ELECTRONIC SUPPLEMENTARY INFORMATION (ESI) FOR:

Hybrid Upconversion Nanomaterials for Optogenetic Neuronal Control

Shreyas Shah[†], Jingjing Liu[‡], Nicholas Pasquale[†], Jinping Lai[†], Heather McGowan[‡],

Zhiping P. Pang[‡,], Ki-Bum Lee[†,*]*

† Department of Chemistry & Chemical Biology, Rutgers, The State University of New Jersey, Piscataway, NJ, U.S.A.

‡ Child Health Institute of New Jersey and Department of Neuroscience and Cell Biology, Rutgers-Robert Wood Johnson Medical School, New Brunswick, NJ, U.S.A.

***Correspondence:**

Prof. Ki-Bum Lee
Tel: 848-445-2081
Fax: 732-445-5312
Email: kblee@rutgers.edu

Prof. Zhiping P. Pang
Tel: 732-235-8074
Fax: 732-235-8612
Email: pangzh@rwjms.rutgers.edu

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SUPPLEMENTARY SECTION 1. EXPERIMENTAL METHODS

A. Materials. Thulium(III) acetate hydrate (99.9%), ytterbium(III) acetate tetrahydrate (99.9%), yttrium(III) acetate hydrate (99.9%), gadolinium(III) acetate hydrate (99.9%), oleic acid (technical grade, 90%), ammonium fluoride (99.99+%), sodium hydroxide (99.9%) and ethanol (99%) were purchased from Sigma-Aldrich. All chemicals were used as received, without any further purification.

B. Synthesis of core $NaYF_4$: 30 mol % Yb^{3+}, 0.2 mol % Tm^{3+} nanoparticles ($NaYF_4$:Yb^{3+}/Tm^{3+}). $Y(CH_3CO_2)_3$ * xH_2O (1.396mmol), $(YbCH_3CO_2)_3$ * xH_2O(0.6mmol), and $Tm(CH_3CO_2)_3$ * xH_2O(0.004mmol) were added to a 100-mL flask containing 12 mL of oleic acid and 30 mL of 1-octadecene. The mixture was heated to 130°C for 30 min under vacuum to form the lanthanide-oleate complexes and remove water. Then, the solution was cooled down to 50°C under argon. Afterwards, 10 mL of a methanol solution containing 8 mmol of NH_4F and 5mmol of NaOH was injected into the solution and held at this temperature for 30 minutes. Then, after the evaporation of methanol, the solution was heated to 300°C under argon for 90 minutes and cooled down to room temperature. The resulting nanoparticles were precipitated by the addition of ethanol, and collected by centrifugation at 6,000 rpm for 7 minutes. This wash was repeated several times, before redispersing the final product in 10 mL of hexanes.

C. Synthesis of core-shell $NaYF_4$: 30 mol % Yb^{3+}, 0.2 mol % Tm^{3+} @ $NaYF_4$ upconversion nanoparticles (UCNPs) ($NaYF_4$:Yb^{3+}/Tm^{3+}@$NaYF_4$). $Y(CH_3CO_2)_3$ * xH_2O (1.8mmol) was added to a 100-mL flask containing 12mL of oleic acid and 30 mL of 1-octadecene. The mixture was heated to 130°C for 30 min under vacuum to form the lanthanide-oleate complexes and remove water. The solution was cooled down to 80°C under argon, and the hexane dispersion of β-$NaYF_4$:Yb/Tm (30/0.2%) was injected into the solution. The resulting reaction mixture was heated to 90°C to remove hexanes. Then, the solution was cooled down to 50°C under argon. Afterwards, 10 mL of a

methanol solution containing 7.2 mmol of NH_4F and 4.5 mmol of NaOH was injected into the solution and held at this temperature for 30 minutes. Then, after the evaporation of methanol, the solution was heated to 300°C under argon for 90 minutes and cooled down to room temperature. The resulting nanoparticles were precipitated by the addition of ethanol, and collected by centrifugation at 6,000 rpm for 7 minutes. This wash was repeated several times, before redispersing the final product in 10 mL of hexanes.

D. Synthesis of NaYF4: 20 mol % Yb^{3+}, 0.2 mol % Tm^{3+}, 30 mol % Gd^{3+} upconversion nanorods (UCNRs). UCNRs were synthesized based on a previous report.[1] Briefly, 0.3 g NaOH was added to 1.5 mL DI water and mixed with 5 mL of ethanol and 5 mL of oleic acid under stirring. To the resulting mixture, 1 mL of NH_4F (2 M) and 2 mL of $RECl_3$ (0.2 M, RE= Y, Yb, Gd, and Tm) was added. The solution was transferred into a 20-mL Teflon-lined autoclave and heated at 200ºC for 2 h. The obtained UCNPs were collected by centrifugation at 3,500*g* for 5 mins, washed with water and ethanol several times and re-dispersed in cyclohexane for storage.

E. Characterization of upconversion nanomaterials. Fluorescence spectra were recorded to confirm the emission profile on a Varian Cary Eclipse fluorescence spectrophotometer using an external NIR 980-nm laser source (High Power Fiber Coupled Diode Laser System; Changchun New Industries, FC-W-980) as an excitation light source. Transmission electron microscopy (TEM) images were obtained on a Phillips CM12 operating at 80kV. Samples were prepared by dropcasting a 1 wt% solution of the as-synthesized UCNPs onto carbon-coated copper TEM grids. Powder X-ray diffraction (PXRD) patterns were recorded on a Rigaku Ultima IV X-ray diffractometer, using Cu K_α radiation (λ = 1.5406 Å). A graphite monochromator was used and the generator power settings were set to 40 kV and 44 mA. Data were collected between 2θ of 10-70° with a step size of 0.02° and a scan speed of 1.5 deg/min. Time-resolved upconversion fluorescence of UCNP were collected on a

Edinburgh FLS920 fluorescence spectrometer with a external continuous 980-nm NIR LED laser diode (1.5 W), which was coupled with a chopper to modulate the excitation into pulse mode.

