Supporting Information

Poly(ε-caprolactone) nanocapsule carriers with sustained drug release: Single dose for long-term glaucoma treatment

Chih-Hung Lee,[‡a] Yu-Jia Li,[‡a] Chih-Ching Huang,[*b,c,d] and Jui-Yang Lai[*a,e,f]

[a]Institute of Biochemical and Biomedical Engineering, Chang Gung University, Taoyuan 33302, Taiwan

[b]Department of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung 20224, Taiwan

[c]Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 20224, Taiwan

[d]School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

[e]Department of Ophthalmology, Chang Gung Memorial Hospital, Taoyuan 33305, Taiwan

[f]Department of Materials Engineering, Ming Chi University of Technology, New Taipei City 24301, Taiwan

[‡]These authors contributed equally.

Correspondence: Professor Chih-Ching Huang, Department of Bioscience and Biotechnology, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 20224, Taiwan; Tel.: 011-886-2-2462-2192 ext. 5517; Fax: 011-886-2-2462-2320; E-mail: huanging@ntou.edu.tw; Professor Jui-Yang Lai, Institute of Biochemical and Biomedical Engineering, Chang Gung University, 259 Wen-Hwa 1st Road, Taoyuan 33302, Taiwan; Tel.: 011-886-3-211-8800 ext. 3598; Fax: 011-886-3-211-8668; E-mail: jylai@mail.cgu.edu.tw

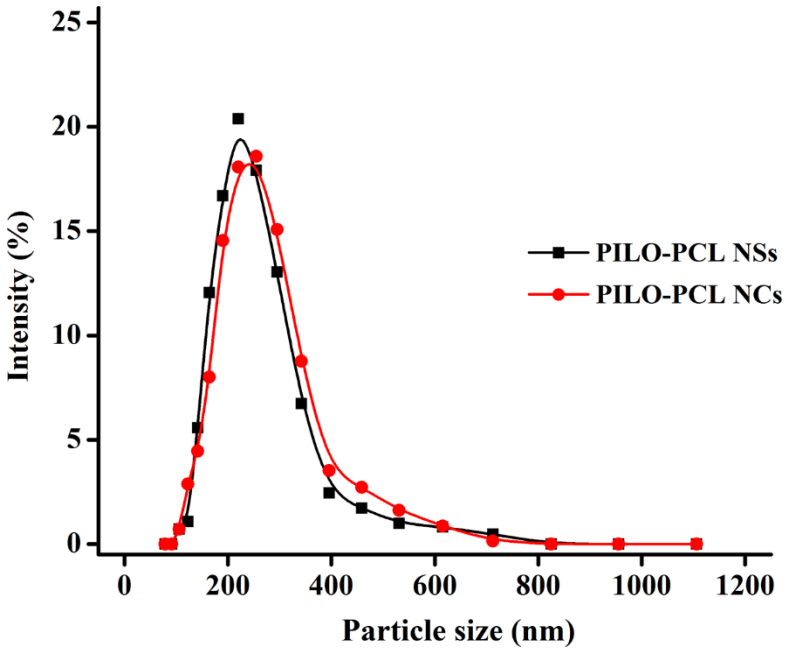

Figure S1. DLS spectra of PILO-PCL NS and PILO-PCL NC dispersions in PBS.

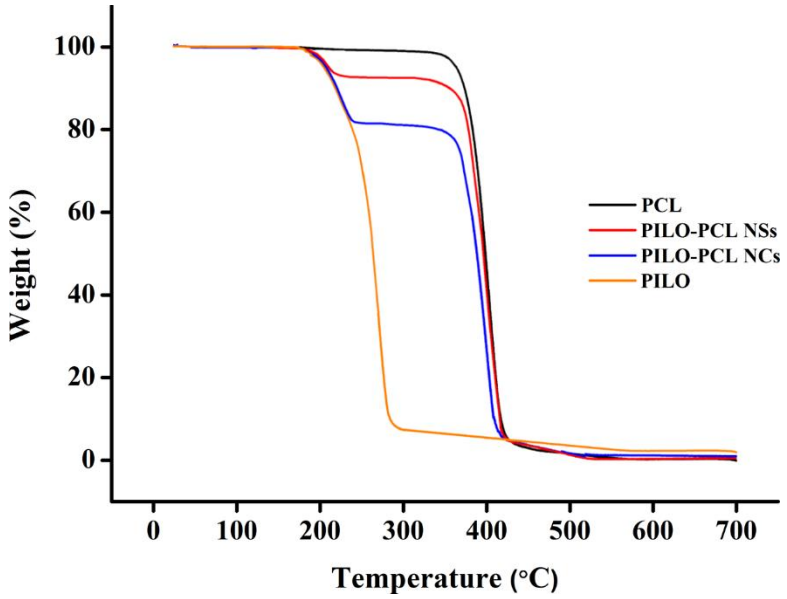

Figure S2. Thermogravimetric analysis (TGA) of PCL, pilocarpine and different forms of pilocarpine-loaded PCL NPs.

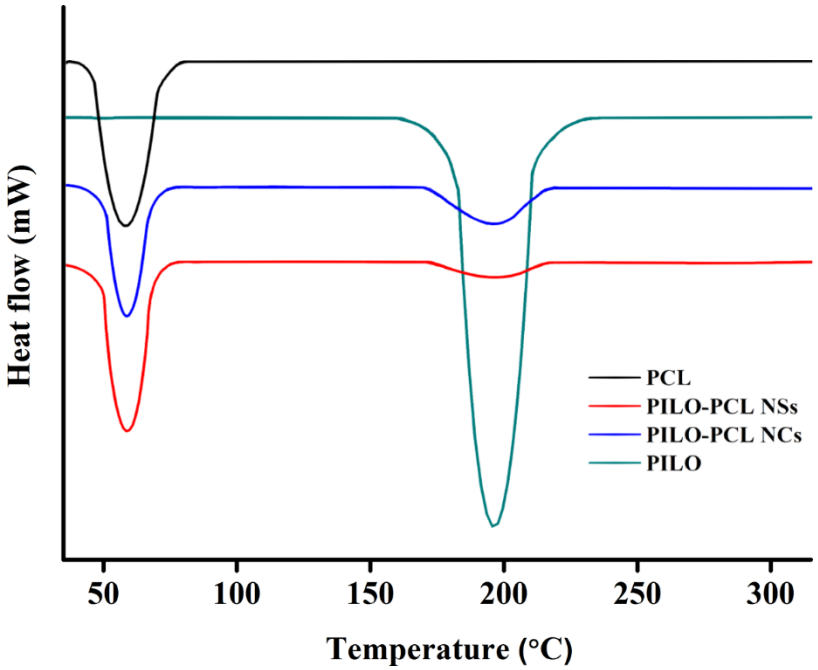

Figure S3. Differential scanning calorimetry (DSC) thermograms of PCL, pilocarpine and different forms of pilocarpine-loaded PCL NPs.

