Supporting Information

Impact of Surface Polyethylene Glycol (PEG) Density on Biodegradable Nanoparticle Transport in Mucus *ex vivo* and Distribution *in vivo*

Qingguo Xu,[▲,#] Laura M. Ensign,[▲,#,†] Nicholas J. Boylan,[#,†] Arne Schön,[▼] Xiaoqun Gong,[▲,#,►,+] Jeh-Chang Yang,[†] Nicholas W. Lamb,[▲,#] Shutian Cai,[†] Tao Yu,[‡,#] Ernesto Freire,[▼] Justin Hanes[▲,#,†,‡,◄,*]

[▲]Department of Ophthalmology, The Wilmer Eye Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA

[#]Center for Nanomedicine, The Johns Hopkins University School of Medicine, MD 21231, USA

[†]Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, Baltimore, MD 21218, USA

[►]Institute of Nanobiotechnology, School of Materials Science and Engineering, Tianjin University, Tianjin 300072, PR China

+ School of Life Science, Tianjin University, Tianjin 300072, PR China

[▼]Department of Biology, The Johns Hopkins University, Baltimore, MD 21218, USA

‡ Department of Biomedical Engineering, The Johns Hopkins University, Baltimore, MD 21205, USA

[◄] Departments of Environmental Health Sciences, Oncology, Neurosurgery, and Pharmacology & Molecular Sciences, The Johns Hopkins University, Baltimore, MD 21231, USA

Table S1. Characteristics of PEG-containing block copolymers

Block polymer	PEG (kDa)	LA:GA [a]	PEG content [b] (%)	Mn[c] (kDa)	Mn[d] (kDa)	Mw[d] (kDa)	PDI[d]
PLGA-PEG$_{5k}$, 25%	5	78:22	25.2	19.9	15.1	21.9	1.75
PLGA-PEG$_{5k}$, 20%	5	49:51	21.4	23.4	25.4	38.7	1.52
PLGA-PEG$_{5k}$, 10%	5	55:45	9.8	50.8	40.5	71.0	1.45

[a] The molar ratio of LA:GA was measured by comparing the ^{1}H NMR integral intensity at 5.22 ppm (-CH- on lactide), 1.59 ppm (-CH_3 on lactide) and 4.83 ppm (-CH_2- on glycolide), as shown in Figure. S3.
[b] PEG content in the block copolymers were determined by ^{1}H NMR.
[c] PLGA-PEG molecular weight (Mn) was determined by ^{1}H NMR through comparing the integral at 5.22 ppm (-CH- in lactide), 1.59 ppm (-CH_3 on lactide), 4.83 ppm (-CH_2- in glycolide) and 3.65 ppm (-CH_2CH_2- in PEG) and by taking into account of the known Mn of PEG. For PCL-PEG, integrals at 4.06 ppm (-O-CH_2-) and 2.31 ppm (-CH_2-CO-) were analyzed.
[d] Mn, Mw and polydispersity (PDI) were measured by GPC.

Table S2. Characteristics of PLGA-PEG$_{5k}$ nanoparticles prepared by nanoprecipitation method

Target PEG content (wt%)	ζ-potential (mV)	D (nm)	Total PEG content (wt%)	Surface PEG content (wt%)	[Γ] (chains/ 100 nm^2)	[Γ]/[Γ*]
10	-3.4±0.4	117±1.4	6.5±0.1	5.8±0.2	15.8±0.6	3.6±0.1