F. Generation of PLGA-UCNP hybrid scaffolds. Ester-terminated poly (lactic-co-glycolic acid) (85:15 PLGA, 0.55-0.75 IV; Durect Corporation) was dissolved in chloroform to make a ~9 wt% solution. A stock solution of UCNPs (or UCNRs) (~150 mg/mL) was made by dispersing in chloroform, followed by sonication. Pre-mixtures of PLGA-UCNP were made by adding dilutions of the PLGA solution and UCNP solution in 0.6-mL Eppendorf tubes and vortexing. Each PLGA-UCNP aliquot (125 μL) contained a total of about 5.5 mg of PLGA and 0-15 mg of UCNPs (or UCNRs). Circular glass coverslips (Ø12mm) were rinsed with ethanol and the surface was entirely covered with the PLGA-UCNP aliquot. Thin films were generated by spin-coating at 3500 RPM for 30 seconds. The amount of UCNP loaded within the scaffold was determined by measuring the mass of the glass coverslip before and after coating. The substrates were sterilized in a culture hood by rinsing with 70% ethanol for 5 mins, followed by UV treatment for 2 hrs, and rinsed with sterile 1xPBS prior to neuronal culture.

G. Neuronal culture. Mouse hippocampal culture was prepared as described elsewhere.[2, 3] Briefly, primary hippocampal neurons were isolated from P0 pups of wild-type (WT) mice and dissociated by papain digestion. The sterilized substrates prepared earlier were coated with Matrigel and seeded with the dissociated neurons. The neurons were cultured *in vitro* for 14-15 days in MEM (Gibco) supplemented with B27 (Gibco), glucose, transferrin, fetal bovine serum and Ara-C (Sigma). All animal experiments were performed under the approval of Rutgers Institutional Animal Care and Use Committee.

H. Lentivirus packaging and infection of neuronal cultures. The packaging of lentiviruses and the infection of neurons with lentiviruses have been described.[3, 4] Briefly, the ChIEF-Channelrhodopsin-2 (ChR2)-tdTomato lentiviral expression vector and three helper plasmids, the pRSV-REV, pMDLg/pRRE and vesicular stomatitis virus G protein (VSVG) were co-transfected into human embryonic kidney (HEK) 293T cells (ATCC, VA), at 6, 2, 2 and 2 μg of DNA per 25 cm^2 culture area respectively. Supernatant with viruses was collected 48 hours after transfection and was concentrated by ultracentrifugation (35,000 rpm for 2 hours). Hippocampal neuronal cultures were infected at 4-5 DIV (*days in vitro*) and used for electrophysiological analysis at 14-15 DIV. All steps were performed under level II biosafety conditions. The efficient expression of ChR2 was confirmed using confocal microscopy (Zeiss LSM 700).

I. Optical stimulation of neuronal cultures. Blue light (470 nm, 250 mW) was generated using Thorlabs collimated LED4C24. The LED was directly attached with an Olympus BX51 microscope via a C-mount adaptor. The light intensity could be adjusted via DC4100 driver, which was synchronized by Clampex10 via a TTL. NIR light (980 nm, 1-2 W) was delivered using an external laser source (High Power Fiber Coupled Diode Laser System; Changchun New Industries, FC-W-980). Where train stimuli were desired, the device was externally triggered by Clampex 10 via a TTL. The NIR light was guided by a multimode patch cable (Ø400 μm; Thorlabs, Cat. #M79L01) comprising of a ceramic ferrule end integrated with a fiber optic cannula (Ø2.5 mm, 20 mm fiber length; Thorlabs, Cat. #CFMC14L20). The fiber optic was positioned ~100 μm from the recorded cell.

J. Electrophysiology. Electrophysiology was performed as described previously.[3, 5] Briefly, the patch pipettes were pulled from borosilicate glass capillary tubes (Warner Instruments, Cat. #64-0793) using a PC-10 pipette puller (Narishige). The resistance of pipettes filled with intracellular

solution varied between 4-5 MOhm. After formation of the whole-cell configuration and equilibration of the intracellular pipette solution, the series resistance was adjusted to 8-10 MOhm. Neuronal activities were monitored with a Multiclamp 700B amplifier (Molecular Devices). Data were acquired using Clampex 10 data acquisition software and analyzed using Clampfit (Molecular devices). The whole-cell pipette solution contained (in mM): 123 K-gluconate, 10 KCl, 1 $MgCl_2$, 10 HEPES, 1 EGTA, 0.1 $CaCl_2$, 1 K_2ATP, 0.2 Na_4GTP, and 4 glucose, pH adjusted to 7.2 with KOH. The bath solution contained (in mM): NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, HEPES 10, and glucose 10, pH 7.4. Synaptic responses were blocked using (in μM): CNQX 20, APV 50, and Picrotoxin 20. Voltage traces to record APs were acquired using 470-nm light and 980-nm light. Whole-cell current responses were collected under a holding potential of -60 mV.

K. Scanning electron microscopy. At 14 DIV, the cultured substrates were fixed with 4% formaldehyde for 15 mins, followed by ethanol drying and critical pointing drying. The dehydrated samples were then gold sputtered (20-nm coating) and imaged using the Zeiss Sigma field emission scanning electron microscope (FE-SEM). Substrates that were not used for cell culture were directly gold sputtered and imaged using the FE-SEM.

References

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2. A. Maximov and T. C. Sudhof, *Neuron*, 2005, **48**, 547-554.
3. Z. P. Pang, P. Cao, W. Xu and T. C. Sudhof, *J. Neurosci.*, 2010, **30**, 4132-4142.
4. J. Tang, A. Maximov, O. H. Shin, H. Dai, J. Rizo and T. C. Sudhof, *Cell*, 2006, **126**, 1175-1187.
5. A. Maximov, Z. P. Pang, D. G. Tervo and T. C. Sudhof, *J. Neurosci. Methods*, 2007, **161**, 75-87.

SUPPLEMENTARY FIGURES

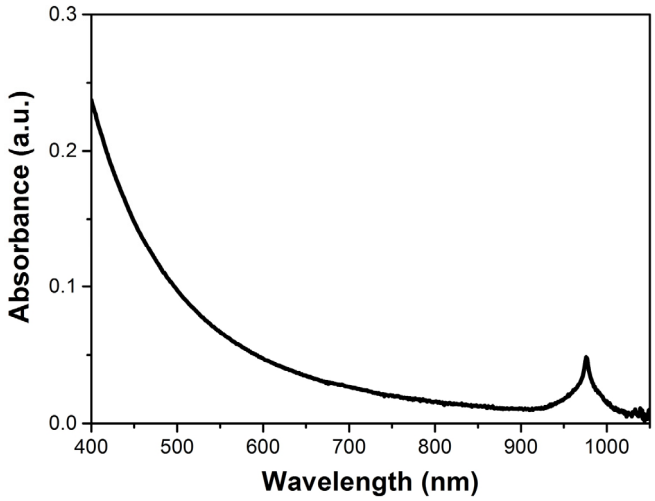

Figure S1. Absorbance spectrum of UCNPs. The absorbance spectrum of the UCNPs shows a peak value at about ~980 nm, corresponding to the $^2F_{7/2} \rightarrow {}^2F_{5/2}$ transition of Yb^{3+} ions.