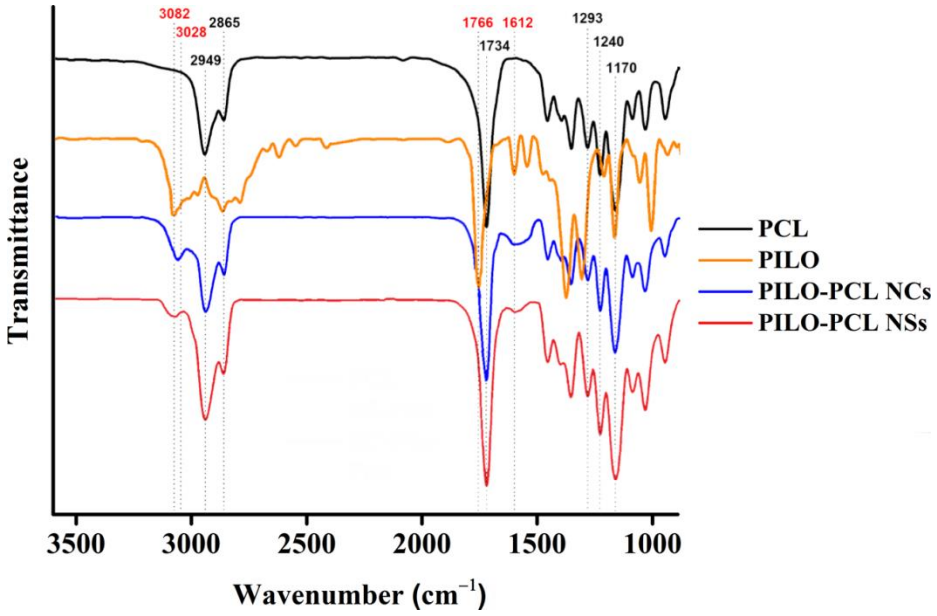

Figure S4. FT-IR spectra of PCL, pilocarpine and different forms of pilocarpine-loaded PCL NPs.

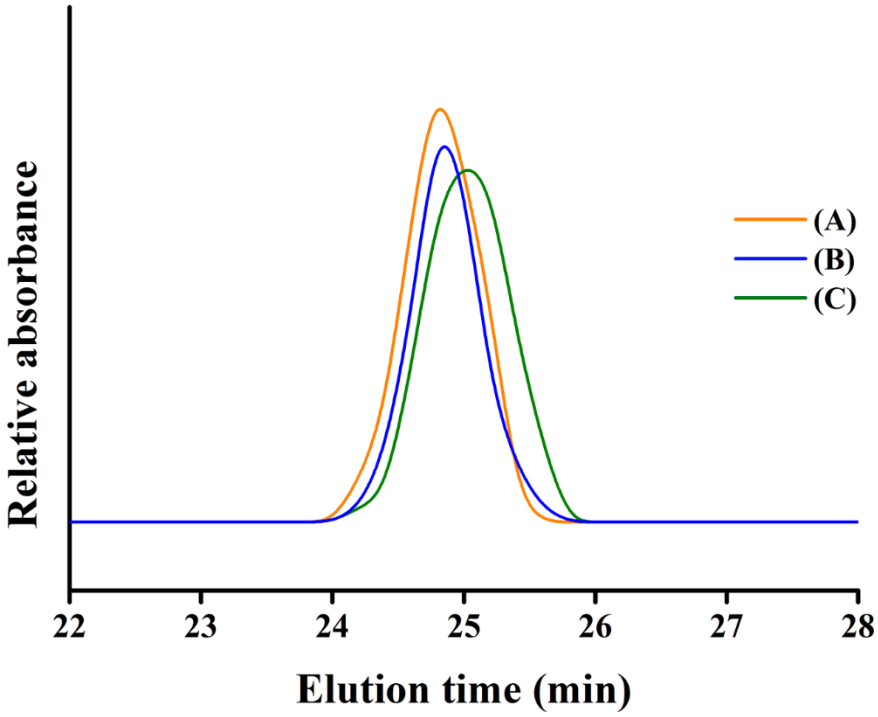

Figure S5. GPC traces of PILO-PCL NCs at different time points in BSS buffer: (A) 0, (B) 42, and (C) 70 days.

