Supporting information

Taylor Dispersion Analysis and Release Studies of β-Carotene-Loaded PLGA Nanoparticles and Liposomes in Simulated Gastrointestinal Fluids

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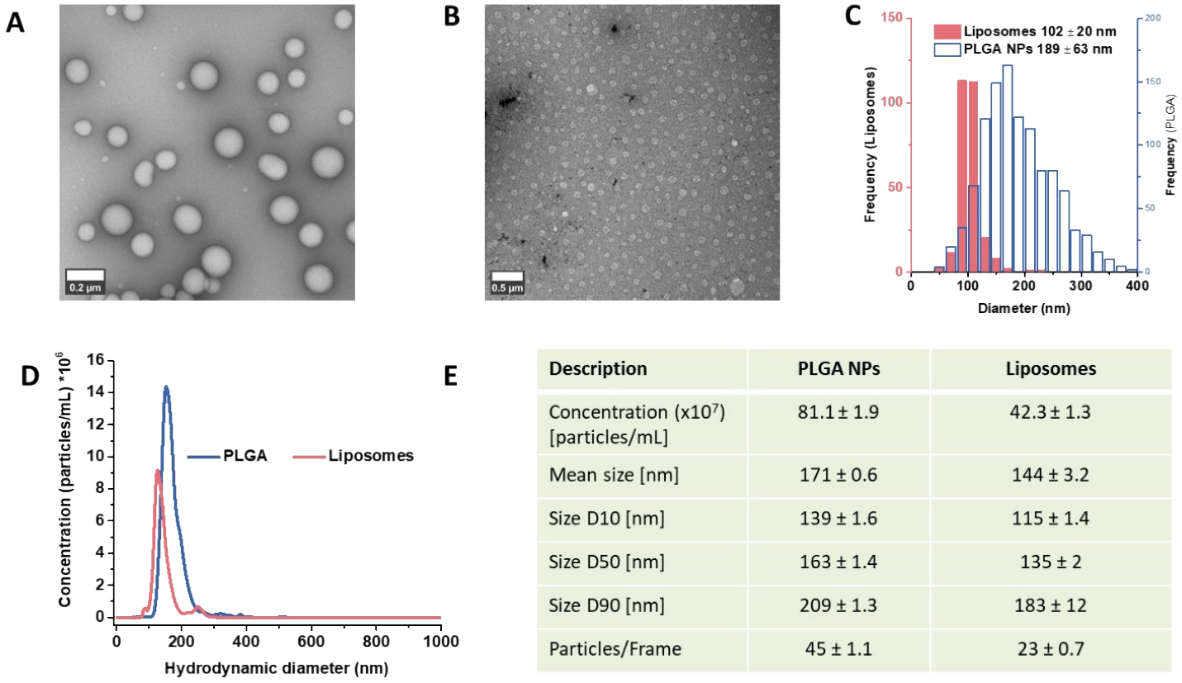

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Description	PLGA NPs	Liposomes
Concentration (x10^7) [particles/mL]	81.1 ± 1.9	42.3 ± 1.3
Mean size [nm]	171 ± 0.6	144 ± 3.2
Size D10 [nm]	139 ± 1.6	115 ± 1.4
Size D50 [nm]	163 ± 1.4	135 ± 2
Size D90 [nm]	209 ± 1.3	183 ± 12
Particles/Frame	45 ± 1.1	23 ± 0.7

Fig. S1. Complementary characterization of the prepared PLGA NPs and liposomes. Transmission electron microscope (TEM) micrographs of (**A**) PLGA NPs and (**B**) liposomes in water following UranyLess negative staining. (**C**) Size distribution of NPs obtained from TEM image processing, n=270 for liposomes and 1116 for PLGA NPs. (**D**) Size distribution of the particles as determined by nanoparticle tracking analysis (NTA). (**E**) Table summarizing the NTA results. All particles were resuspended in water for PLGA NPs and PBS for liposomes. The concentration values provided in the table correspond to PLGA nanoparticles diluted 7,500-fold, and liposomes diluted 12,500-fold. Both samples were prepared using Milli-Q water as the diluent.

