Supporting Information for

Semiconductor Nanorod-Carbon Nanotube Biomimetic Films for Wire-Free Photostimulation of Blind Retinas

Lilach Bareket,[†,‡] Nir Waiskopf,[§,∥] David Rand,[†,‡], Gur Lubin,[†,‡] Moshe David-Pur,[†,‡] Jacob Ben-Dov,[†,‡] Soumyendu Roy,[†,‡] Cyril Eleftheriou,[#] Evelyne Sernagor,[#] Ori Cheshnovsky,[⊥,‡] Uri Banin,[§,∥] and Yael Hanein[†,‡]*

[†]School of Electrical Engineering, [‡]Tel Aviv University Center for Nanoscience and Nanotechnology, and [⊥]School of Chemistry, Tel Aviv University, Tel Aviv 69978, Israel

[§]Institute of Chemistry, and [∥]Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

[#]Institute of Neuroscience, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

Cross Section SEM Imaging of CdSe/CdS-GSH NR-CNT Film

Penetration of CdSe/CdS-GSH NRs into three dimensional CNT matrix is demonstrated using cross-section SEM imaging (Figure. S1). NRs appear as elongated bright elements on the CNTs.

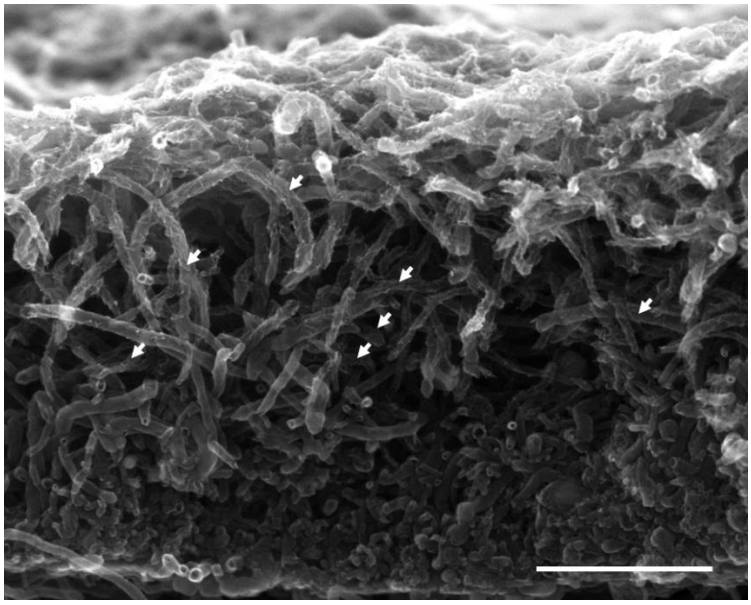

Figure S1. Penetration of NRs into 3D CNT matrix. SEM image of a NR-CNT film; scale bar is 500 nm.

Photocurrent Characterization of CdSe/CdS-GSH NR-CNT Films

A schematic drawing demonstrating equivalent electric circuit model of the photoresponsive films with neuronal tissue is presented in Figure. S2a. The model depicts the electrochemical interface resistance and capacitance of the CNT electrode (R_e and C_e) and of the reference electrode (R_{ref} and

C_{ref}), as well as the solution resistance (R_s) and the photosensitive interface described as a current source and a diode.