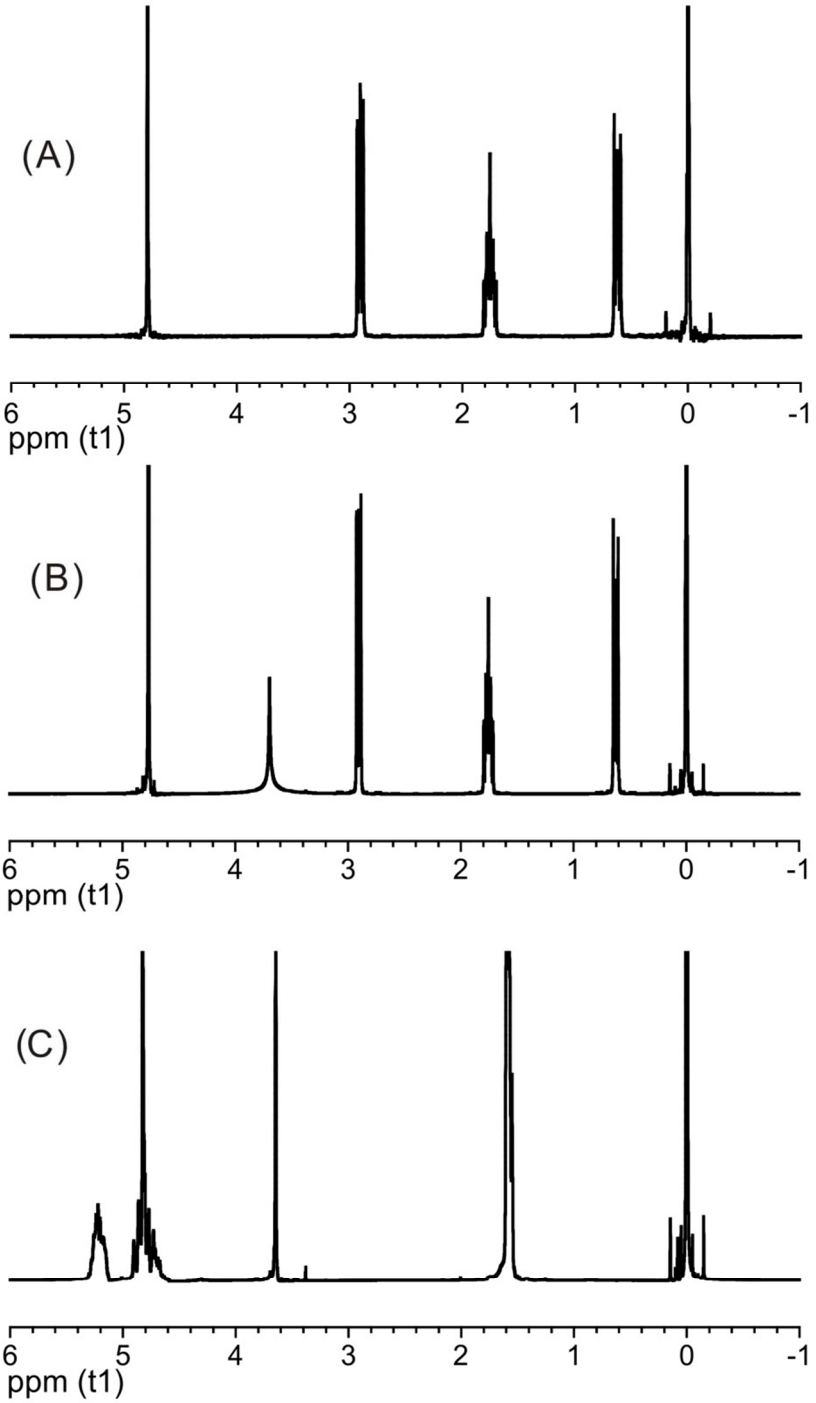

Figure S1. Representative ^{1}H NMR spectra of (A) PLGA (0% PEG) nanoparticles and (B) PLGA-$PEG_{10\%}$ nanoparticles suspended in D_2O with 1 wt% DSS as internal standard. Peaks at 2.91, 1.76, 0.65 and 0 ppm are from DSS. PEG on the nanoparticle surface shows broadened peak around 3.65 ppm, however, no peaks were observed from PLGA nanoparticles at 3.65 ppm. (C) ^{1}H NMR spectra of lyophilized PLGA-$PEG_{10\%}$ nanoparticles dissolved in $CDCl_3$ with TMS as internal standard and PEG shows sharp peak around 3.65 ppm.