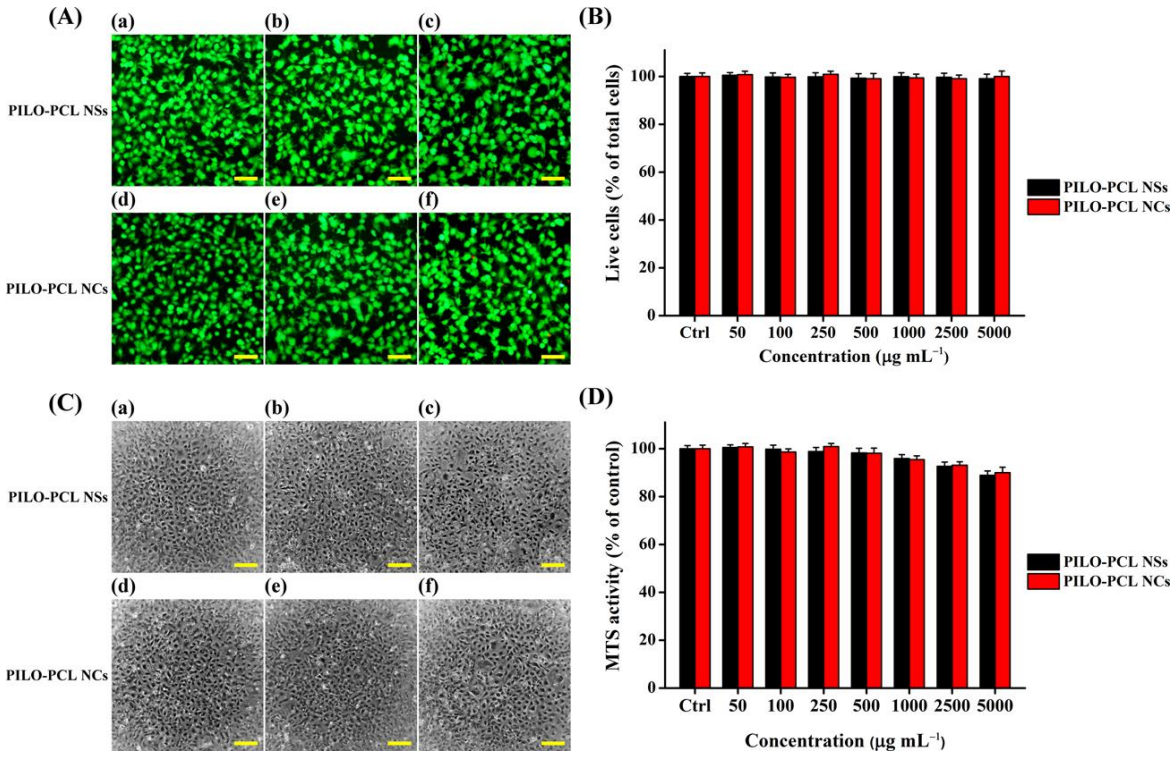

Figure S6. (A) Live/dead cell staining of calcein-AM/ethidium homodimer-1 for BCE cells treated with PILO-PCL NSs (a–c) or PILO-PCL NCs (d–f) at concentrations of (a, d) 0, (b, e) 500, and (c, f) 5000 μg mL^{-1} in culture medium at 37 ˚C for 2 days. (B) Live cell ratio of BCE cells after treatment with various concentrations of PILO-PCL NSs or PILO-PCL NCs for 2 days. (C) Phase-contrast microscopic images of BCE cells treated with PILO-PCL NSs (a–c) or PILO-PCL NCs (d–f) at concentrations of (a, d) 0, (b, e) 500, and (c, f) 5000 μg mL^{-1} in culture medium at 37 ˚C for 2 days. (D) MTS cell proliferation assay of BCE cells after treatment with various concentrations of PILO-PCL NSs or PILO-PCL NCs for 2 days. The cell medium-treated groups (a, d) served as control (Ctrl). Scale bars: 100 μm. The error bars in (B) and (D) represent the standard deviation of five repeated measurements.

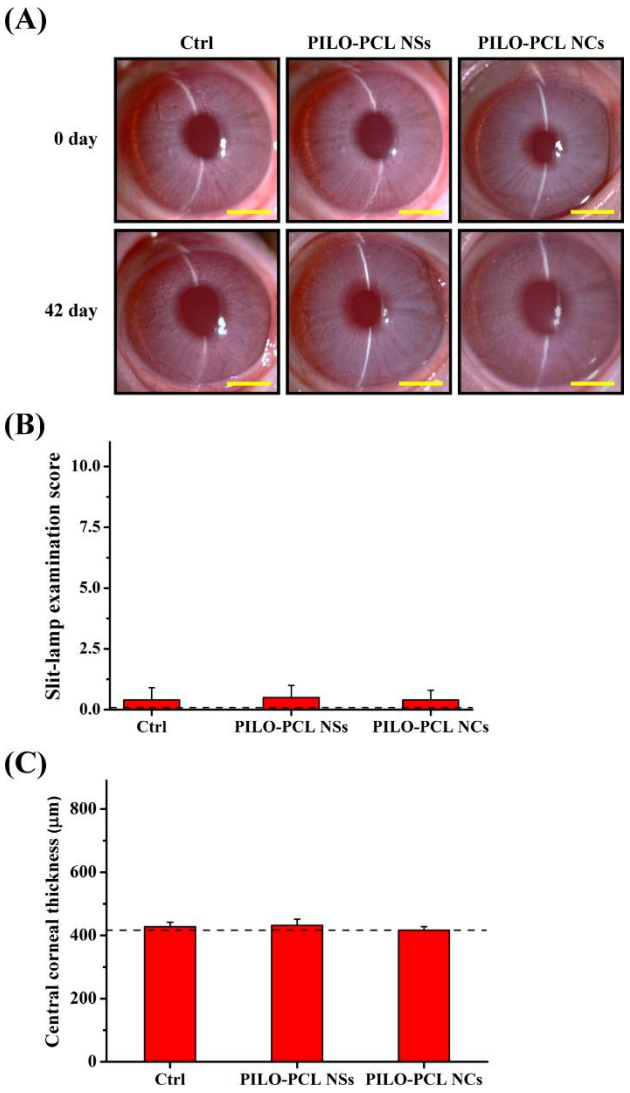

Figure S7. (A) Representative slit-lamp biomicroscope images of rabbit eyes 0 and 42 days after injection of 20 μL BSS buffer, PILO-PCL NSs or PILO-PCL NCs in the ocular anterior chamber. The concentration of drug-containing NPs was 500 μg mL^{-1} in the anterior chamber of the eyes. The rabbits receiving BSS without PCL NPs serve as the Ctrl group. Scale bars: 4 mm. (B) Slit-lamp examination scores of rabbit eyes 42 days after injection of BSS buffer, PILO-PCL NSs or PILO-PCL NCs in the ocular anterior chamber. The dashed line represents the preoperative score. (C) Central corneal thickness of eyes from various groups at day 42. The dashed lines denote the baseline values in the preoperative eyes. Error bars in (B) and (C) represent the standard deviation of six repeated measurements.