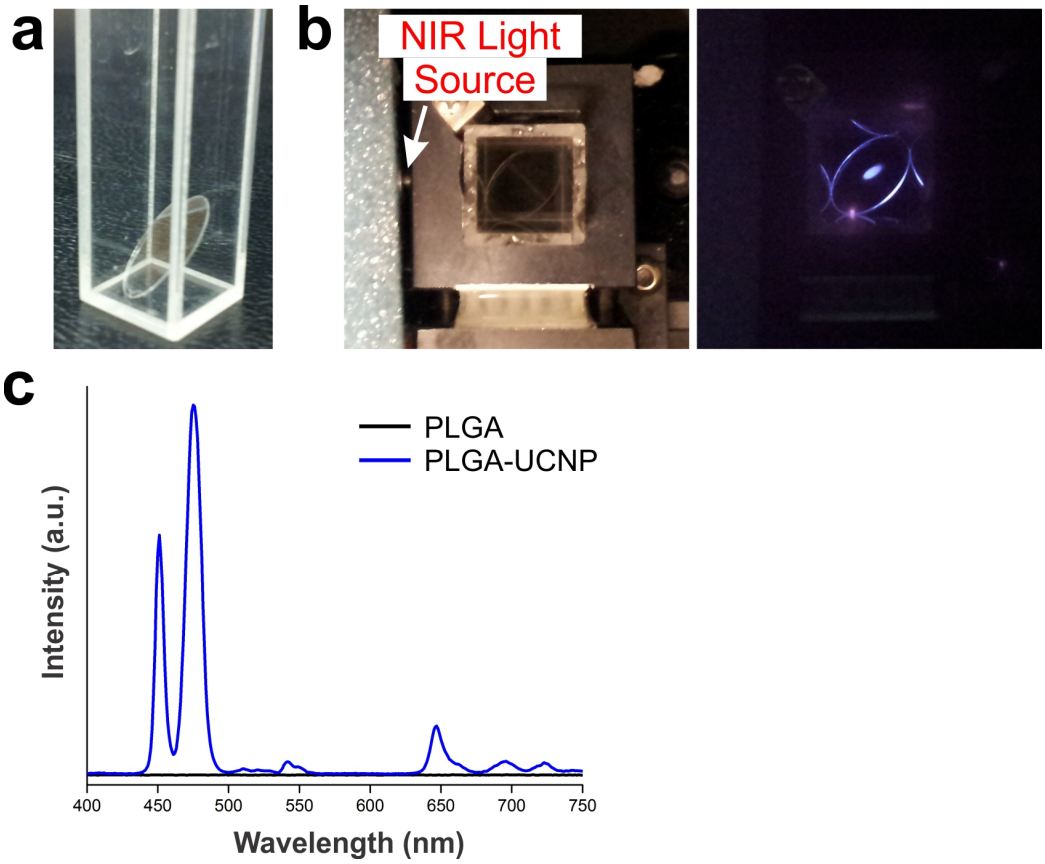

Figure S2. Measuring luminescence of PLGA-UCNP hybrid scaffolds under near-infrared light (NIR) excitation. (a) Picture of a PLGA-UCNP hybrid scaffold coated on a glass coverslip, inserted in a standard quartz cuvette. (b) Fluorometer setup to measure luminescence spectra from PLGA-UCNP hybrid scaffold. (c) Luminescence spectra of PLGA and PLGA-UCNP under NIR light excitation at 980 nm.

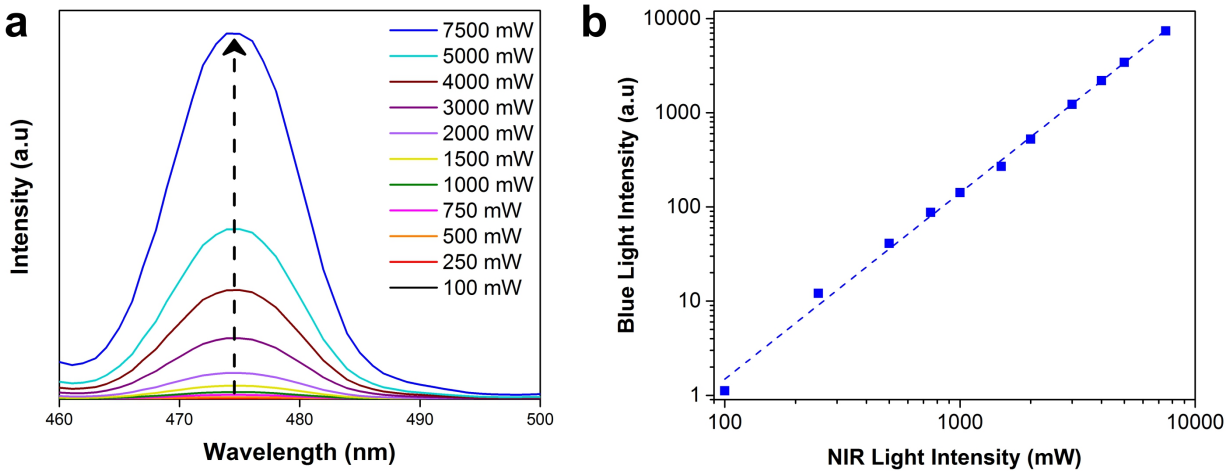

Figure S3. Relationship between NIR light intensity and emitted blue light. (a) Luminescence spectra of PLGA-UCNP hybrid scaffolds under varying 980-nm NIR light excitation power. (b) Emitted blue light dependence on NIR excitation intensity plotted on a log-log scale, showing a linear slope.

