efficacy at various DOX/PNE mass ratio.

[1] Nakatsuka, N., Hasani-Sadrabadi, M. M., Cheung, K. M., Young, T. D., Bahlakeh, G., Moshaverinia, A. & Andrews, A. M. (2018). Polyserotonin nanoparticles as multifunctional materials for biomedical applications. ACS nano, 12(5), 4761-4774.