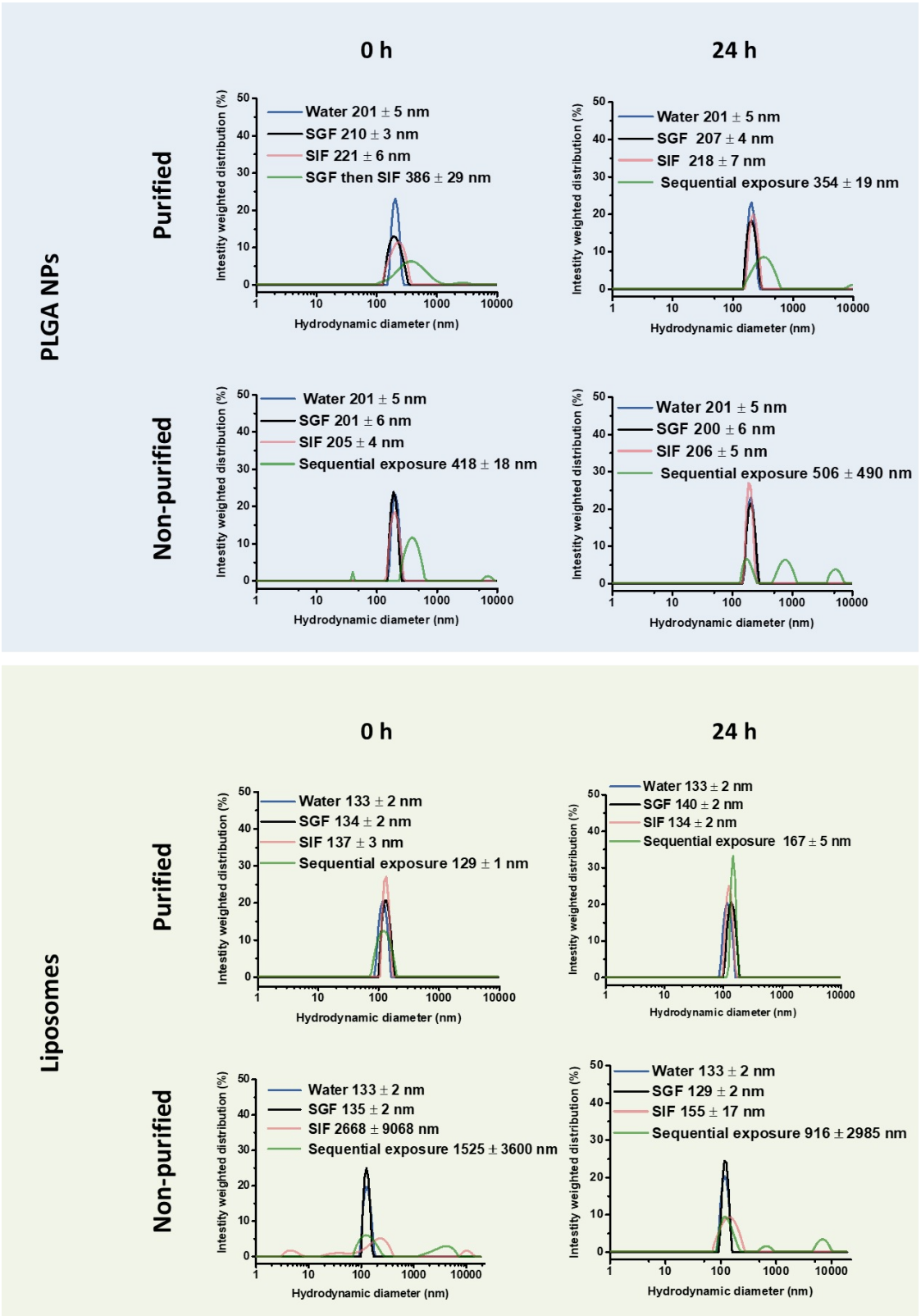

Fig. S2. DLS results for PLGA NPs and liposomes at 0 h and 24 h in water, simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and sequential exposure to SGF followed by SIF. NPs were diluted in milli-Q water for analysis