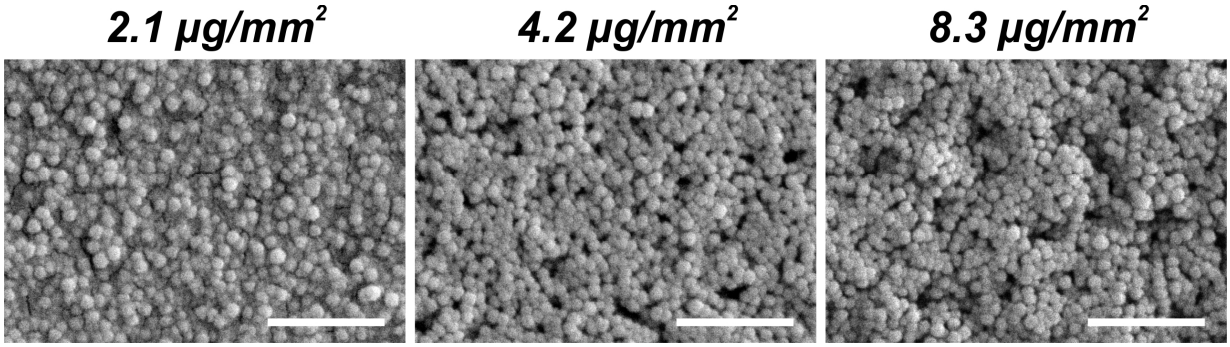

Figure S4. Scanning electron microscopy (SEM) of hybrid scaffolds with varying concentration of UCNPs. SEM images depicting the surface of hybrid scaffolds containing varying concentration of UCNPs ranging from 2.1 µg/mm^2 to 8.3 µg/mm^2. Scale bars: 500 nm.

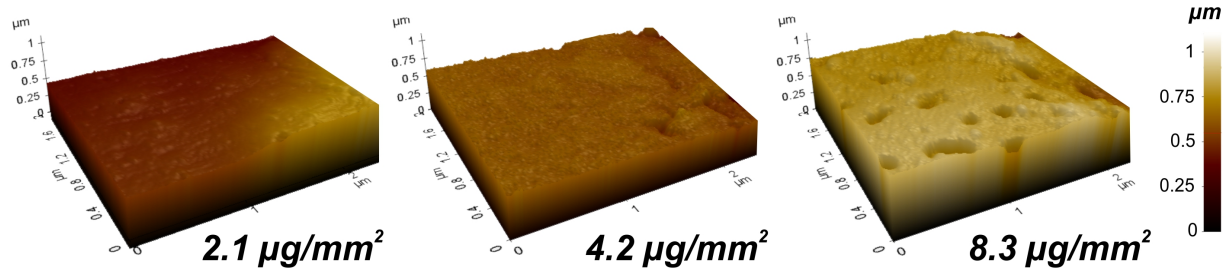

Figure S5. Atomic force microscopy (AFM) images of hybrid scaffolds with varying concentration of UCNPs. AFM images depicting the height profile and thickness of hybrid scaffolds containing varying concentration of UCNPs ranging from 2.1 µg/mm^2 to 8.3 µg/mm^2.

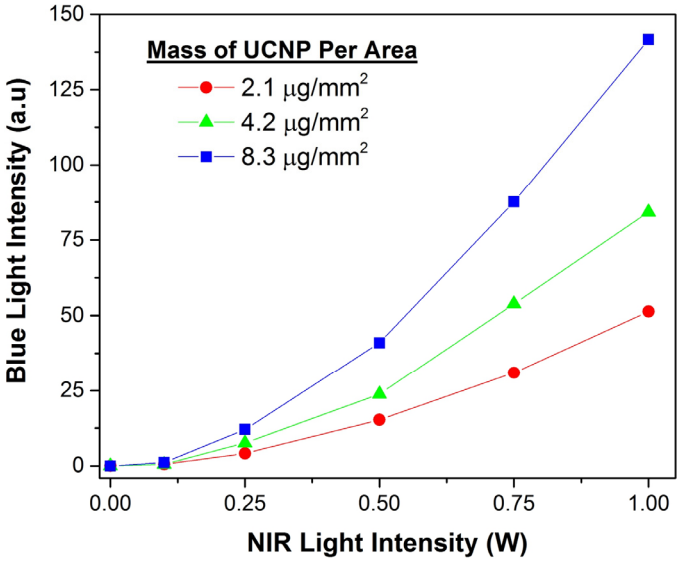

Figure S6. UCNP loading-dependent emission output from hybrid scaffolds. Integrated area of the 475-nm emission peak of PLGA-UCNP hybrid scaffolds with varying UCNP loading, under illumination with 980-nm NIR light excitation power ranging from 0 to 1 W.

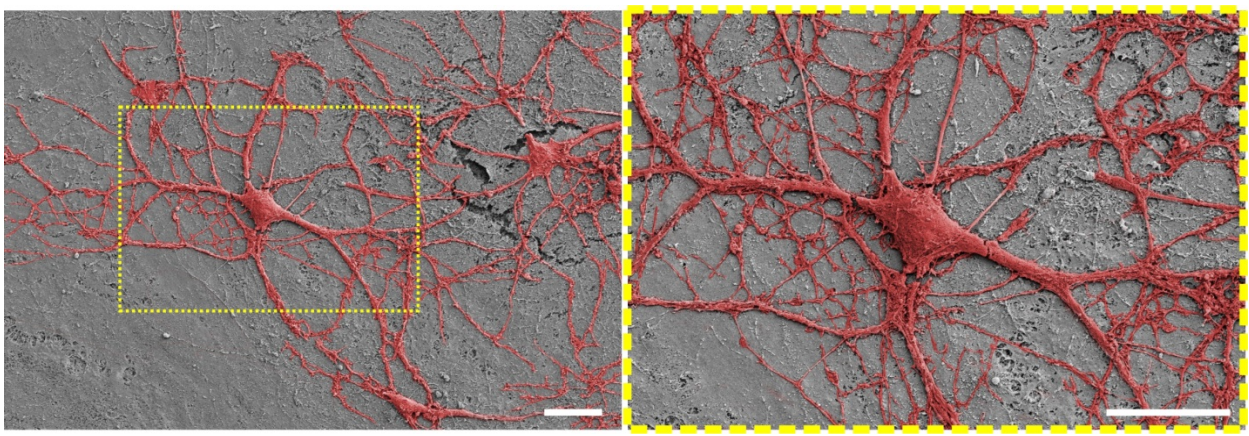

Figure S7. Large-scale scanning electron microscopy (SEM) image of cultured ChR2-infected neurons. SEM image depicts the ChR2-infected hippocampal neurons (pseudocolored *red* for contrast) grown on the hybrid polymer-UCNP substrate at 14 days *in vitro*. The large-scale image shows the attachment and spreading of neurons on the biocompatible surface. Scale bars: 20 µm.