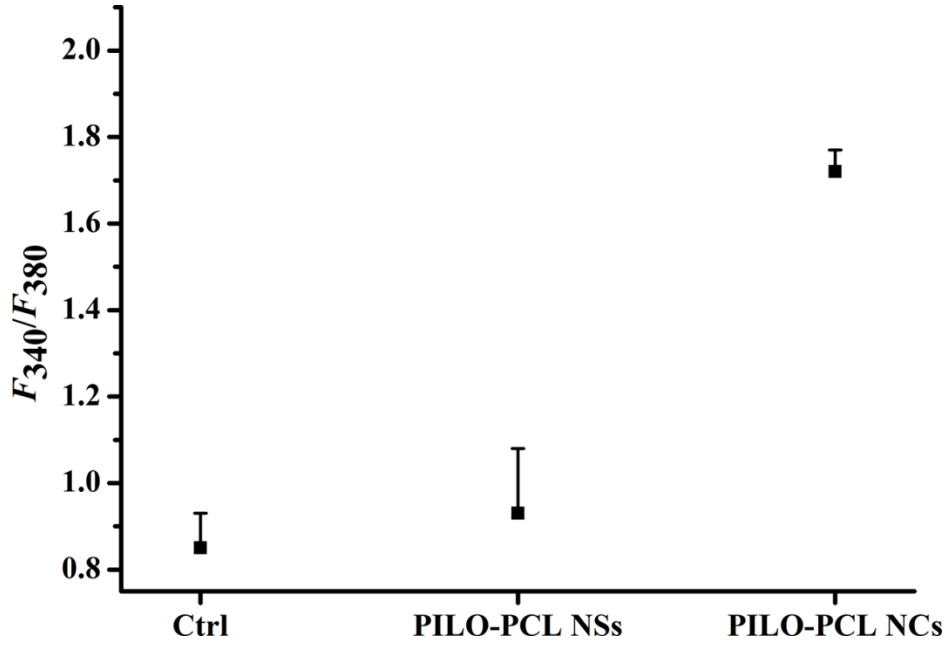

Figure S8. Intracellular calcium levels of rabbit ciliary smooth muscle cells after a 7 days of treatment with PILO-PCL NSs or PILO-PCL NCs. The intracellular calcium level of cells stained by Fura-2 AM. Fura-2 AM itself does not respond to calcium. However, once inside the cells, it is readily hydrolyzed to Fura-2 by nonspecific esterases. The Fura-2 indicator is typically excited at 340 nm and 380 nm, and the ratio of the fluorescent intensities at 510 nm, corresponding to the two excitations, is used to calculate the intracellular calcium level. The level of free intracellular calcium is proportional to the ratio of fluorescence (F_{340}/F_{380}) at excitations of 340 nm to 380 nm.

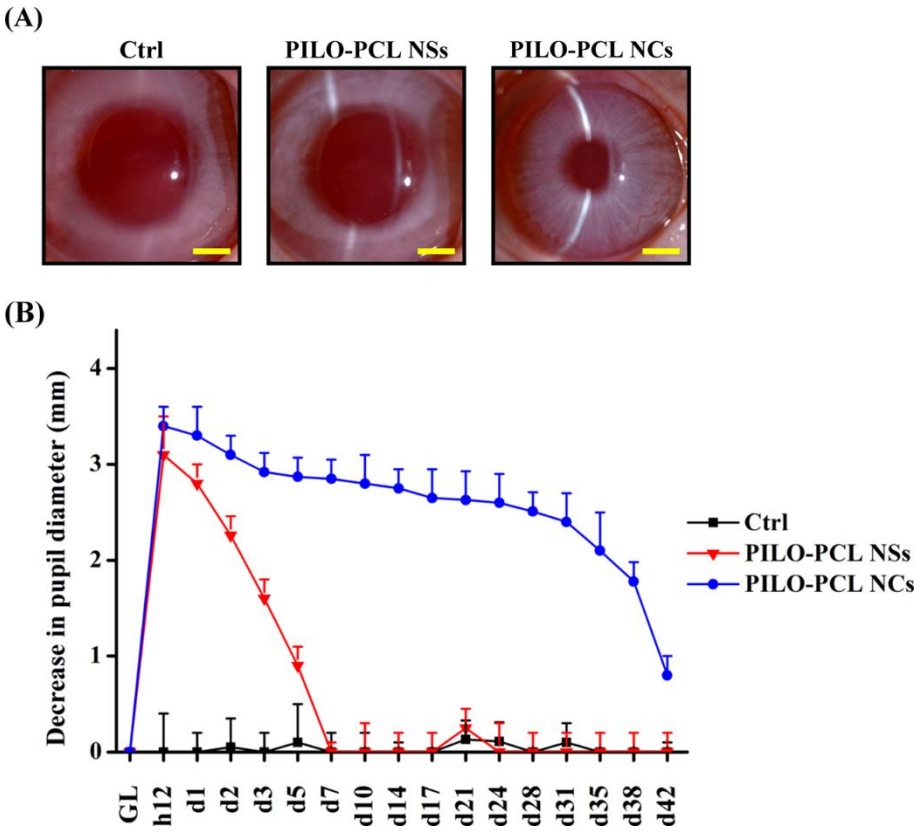

Figure S9. (A) Slit-lamp biomicroscope images (front view) of glaucomatous rabbit eyes injected with BSS, PILO-PCL NSs or PILO-PCL NCs after 42 days and (B) time course decrease in pupil diameters of glaucomatous rabbit eyes after different treatments. The scale bar is 4 mm. Glaucoma (GL)-induced rabbit eyes receiving BSS buffer served as the control group (Ctrl). Follow-up time point: day (d). The error bars in (B) represent the standard deviation of six repeated measurements.

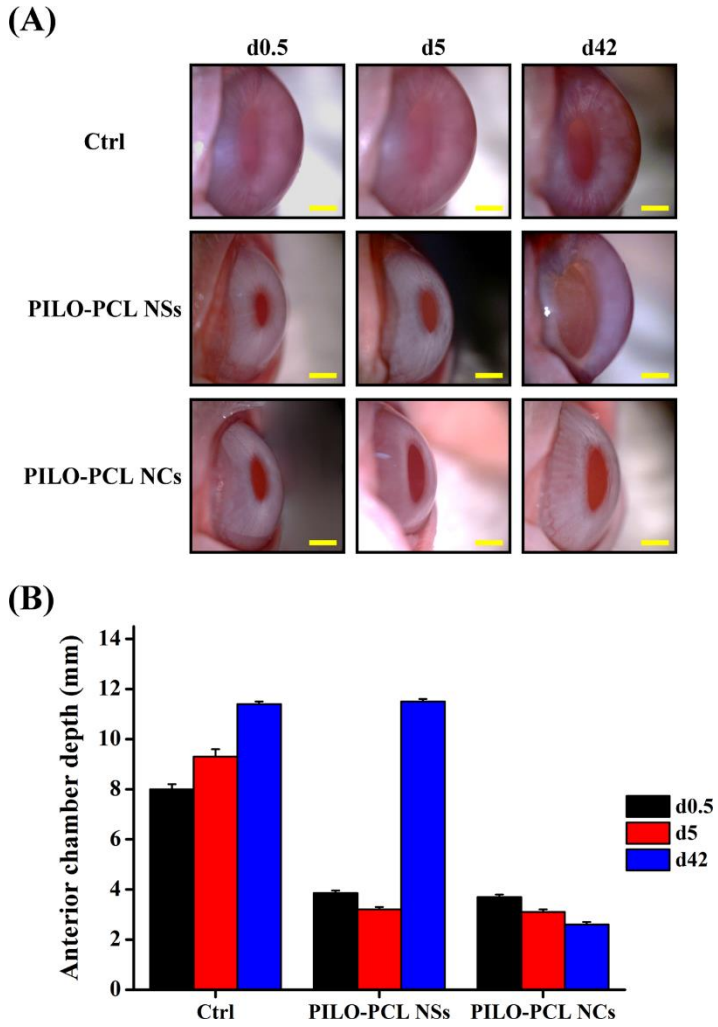

Figure S10. Time-course slit-lamp biomicroscope images (side view) and (B) anterior chamber depth of glaucomatous rabbit eyes injected with BSS, PILO-PCL NSs or PILO-PCL NCs at day 0.5 (d0.5), day 5 (d5) and day 42 (d42). The BSS buffer-treated group served as the control (Ctrl). The scale bar is 4 mm. The error bars in (B) represent the standard deviation of six repeated measurements.