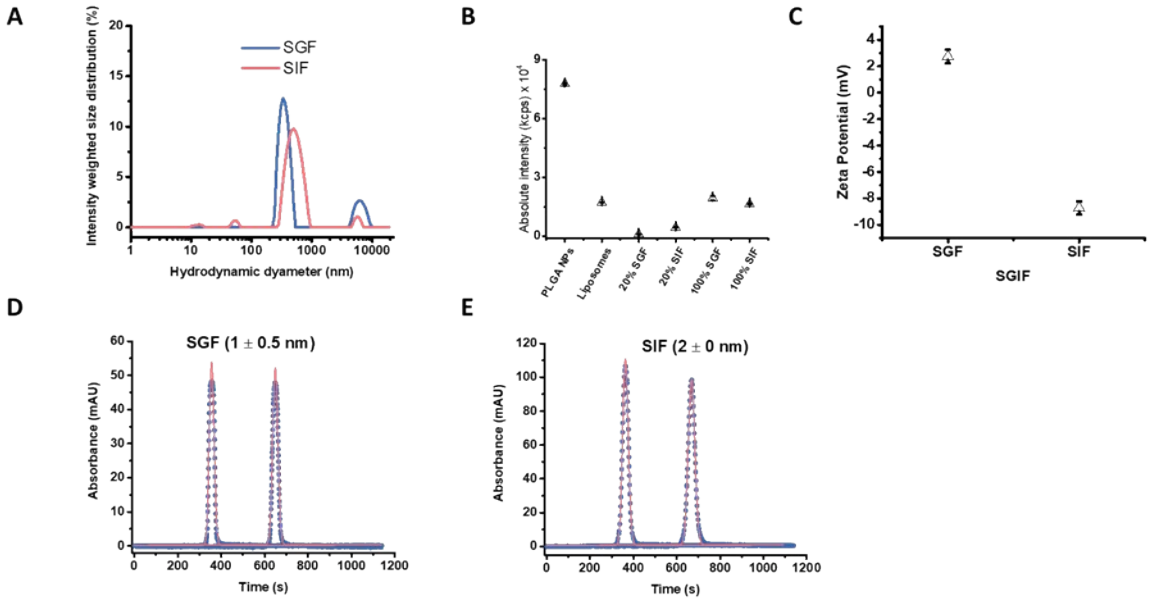

Fig. S3. (**A**) Dynamic light scattering of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) diluted in milli-Q water. (**B**) Absolute scattering intensities of NPs and simulated gastrointestinal fluids. (**C**) Zeta potential of diluted SGF and SIF at room temperature. (**D**) Taylorgrams of SGF and (**E**) SIF using water as eluent. The hydrodynamic diameter was determined after fitting the data using the Taylor-Aris model.[1-3] The results are expressed as average n=5, ± standard deviation. ●●●experimental data values ▬fitted data values.