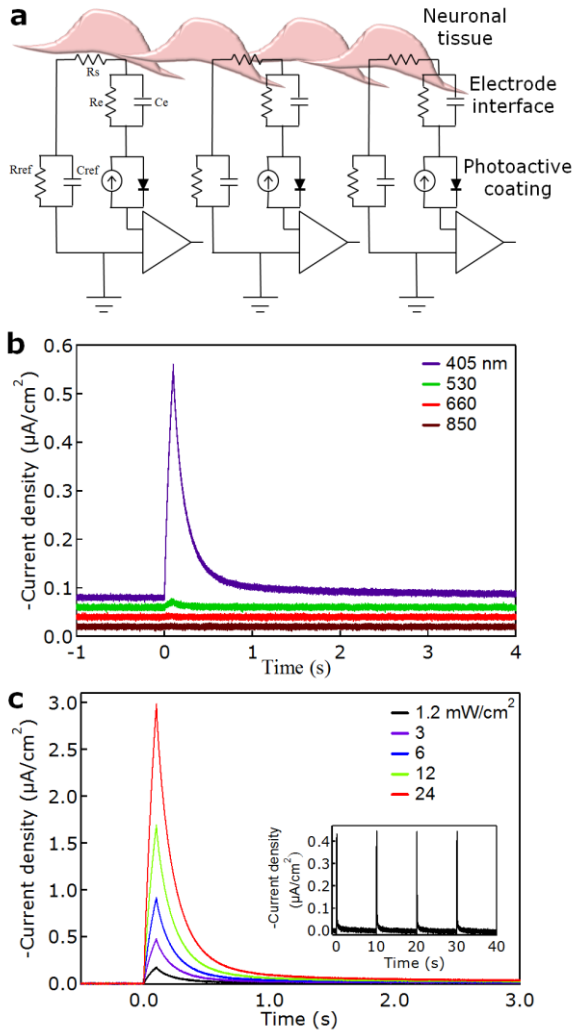

Figure S2. Photocurrent characterization of CdSe/CdS-GSH NR-CNT films. (a) An equivalent electrical circuit of the CdSe/CdS-GSH NR-CNT electrodes with recording amplifiers and neuronal tissue. (b) Photocurrent versus time under 3 mW/cm^2 illumination (for 100 ms) at different wavelengths (405, 530, 660 and 850 nm) recorded from CdSe/CdS-GSH NR-CNT electrode. **(**c) Photocurrent versus time recorded from CdSe/CdS-GSH NR-CNT electrode following 405 nm illumination (100 ms) at different intensities (1.2, 3, 6, 12 and 24 mW/cm^2), and following repeated 100 ms pulses (inset) at intensity of 3 mW/cm^2. CNT electrode surface area was 1 cm^2 and illumination spot size was 0.46 cm^2.

The sensitivity of CdSe/CdS-GSH NR-CNT films to different illumination wavelengths is demonstrated in Figure. S2b. When CdSe/CdS-GSH NR-CNT electrodes were illuminated using 530, 660 and 850 nm light sources, where NRs absorption is very low (see Figure. 1d), almost no photocurrent was observed, confirming the role of the NRs in eliciting charge separation at the interface. The dependence of photocurrent on illumination intensity was also tested. Figure. S2c demonstrates increase in photocurrent with increase in illumination intensity. Repeated stimulations

at moderate light intensities did not hamper the reliability of the photocurrent response (Figure. S2c, inset).

In Vitro Tests

The neuronal activity of E14 embryonic chick retinas in response to light stimulation is demonstrated in Figure. S3. Retinas were placed with RGCs facing down on either pristine TiN MEAs (n=19), CdSe/CdS-GSH NR-TiN MEAs (n=3), or CdSe/CdS-GSH NR-CNT MEAs (n=4). While illumination of the pristine TiN MEA with 30 s long consecutive light (405 nm, 8 mW/cm^2) pulses evoked delayed responses with latencies typical to that of ipRGCs (Figure. S3-a1), no responses were detected when illuminating with 100 ms pulses (Figure. S3-a2). In contrast, 100 ms light pulses of the same wavelength and intensity clearly evoked short latency responses in retinas placed on CdSe/CdS-GSH NR coated MEAs, both TiN MEA (Figure. S3-b1 and S3-b2) and CNT MEA (Figure. S3-c1 and S3-c2).