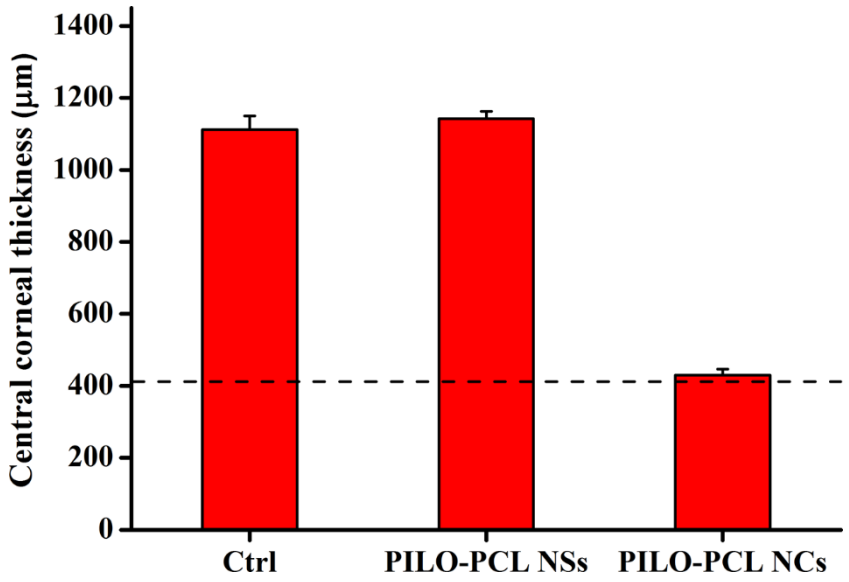

Figure S11. Central corneal thickness of glaucomatous rabbit eyes injected with BSS, PILO-PCL NSs or PILO-PCL NCs after 42 days. The BSS buffer-treated group served as control (Ctrl). The dashed line denotes the baseline values in preoperative eye. The error bars represent the standard deviation of six repeated measurements.

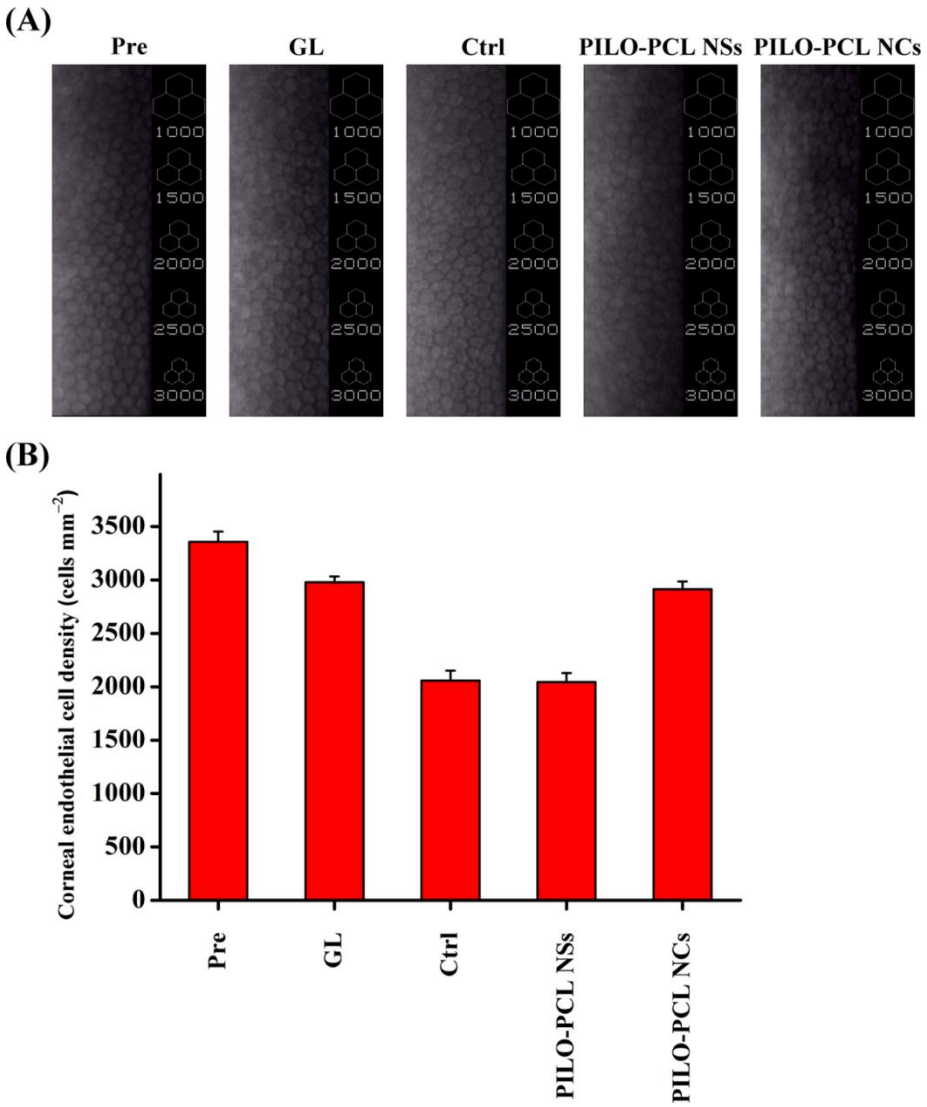

Figure S12. (A) Representative specular microscopic images of corneal endothelium cells and (B) corneal endothelial cell densities of normal rabbit [preoperative (Pre) group] and glaucomatous eye (GL group) rabbits 42 days after anterior chamber injection of BSS (Ctrl group), PILO-PCL NSs or PILO-PCL NCs. The error bars in (B) represent the standard deviation of six repeated measurements.