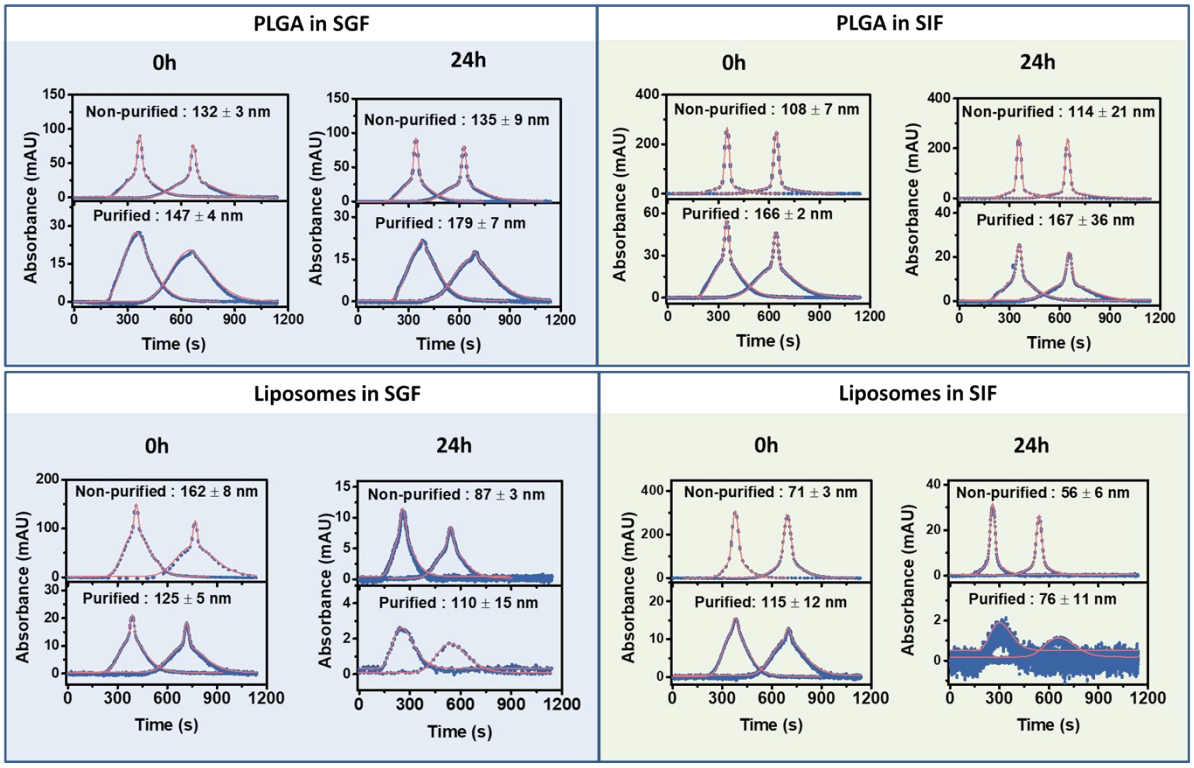

Fig. S4. Taylorgrams of purified and non-purified PLGA NPs and liposomes after incubation in pure SGF and pure SIF for 0 and 24 hours. Pure SGF and pure SIF were used as eluents for the non-purified NPs, while water was used for the purified NPs. The hydrodynamic diameter was determined after fitting the data using the Taylor-Aris model.[1-3] The results are expressed as average n=5, ± standard deviation. ●●●experimental data values ▬fitted data values.

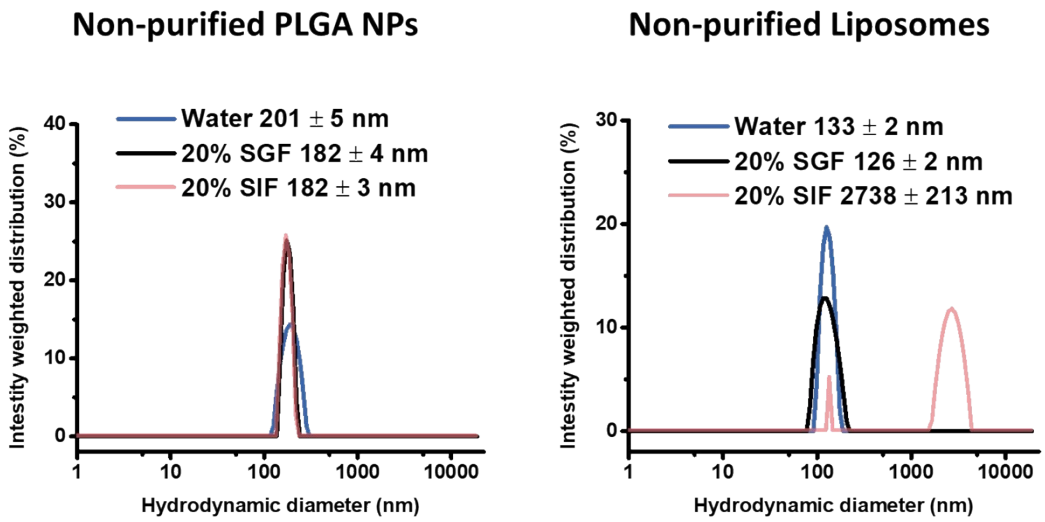

Fig. S5. DLS data was obtained on non-purified NPs and dispersed in 20 vol.% SGF and SIF at t = 0 h.

Taylor dispersion analysis

The Taylorgram of particles of hydrodynamic radius r_H can be expressed by two factors: $A(t) = A_0 a(t)$. A_0 is the amplitude of the absorbance profile, which is quantified by particle type, particle concentration and capillary radius Y. $a(t)$ is a function of time and hydrodynamic radius, and when particles are dispersed in a cylindrical channel[4-6]

$$a(t) = \frac{1}{\sqrt{\pi \cdot \kappa \cdot t}} e^{-\frac{(t-t_0)^2}{\kappa \cdot t}}$$

where $\kappa = r_H \pi \eta Y^2 / (2 k_B T)$, T temperature, η viscosity of the fluid, k_B Boltzmann constant, and $t_0 = \frac{x}{v}$ the so-called residence time defined by the distance between detection and injection points and the mean velocity of the flow (v).

Eq 1 represents the ideal experimental scenario where 1) the detector response is linear, 2) the relationship between particle concentration and optical absorbance is accounted for by the Lambert-Beer law, 3) inter-particle interactions are negligible, 4) the injected volume and the volume of detection are small compared to the capillary volume between the injection and the detection points, and 5) the length of the overall pressure ramp is short compared to the residence time.

In this experiment, two detection points were used, as it rendered the analysis more robust,[7, 8] and in the analysis, a bimodal form of Eq 1 was regressed to the experimental data obtained at the two detection windows: $a(t) = a_A(t) + a_B(t)$. The modes interpret the particles and the complex medium suspending the particles. From the mode corresponding to the particles, the hydrodynamic radius was estimated via:

$$r_H = \frac{4 k_B T V_2 - V_1}{\pi \eta Y^2 t_2 - t_1}$$

where t_1 and t_2 are the two residence times, and V_1 and V_2 are the temporal variance of the Taylogram corresponding to the particles.