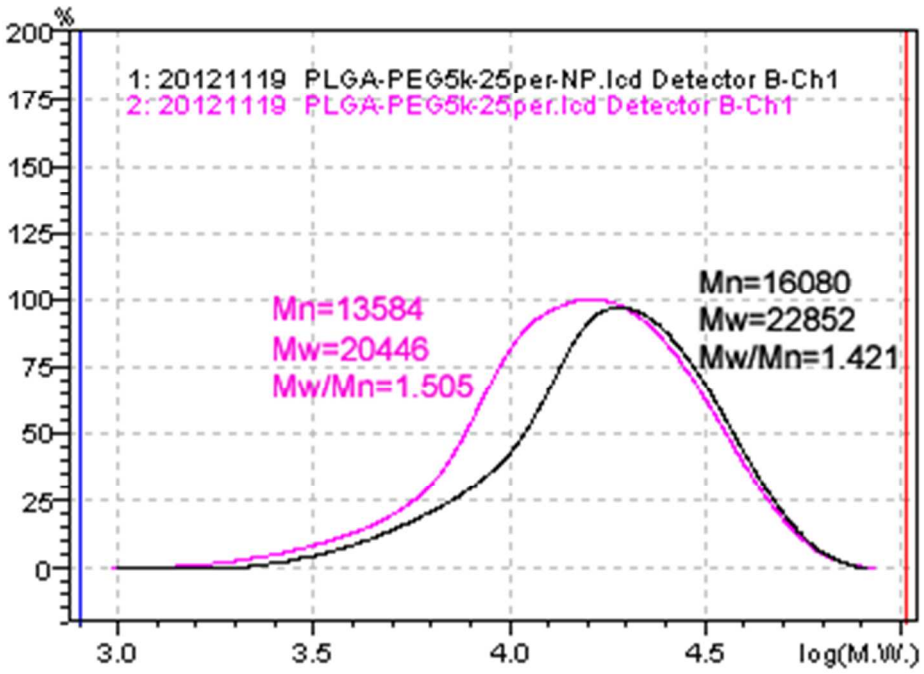

Figure S2. GPC results of PLGA-PEG_{5k} (25 wt% PEG) raw materials (pink color) and the nanoparticles prepared from PLGA-PEG_{5k} (25 wt% PEG) by the emulsification method (black color)

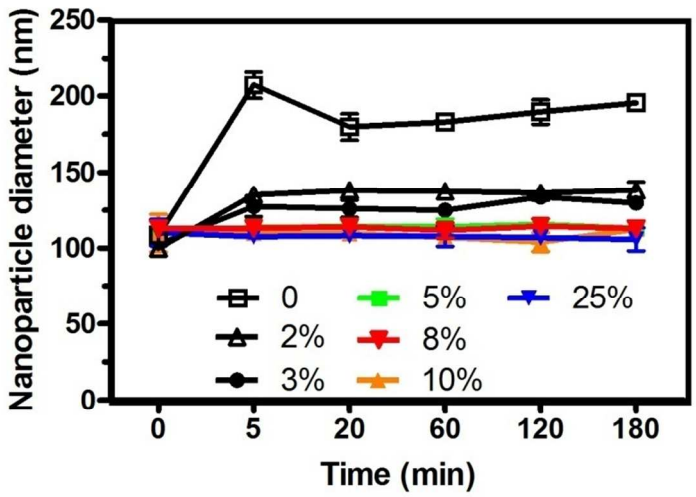

Figure S3. Nanoparticle stability over time *in vitro* in mucin solution. PLGA nanoparticles with different PEG content (0, 2%, 3%, 5%, 8%, 10% and 25% PEG) were incubated with 10 mg/ml mucin solution at 37^{o}C under gentle stirring. At each time point, an aliquot of the nanoparticle suspension was removed to measure the hydrodynamic diameter using dynamic light scattering.