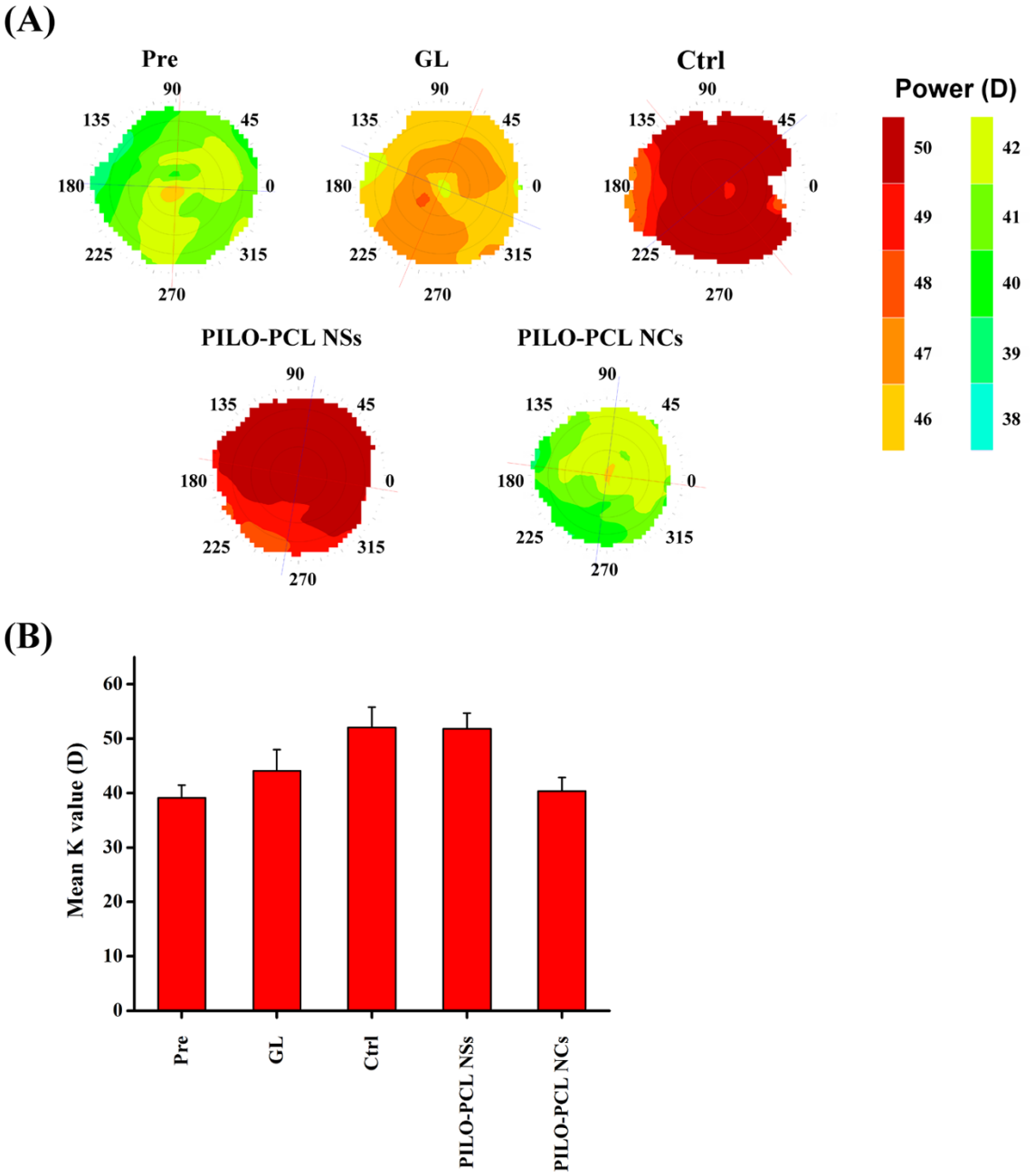

Figure S13. (A) Representative topographic corneal profile and (B) mean K values of normal rabbit [preoperative (Pre) group] and glaucomatous eye (GL group) rabbits 42 days after anterior chamber injection of BSS (Ctrl group), PILO-PCL NSs or PILO-PCL NCs. The BSS buffer-treated group served as the control (Ctrl). The error bars in (B) represent the standard deviation of six repeated measurements.

