Exploiting nano-in-micro technologies to couple PLGA-hydroxyl-FK866 nanoparticles to hydrogel network for local drug release

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SUPPLEMENTARY INFORMATION

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SI1. Properties of Rho-PLGA nanoparticles

Rhodamine-labelled PLGA nanoparticles (Rho-PLGA NPs) were prepared by microfluidics system, using 5 mg/mL rhodamine-PLGA in acetonitrile and 1% PVA in water with FRR 1/9, total flow rate 200 µL/min, temperature was set as 25 °C. Of note, hydroxyl-FK866-PLGA was prepared using PLGA (RG752H, lactide:glycolide 75:25, Mw 4000-15000, Sigma-Aldrich, UK). Size and ζ-potential were measured by DLS as reported in the main manuscript.

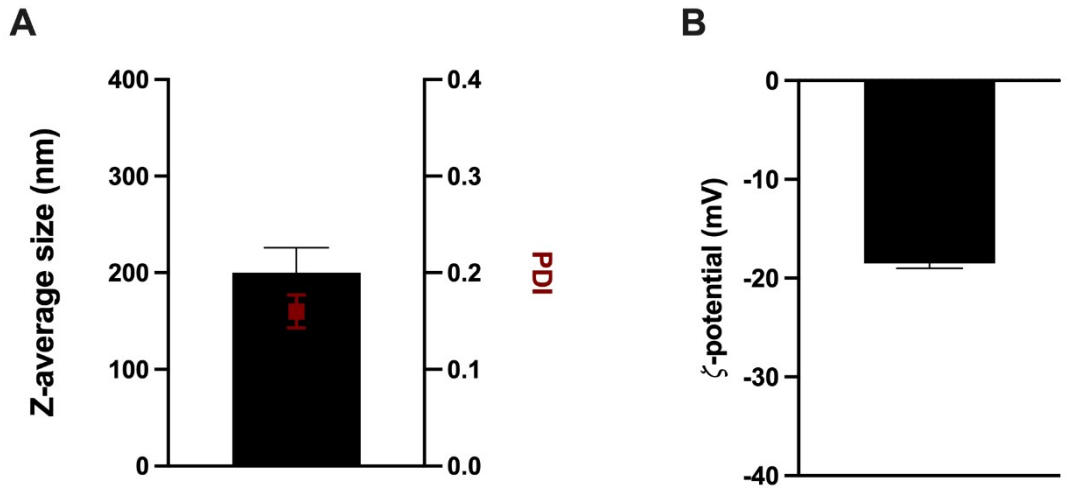

Figure S1. Characterisation of rhodamine-labelled PLGA nanoparticels (Rho-PLGA NPs): **A)** Size; **B)** ζ-potential. Data reported as mean ± st.dev. of N=3 independent experiments.

SI.2 Physico-chemical properties of hydroxyl-FK866-PLGA nanoparticles

Hydroxyl-FK866-PLGA nanoparticles were prepared using 5 mg/mL hydroxyl-FK866-PLGA (**Figure S2A**) in acetonitrile, 1% w/v PVA (aq.) as the aqueous phase with total flow rate = 200 µL/min, flow rate ratio = 2:3 and temperature set as 25 °C; as reported in our previous work (https://doi.org/10.1016/j.bioadv.2023.213649).

As described in the main manuscript the highest concentration of chitosan (CS) was used to coat hydroxyl-FK866-PLGA. Briefly, chitosan solution was prepared at a concentration of 0.1% w/v in 4.6 mM HCl. Z-average size, PDI and ζ-potential (**Figure S2B** and **S2C**) were measured by DLS as reported in the methodology section in the main manuscript.