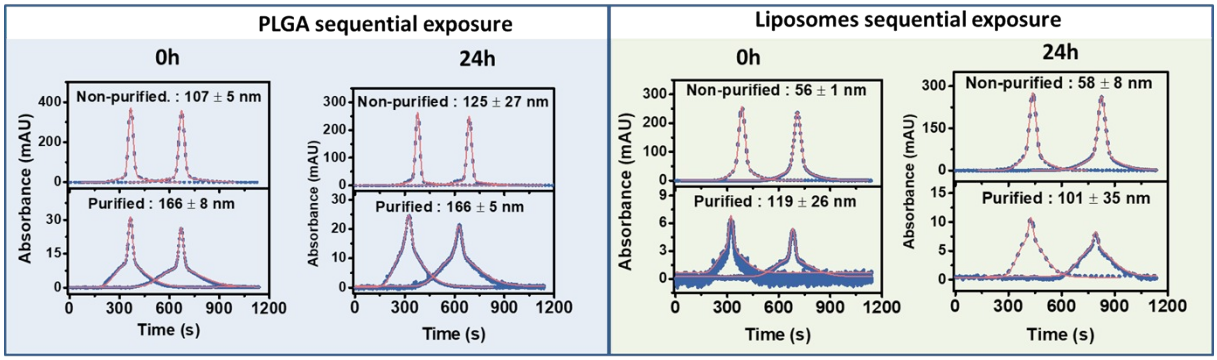

Fig. S6. Taylorgrams of purified and non-purified PLGA NPs and liposomes subjected to sequential exposure, first to 100% SGF for 2 h followed by 100% SIF for 24 hours. For the purified samples, after each incubation time-point, NPs were purified by centrifugation, resuspended in Milli-Q water, and characterized by TDA. Pure SGF and pure SIF were used as eluents for the non-purified NPs, while water was used for the purified NPs. The hydrodynamic diameter was determined after fitting the data using the Taylor-Aris model.[1-3] The results are expressed as average n=5, ± standard deviation. ●●●experimental data values ▬fitted data values.

Table S1. Absorbance value for antioxidant activity of βC in SGIF

Sample description	Absorbance (a.u)
βC in SGF with DPPH	0.3231 ± 0.0016
SGF alone	0.0044 ± 0.0000
SGF with DPPH	0.5864 ± 0.0048
βC in SIF with DPPH	0.3765 ± 0.0003
SIF alone	0.2150 ± 0.0006
SIF with DPPH	0.9706 ± 0.0046
Control (βC with DPPH)	1.0126 ± 0.0007

Table S2. Storage stability of PLGA NPs and liposomes.

	PLGA NPs						Liposomes					
Day	**Size [nm]**		**PdI**		**Zeta potential [mV]**		**Size [nm]**		**PdI**		**Zeta potential [mV]**	
	Empty	Loaded	Empty	Loaded	Empty	Loaded	Empty	Loaded	Empty	Loaded	Empty	Loaded
1	206 ± 1	210 ± 3	0.07 ± 0.02	0.15 ± 0.04	-35 ± 1	-39 ± 1	117 ± 2	122 ± 2	0.08 ± 0.01	0.11 ± 0.01	-42 ± 1	-40 ± 1
5	195 ± 1	198 ± 6	0.05 ± 0.01	0.16 ± 0.04	-45 ± 1	-44 ± 1	116 ± 2	114 ± 1	0.10 ± 0.01	0.08 ± 0.01	-29 ± 3	-30 ± 1
10	190 ± 2	236 ± 7	0.05 ± 0.01	0.16 ± 0.04	-40 ± 0	-36 ± 1	117 ± 2	117 ± 1	0.10 ± 0.01	0.01 ± 0.01	-35 ± 1	-37 ± 1
20	165 ± 4	167 ± 7	0.11 ± 0.05	0.14 ± 005	-43 ± 1	-30 ± 1	116 ± 2	120 ± 2	0.09 ± 0.01	0.12 ± 0.01	-38 ± 2	-34 ± 1
30	177 ± 4	150 ± 3	0.06 ± 0.003	0.12 ± 0.05	-30 ± 1	-35 ± 1	116 ± 3	112 ± 1	0.09 ± 0.01	0.09 ± 0.01	-38 ± 1	-30 ± 1