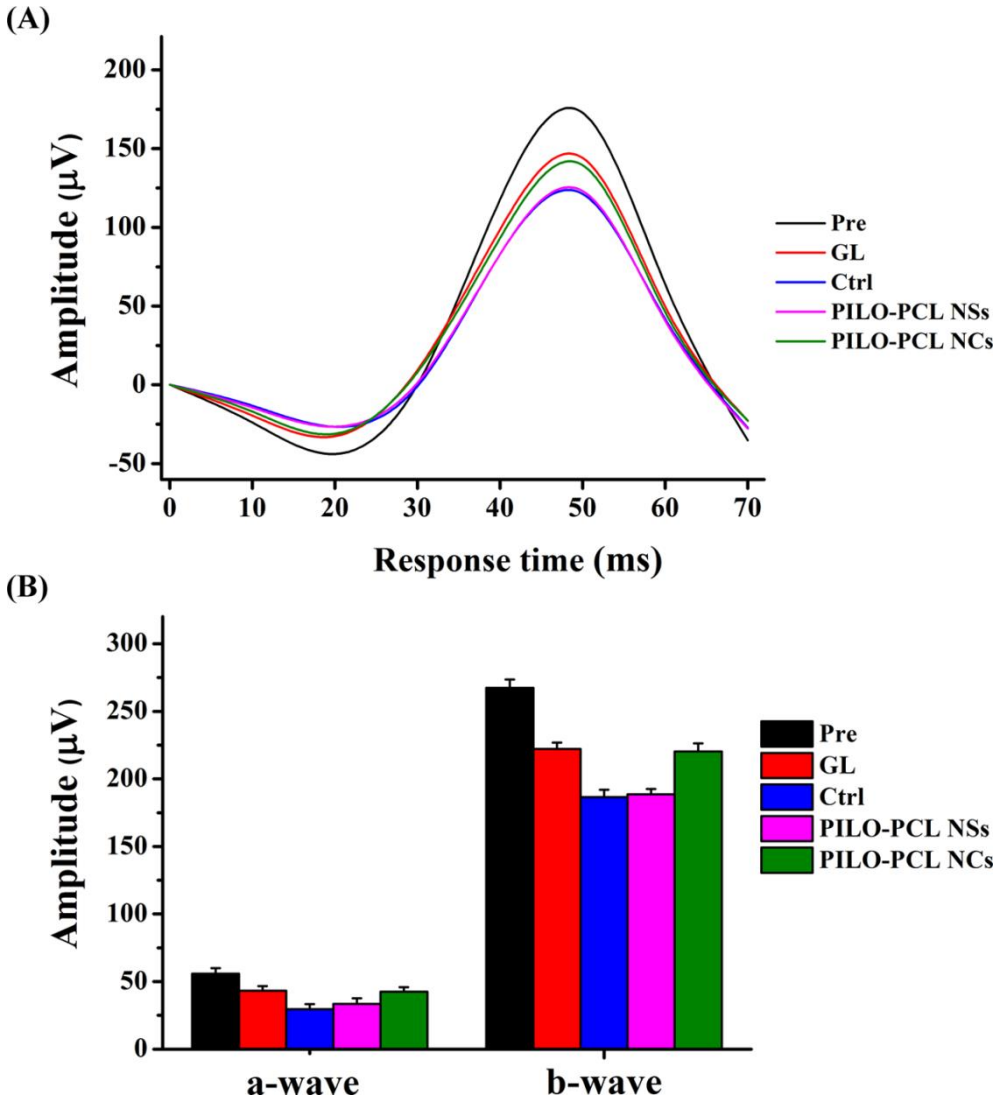

Figure S14. (A) ERG curves and (B) amplitude changes of the a-wave and b-wave of normal rabbit [preoperative (Pre) group] and glaucomatous eye (GL group) rabbits 42 days after anterior chamber injection of BSS (Ctrl group), PILO-PCL NSs and PILO-PCL NCs. The error bars in (B) represent the standard deviation of six repeated measurements.

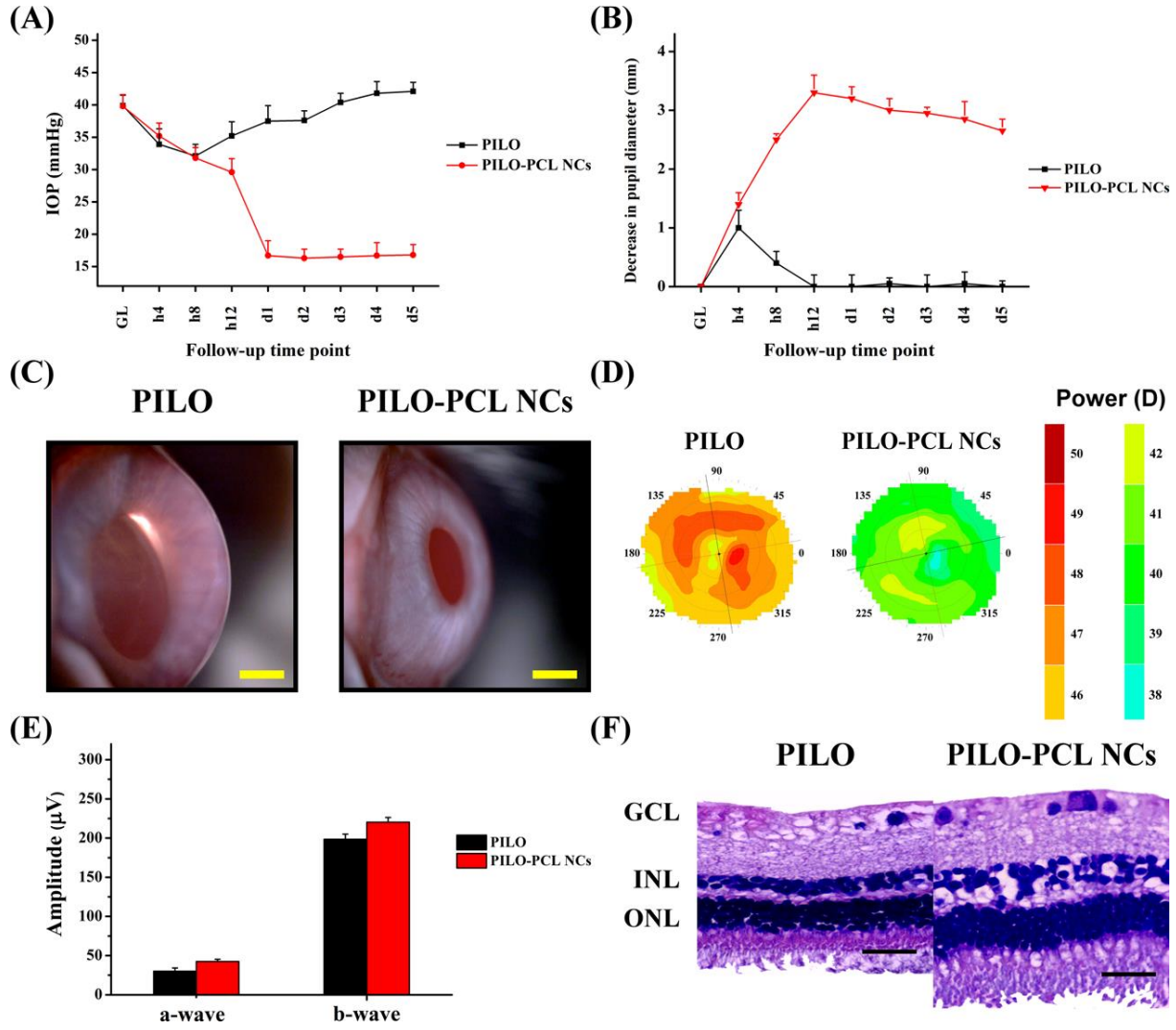

Figure S15. Time-course (A) IOP values and (B) decrease in pupil diameters of glaucomatous eyes (GL) after treatment with 20 μL of free drugs (PILO group) or drug-containing NPs (PILO-PCL NCs). Follow-up time point: hour (h); day (d). (C) Slit-lamp biomicroscope images (side view), (D) topographic corneal profiles, (E) amplitude changes of the a-wave and b-wave, and (F) retina histology images of GL rabbits in PILO and PILO-PCL NCs groups at postoperatively 5 days (d5). GCL: ganglion cell layer, INL: inner nuclear layer, ONL: outer nuclear layer. Scale bars indicate 4 mm (C) and 50 μm (F). Error bars represent the standard deviation of six repeated measurements.