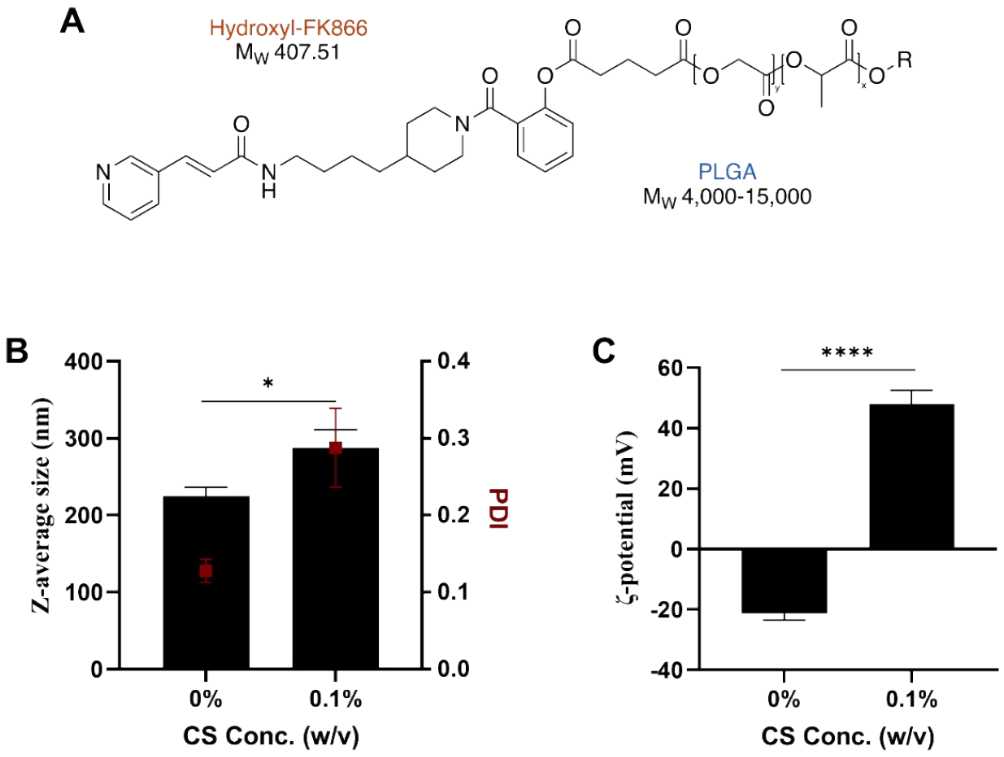

Figure S2. Size and ζ-potential of uncoated and CS-coated hydroxyl-FK866-PLGA NPs. **A)** Structure of hydroxyl-FK866-PLGA conjugate; **B)** Z-average size and PDI **F)** ζ-potential. Data is presented as average ± st.dev. (N=3 independent experiments). Differences were considered significant at $p < 0.05$ (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$).

SI.3 Properties of PLGA nanoparticles: role of chitosan concentration in coating efficiency

Table S1. Physical properties of PLGA NPs and CS/PLGA NPs varying CS concentration during the coating step. PLGA NPs were prepared with microfluidics and then coated as described in the main manuscript. Z-average size, PDI and ζ-potential were measured by DLS, data is presented as average ± st.dev. (N=3 independent experiments).

	PLGA NPs	**CS/PLGA NPs (0.01% w/v CS)**	**CS/PLGA NPs (0.1% w/v CS)**
Z-average size (nm)	207 ± 15	218 ± 4	245 ± 20
PDI	0.118 ± 0.007	0.207 ± 0.024	0.256 ± 0.025
ζ-potential (mV)	-21.90 ± 2.73	+23.00 ± 4.32	+49 ± 1.25

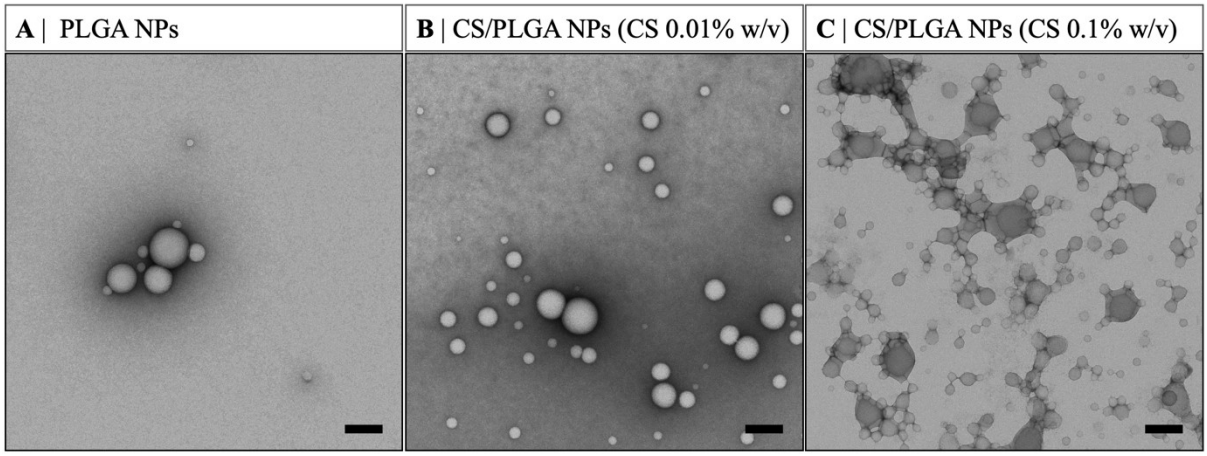

Figure S3. Transmission electron microscopy images of PLGA NPs and CS/PLGA NPs varying CS concentration during the coating step: A) PLGA NPs, B) CS/PLGA NPs using 0.01% w/v/ CS, and C) CS/PLGA NPs using 0.1% w/v/ CS. Scale bars: 200 nm.