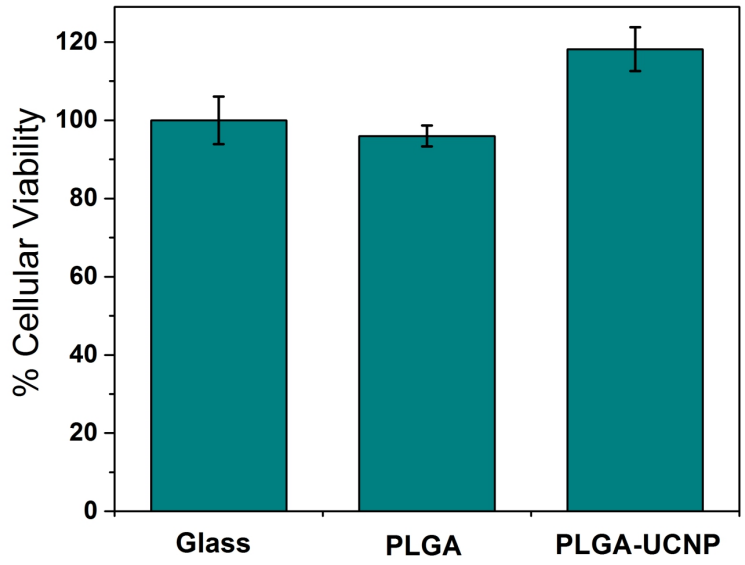

Figure S8. Biocompatibility of hybrid scaffolds. The percentage of viable cells was determined using the PresetoBlue Cell Viability assay for neurons cultured on various substrates after one week of culture. The fluorescence at 590 nm (with 560 nm excitation) was measured and normalized to the control glass substrates.

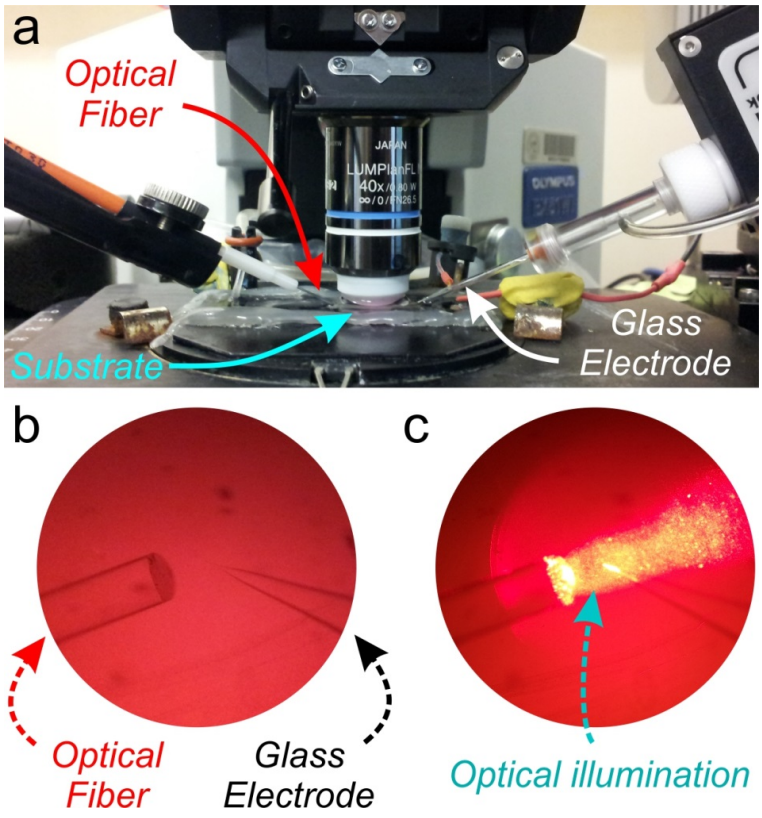

Figure S9. Experimental setup for optical stimulation of neuronal cultures. (a) Microscope setup showing the arrangement of the optical fiber (to deliver 980-nm light), the glass electrode for whole-cell patch clamp recordings and the cultured substrates. (b) Image depicting orientation of the optical fiber and glass electrode through the microscope lens. (c) Image depicting optical illumination alignment using a red-colored guide light.

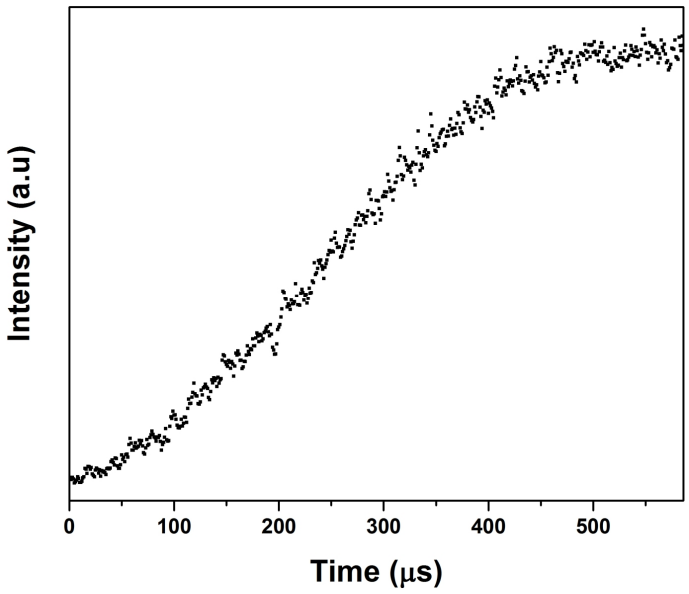

Figure S10. Fluorescence emission rise time of UCNPs. The emission was collected for fluorescence lifetime at 473 nm under 980-nm excitation, showing a rise time of about 310 μs.