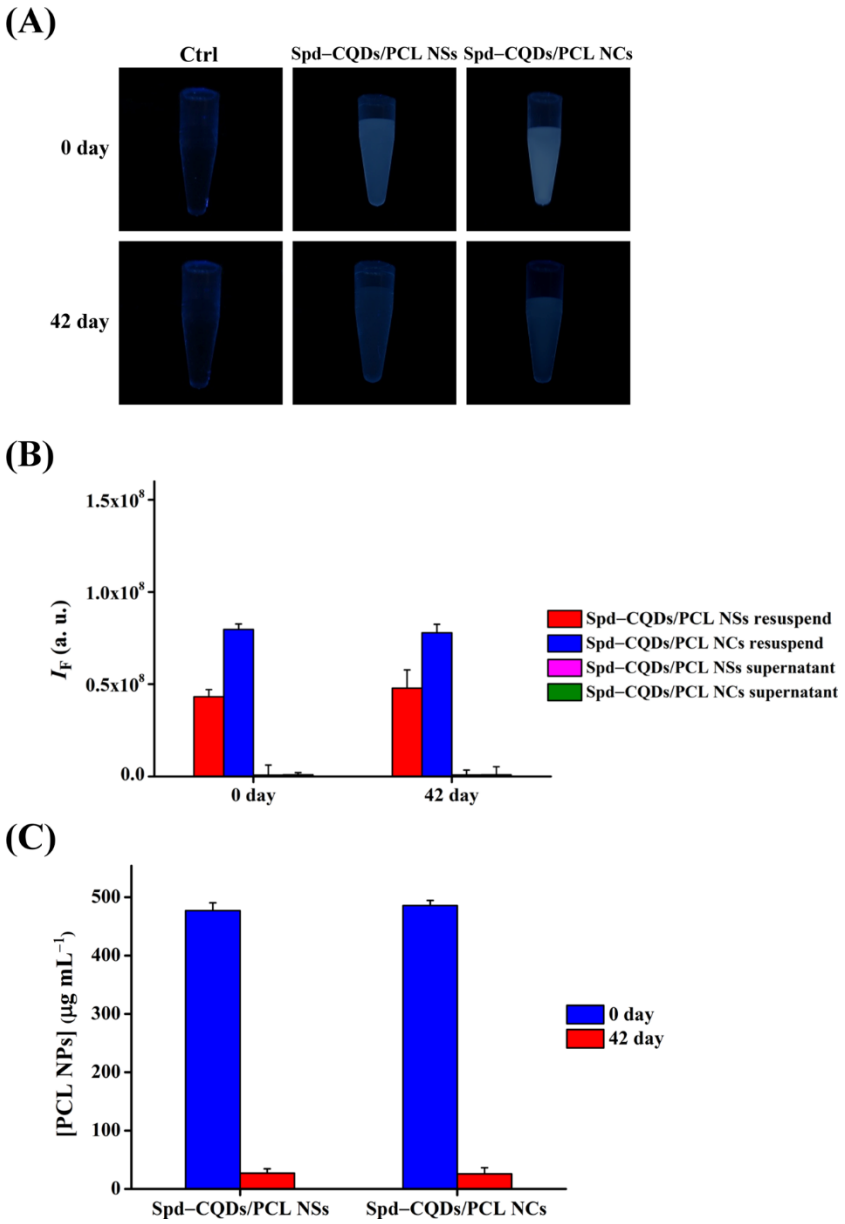

Figure S16. (A) Fluorescence photographs of Spd–CQDs/PCL NPs in aqueous humor (200 μL) collected immediately (0 day) and 42 days post-injection into the rabbits' anterior chamber (500 μg mL^{-1}) upon excitation under a handheld UV lamp (365 nm). (B) Fluorescence intensities of from the resuspended and supernatant solutions of Spd–CQDs/PCL NSs (500 μg mL^{-1}) and Spd–CQDs/PCL NCs (500 μg mL^{-1}) incubated in BSS buffer (200 μL) immediately (0 day) and after 42 days and centrifuged at an RCF of 20,000 *g* for 10 min. (C) Quantification of Spd–CQDs/PCL NPs in aqueous humor immediately and 42 days post-injection. The fluorescence intensities (I_F) are plotted in arbitrary units (a. u.); excitation wavelength: 365 nm.

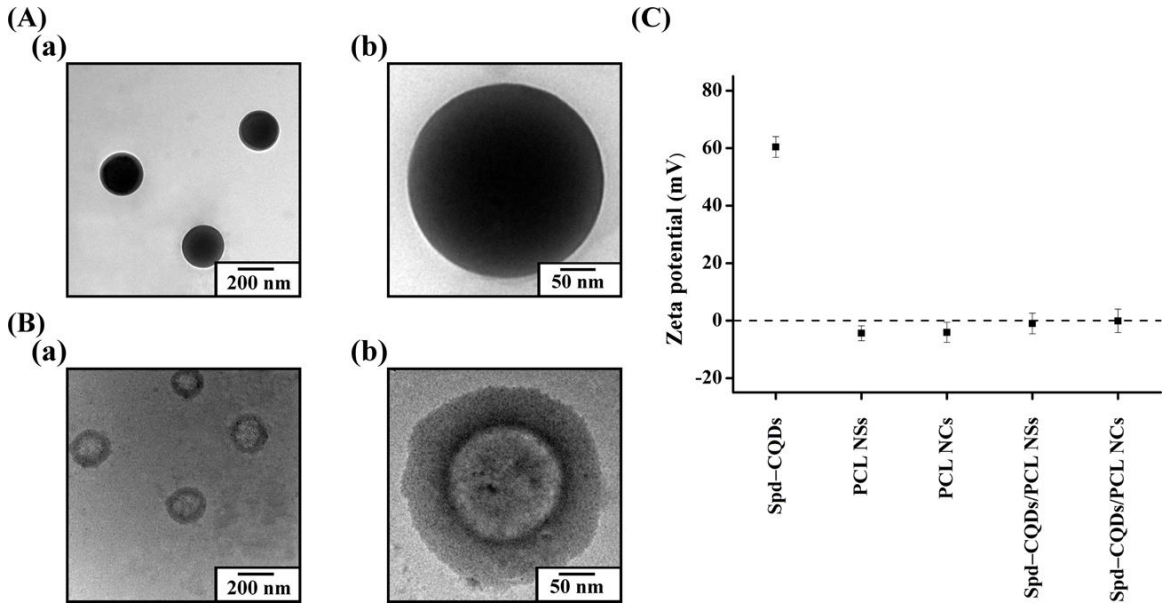

Figure S17. (a) Low- and (b) high-magnification TEM images of (A) Spd−CQDs/PCL NSs and (B) Spd−CQDs/PCL NCs. (C) Zeta potential of Spd−CQDs, and different forms of PCL NPs and Spd−CQDs/PCL NPs. Error bars represent the standard deviation of six repeated measurements.