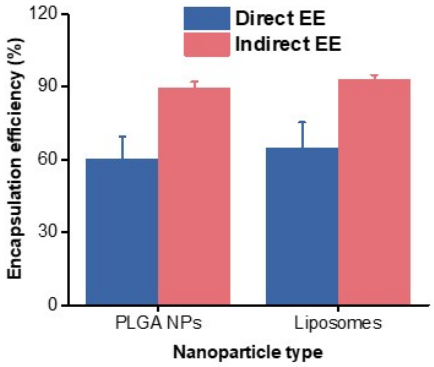

Fig. S7. Direct and indirect encapsulation efficiency (EE) of PLGA NPs and liposomes. Results shows average of n=9, error bar presents the standard deviation.

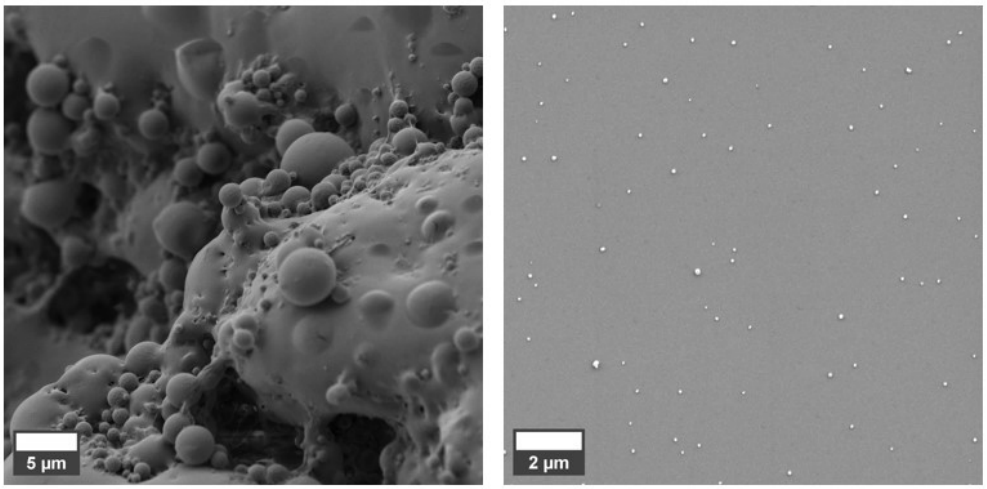

Fig. S8. Scanning electron microscope of PLGA NPs after incubation in the simulated gastrointestinal medium for 168 h.

Quantification of free fatty acids

Free fatty acid was calculated indirectly following the method by Tan et. al [9]. During the intestinal digestion process, at a pre-determined time point, the free fatty acids (FFAs) released from lipid digestion were neutralised by adding 0.1 M NaOH manually to maintain the pH of the solution at 7.5. The volume of NaOH added over time was recorded throughout the digestion. It was assumed that the liposomal phospholipids were hydrolysed and liberated free fatty acids and lyso-phospholipids. Therefore, the percentage of FFAs released (%) during the digestion process was calculated using the following equation:

$$FFA_{(t)} = \frac{V_{NaOH\,(t)} * C_{NaOH} * M_{w.\ lipid}}{m_{lipid}} * 100 \quad (1)$$

where V_{NaOH} (t) is the volume of NaOH solution required to neutralise the FFAs released at given time t, C_{NaOH} is the molarity of the NaOH solution used to titrate the sample (mol/L), $M_{w.lipid}$ is the molecular weight of the lipid (g/mol), and m_{lipid} is the total mass of lipid present in the sample (g).

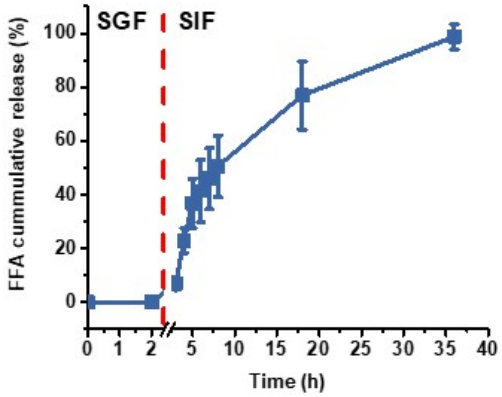

Fig. S9. Free fat acid (FFA, %) from liposomes during 36 h incubation in SGIF. SGF means stimulated gastric fluid, and SIF means stimulated intestinal fluid.

References

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