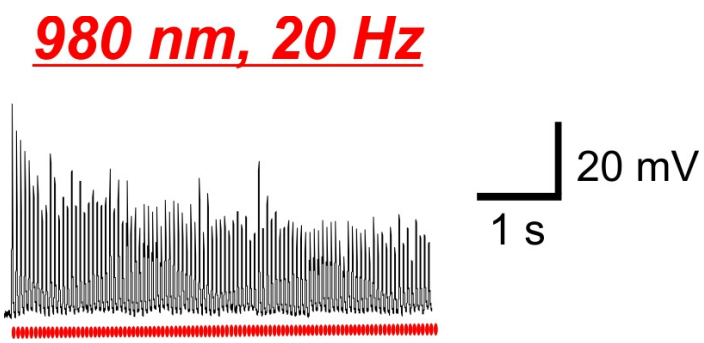

Figure S11. Stimulation with Higher Frequency of 980-nm Light. Representative traces showing action potentials in a current-clamped hippocampal neuron evoked by 20 Hz train of light pulses from 980-nm light (*right*; 1 W, 3 ms pulse width).

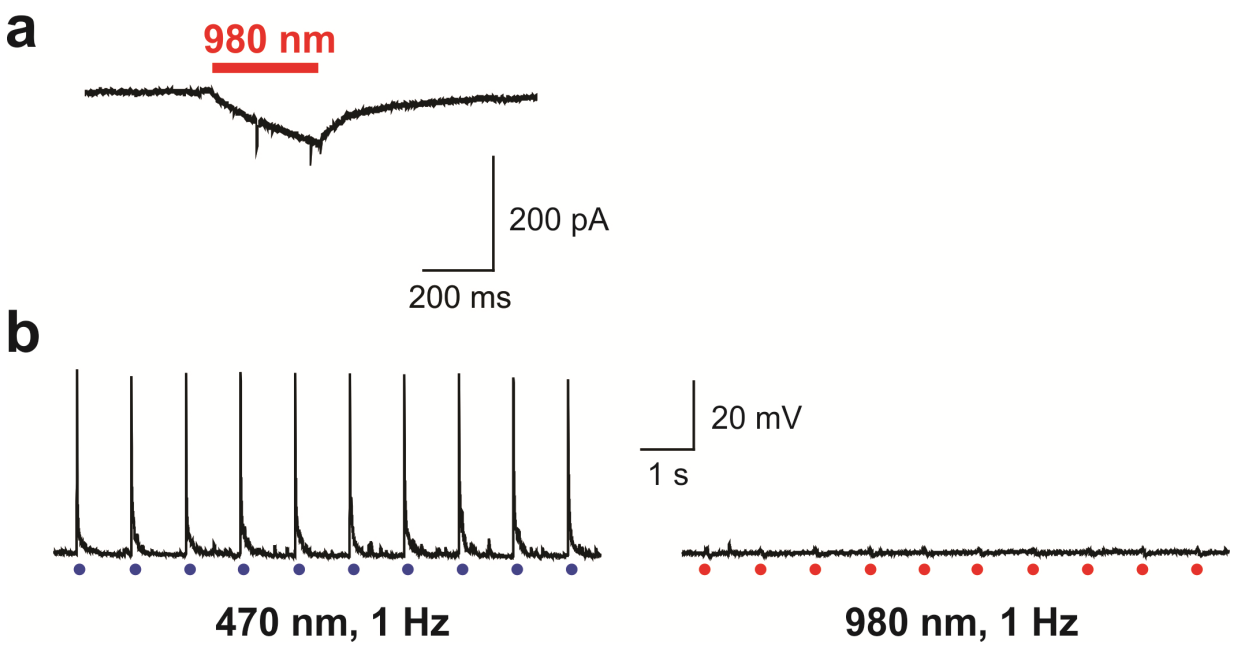

Figure S12. Lack of NIR light response of ChR2-infected neurons cultured on PLGA substrates (w/o UCNPs). (a) Nominal inward current flow in voltage-clamped ChR2-infected hippocampal neurons exposed to 200 ms of 980-nm light (1 W). (b) Representative traces showing nerve impulses (i.e. action potentials) in a current-clamped hippocampal neuron evoked by 1 Hz train of light pulses from 470-nm light (*left*), but none for 980-nm light (*right*). This data demonstrates that the conventional channelrhodopsin-expressing neurons cannot be excited with 980-nm light.