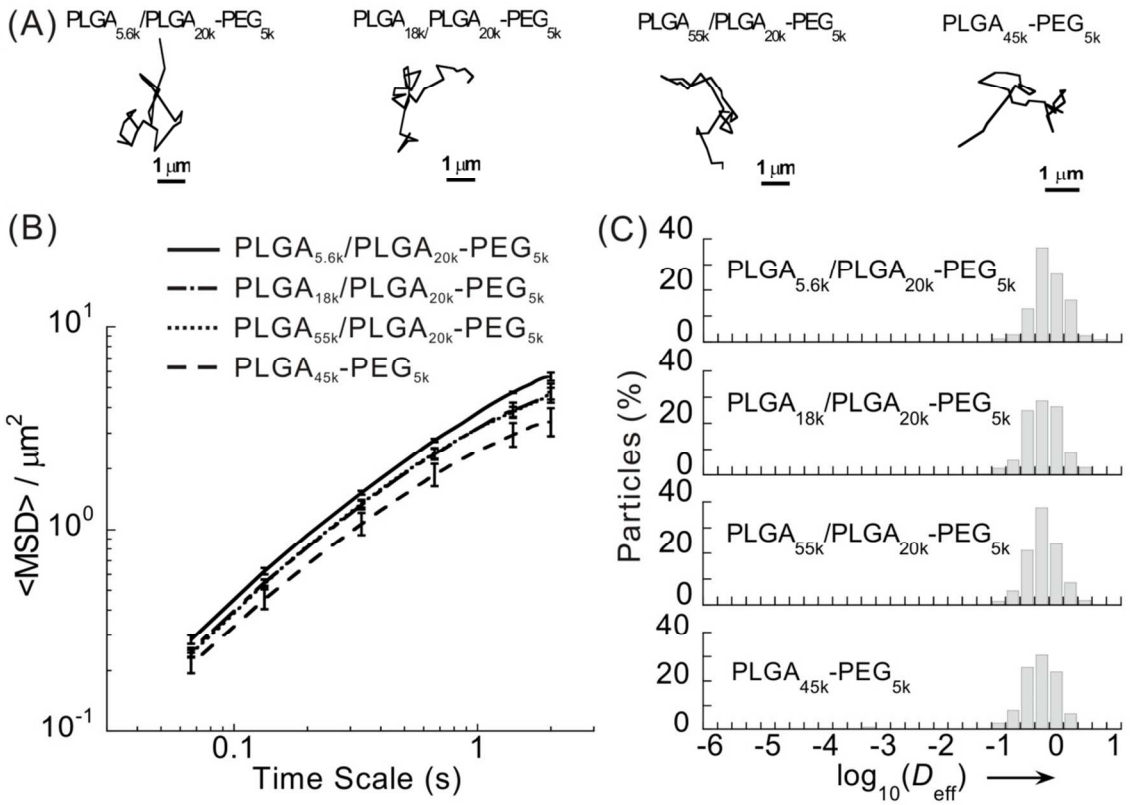

Figure S4. Transport of PTX-loaded PLGA-PEG and PLGA/PLGA-PEG blended nanoparticles in fresh, undiluted CVM. A) Representative trajectories for 3 s of particle motion. B) Ensemble-averaged geometric mean square displacement (<MSD>) as a function of time scale. C) Distributions of the logarithms of individual particle effective diffusivities (D_{eff}) at a time scale of 1 s. Data represent three independent experiments with ≥ 120 nanoparticles tracked for each experiment (mean ± SEM).

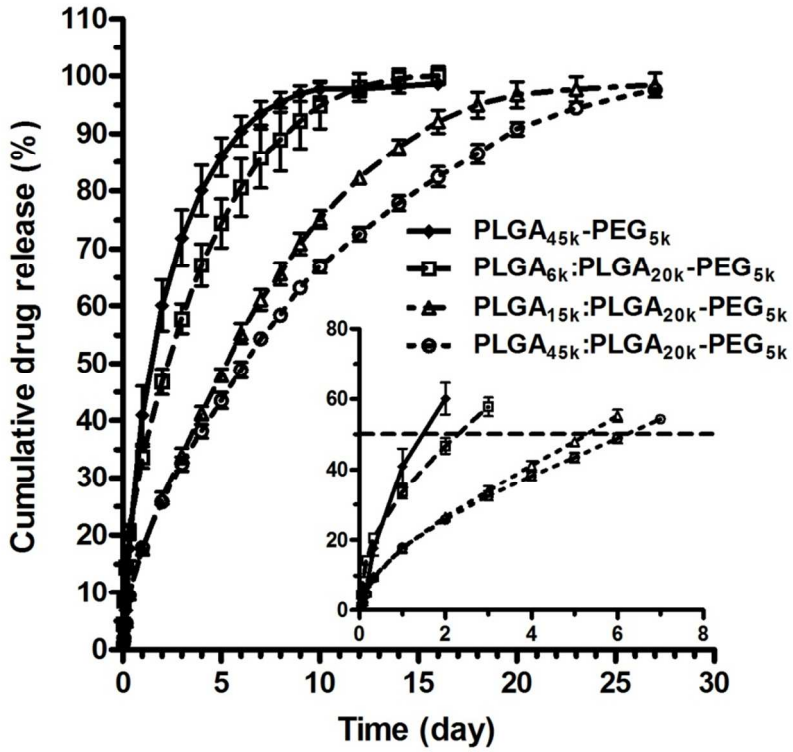

Figure S5. *In vitro* release of PTX from PLGA-PEG and PLGA/PLGA-PEG nanoparticles. The insert highlights the release profiles with extrapolated $T_{1/2}$, the time to release 50% of encapsulated drug.