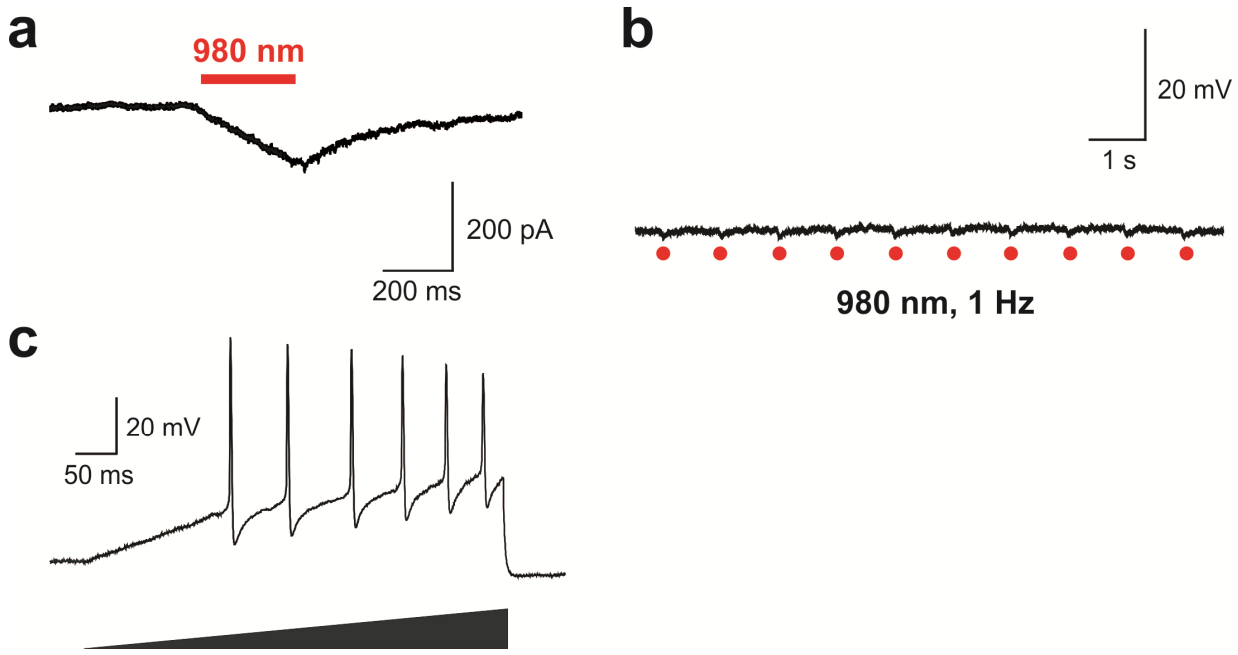

Figure S13. Lack of NIR light response of non-infected neurons cultured on hybrid PLGA-UCNP substrates. (a) Nominal inward current flow in voltage-clamped hippocampal neurons exposed to 200 ms of 980-nm light (1 W). (b) Representative trace shows the lack of nerve impulses induced in a current-clamped hippocampal neuron evoked by 1 Hz train of light pulses from 980-nm light. (c) Action potential trains evoked by a 1.0 pA/ms ramp current from a holding potential of -45 mV. The presence of nerve impulses indicates that the neuron is viable and physiologically-active. This data demonstrates that hippocampal neurons cannot be excited with 980-nm light.

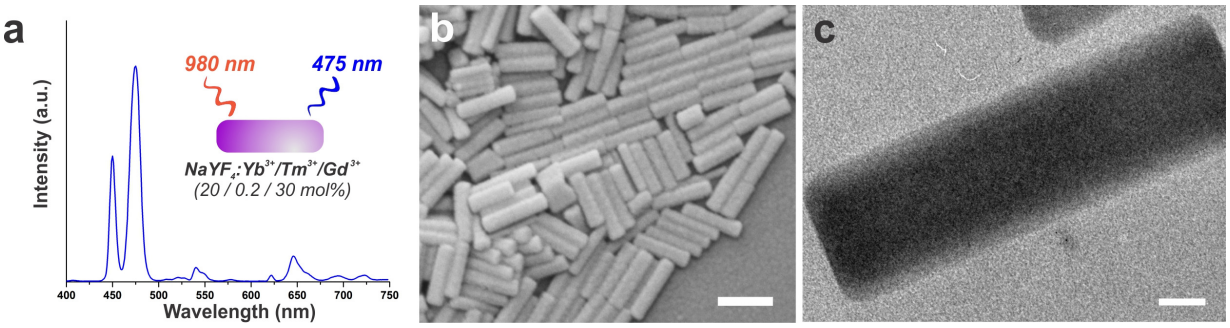

Figure S14. Characterization of blue-emitting upconversion nanorods (UCNRs). (a) Upconversion emission spectrum of the $NaYF_4:Yb^{3+}/Tm^{3+}/Gd^{3+}$ (20/0.2/30 mol%) UCNRs in hexane solution under laser excitation at 980 nm. (b) SEM image of UCNR. Scale bar: 200 nm. (c) TEM image of UCNR. Scale bar: 20 nm. Both SEM and TEM show a UCNR length of about 200-nm and width of 50-nm.

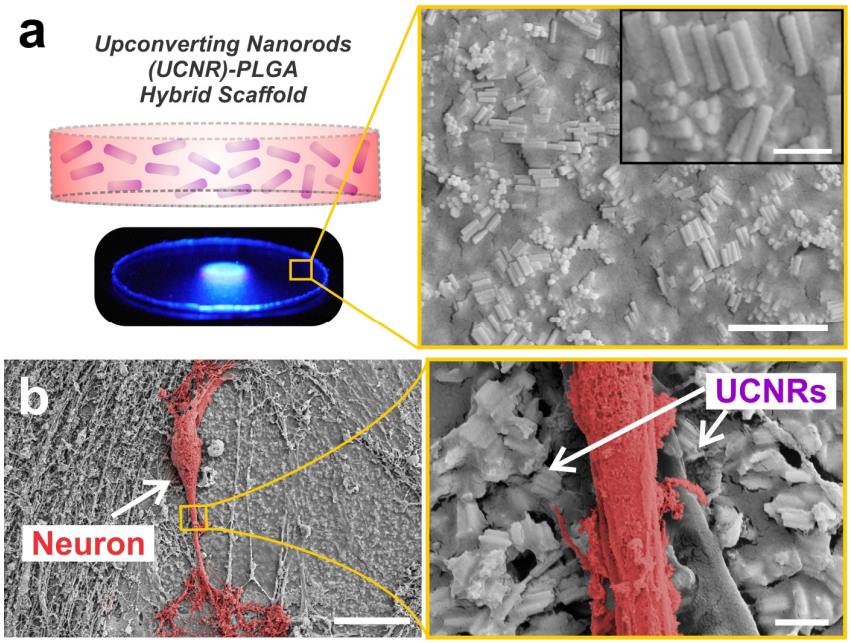

Figure S15. Encapsulation of UCNRs within PLGA films and culture of hippocampal neurons. (a) Scanning electron microscopy (SEM) image shows the distribution of the UCNRs within the PLGA film. Scale bar: 1 µm (*bottom*), 200 nm (*inset*). (b) SEM image of a hippocampal neuron (pseudocolored *red* for contrast) cultured on the polymer-UCNR films at 14 DIV. Scale bar: 20 µm (*left*), 1 µm (*right*).

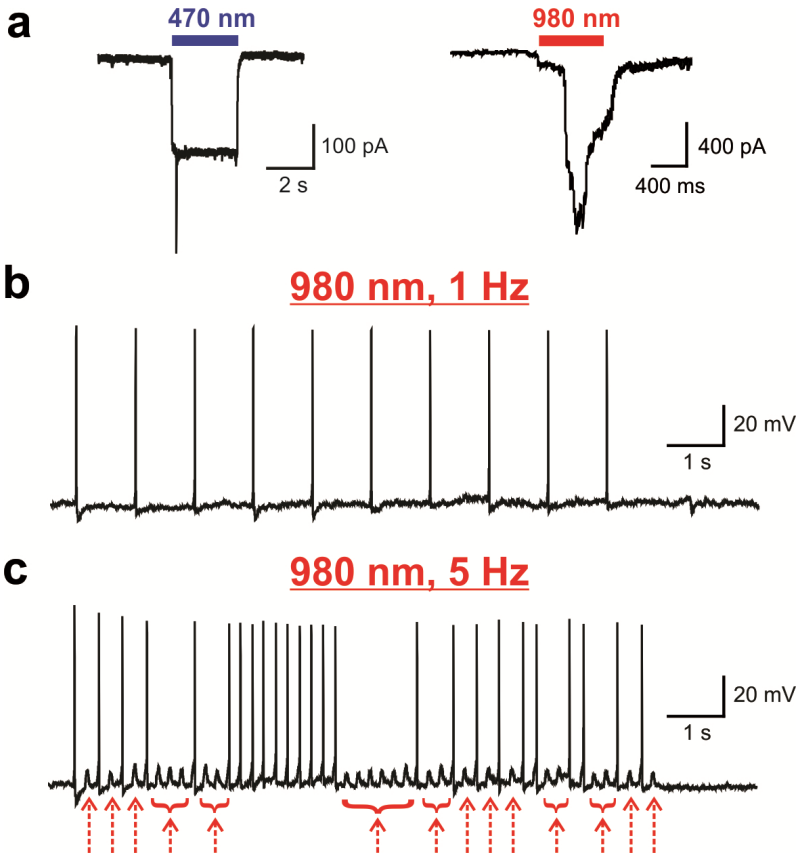

Figure S16. Inconsistent NIR-light response of ChR2-expressing neurons grown on PLGA-UCNR substrates. (a) Inward current flow in voltage-clamped ChR2-infected hippocampal neuron evoked by 470-nm light (*left*; 2.5 mW) and 980-nm light (*right*; 2 W). In comparison, a significant difference in the dynamics of current flow is evident in the two traces. (b) Representative traces in a current-clamped ChR2-infected hippocampal neuron evoked by 1 Hz 980-nm light (2 W, 50 ms pulse duration). At this lower frequency, nerve impulses were generated. (c) Representative traces in a current-clamped ChR2-infected hippocampal neuron evoked by 5 Hz 980-nm light (2 W, 50 ms pulse duration). At this higher frequency, there were inconsistent nerve impulses induced, wherein misfiring was observed (indicated with red arrow) upon NIR irradiation.

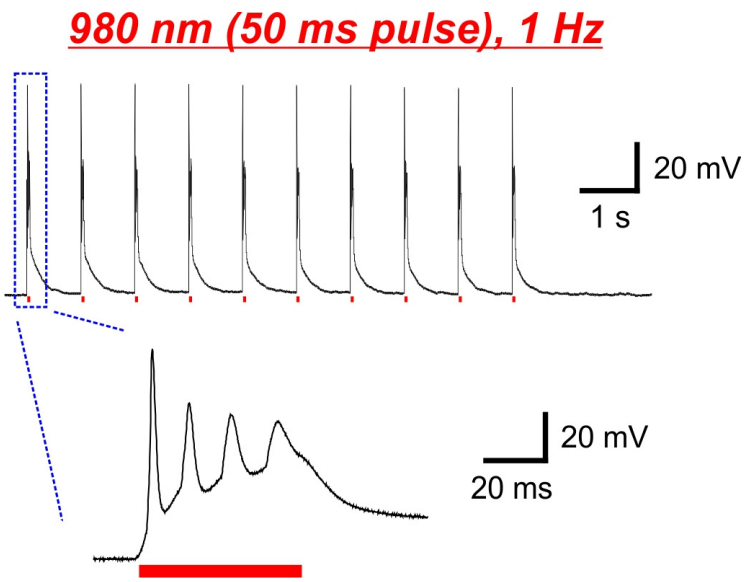

Figure S17. Generation of spikelets using longer NIR pulse duration. Representative trace and magnified action potential of a current-clamped ChR2-infected hippocampal neuron cultured on PLGA-UCNP hybrid scaffolds evoked by 1 Hz 980-nm light (1 W, 50 ms pulse duration).

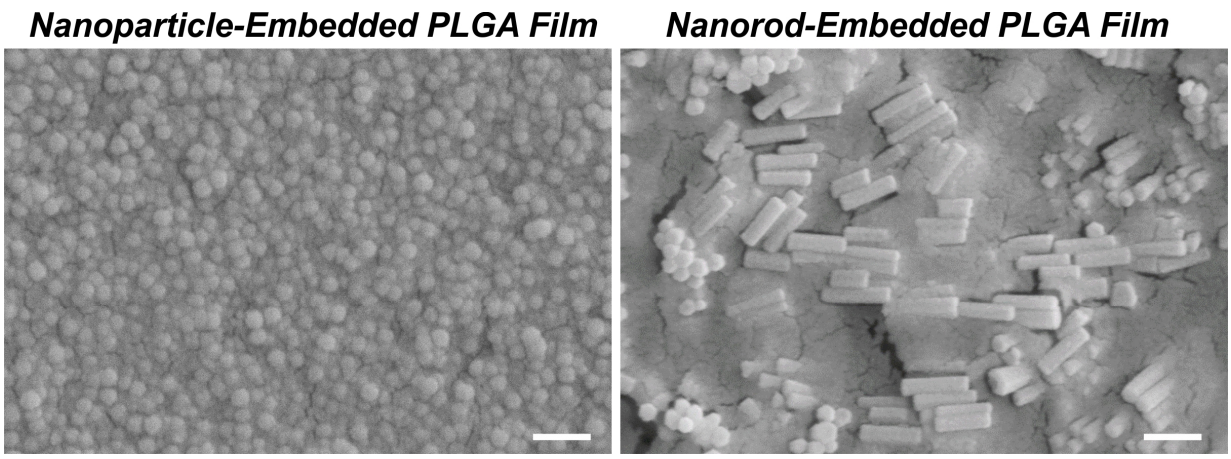

Figure S18. Comparison of UCNP- and UCNR-embedded PLGA films. SEM images depicting the surface of hybrid scaffolds containing UCNPs (*left*) or UCNRs (*right*). UCNPs show better packing and distribution throughout the PLGA film, compared to UCNRs which have varying orientations. Scale bars: 200 nm.

Table 1. UCNP Loading within PLGA-UCNP hybrid scaffolds.

Mass of UCNP Loaded in PLGA Solution Before Spin-Coating (mg)	Total Mass of UCNP Embedded Within PLGA Films (mg)	Mass of UCNP in PLGA Films Per Square Area (μg/mm^2)
5	0.24	2.1
10	0.47	4.2
15	0.94	8.3