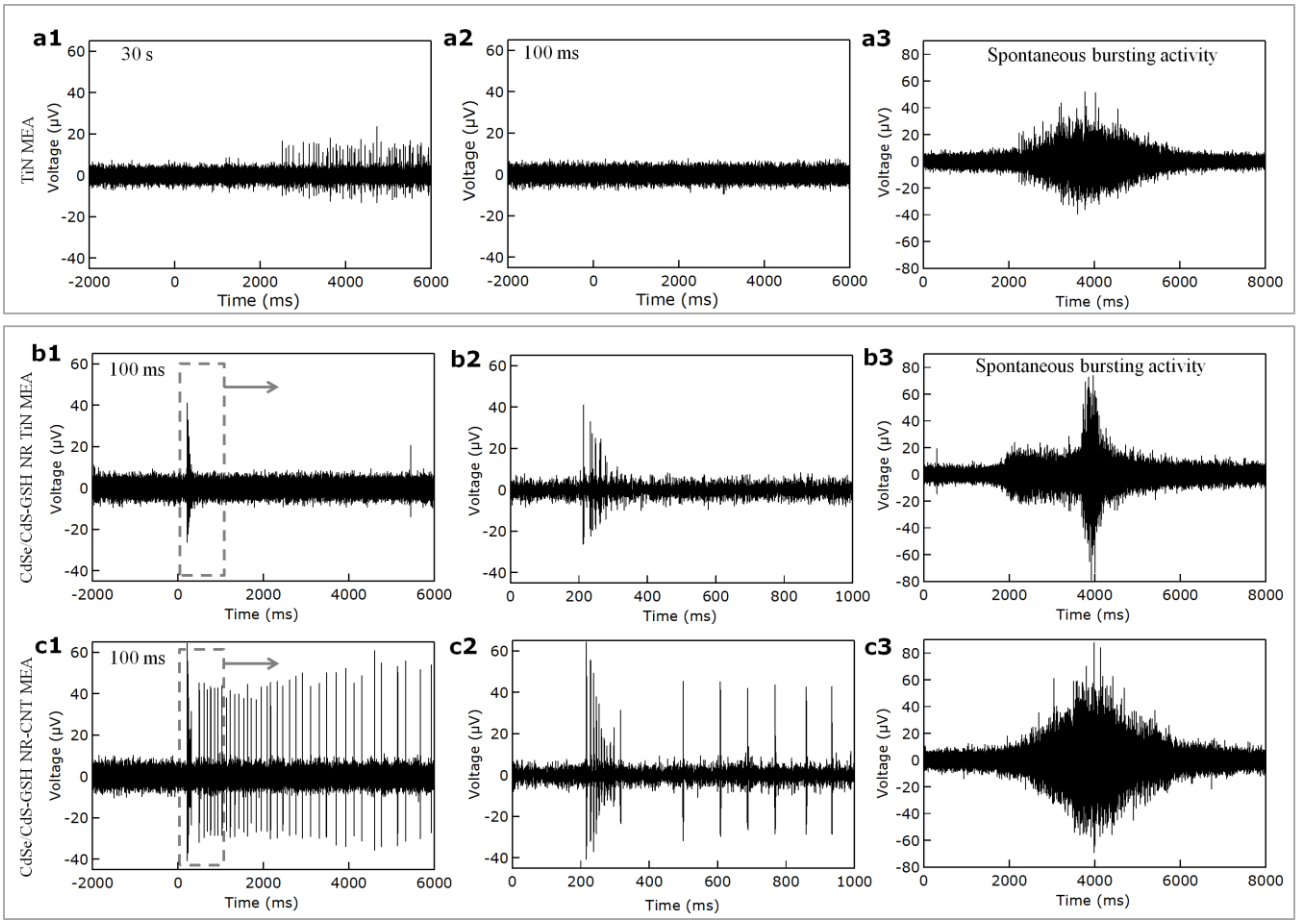

Figure S3. Retina spontaneous activity and photostimulation: Comparison of CdSe/CdS-GSH NR conjugated and pristine MEAs. Representative neuronal activity recordings of E14 chick retinas placed on (a) pristine TiN MEA, **(**b) CdSe/CdS-GSH NR-TiN MEA and (c) CdSe/CdS-GSH NR-CNT MEA**.** Retinas were stimulated using pulsed illumination at wavelength of 405 nm and intensity of 8 mW/cm^2. Light pulses were triggered at time=0. Application of

long duration illumination (30 s) resulted with delayed responses from retinas placed on an unconjugated TiN MEAs (a1). No responses were observed when illuminating with short light pulses of 100 ms (a2). Stimulating with such short light pulses (100 ms) evoked short-latency bursts of neuronal responses in retinas placed on CdSe/CdS-GSH NR-TiN MEA (b1 and b2) and on CdSe/CdS-GSH NR-CNT MEA (c1 and c2), indicating a direct response. This burst is followed by a tonic spiking activity observed from retinas placed on the NR-CNT MEA, indicating an indirect response. Right panels show the spontaneous bursting activity recorded after 24 hr *in vitro* from retinas placed on pristine TiN MEA (a3), NR-TiN MEA (b3) and NR-CNT MEA (c3), demonstrating similar viability.

The RGCs of the developing chick retina exhibit spontaneous synchronized bursting activity resulting in propagating waves, which are most prominent between embryonic days 13-18[1]. Such spontaneous bursts were detected in E14 retinas 24 hours after being placed on either pristine or CdSe/CdS-GSH NR-TiN MEA, as well as on the CdSe/CdS-GSH NR-CNT MEA (Figure. S3-a3, S3-b3 and S3-c3, respectively), validating retina viability on these surfaces. Cell viability assay was used as an additional *in vitro* test. The viability of embryonic rat cortical neurons cultured on CdSe/CdS-GSH NR-CNT patterns (on SiO_2 substrate) was compared to cells cultured on poly-D-lysine coated surfaces (a standard culturing environment for embryonic rat neurons). Neuro-glia cultures were derived from brains of rat embryos (E18) following a procedure reported extensively before[2], and plated onto substrates at a density of 1200 cells/mm^2. Cell viability was verified by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. 0.25 mg/ml MTT (Sigma-Aldrich) was added to each cell culture well containing a sample of CdSe/CdS-GSH NRs-CNT patterns on a SiO_2 substrate, and incubated for 1 hr at 37 °C. Next, the medium was removed and 1.5 ml of dimethyl sulfoxide (DMSO) was added. The absorbance of this solution at 635 nm was subtracted from the absorbance at 535 nm.

Cell viability was monitored at 7, 14 and 21 days *in vitro* (Figure. S4). No significant differences were found in cell viability, indicating that the CdSe/CdS-GSH NR-CNT conjugates did not hinder neuronal viability over this time period.

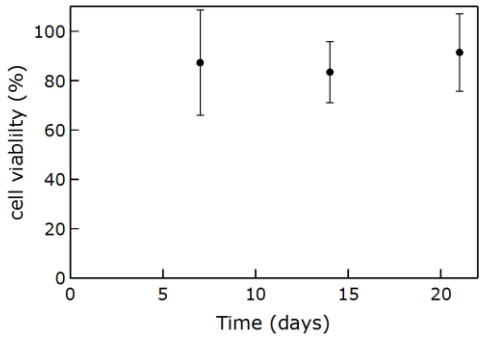

Figure S4. In vitro cell viability assay. The average viability of cortical neurons cultured on CdSe/CdS-GSH NR-CNT patterned films normalized to that of cells cultured on PDL during 21 days *in vitro*.

Optical Properties of CdSe QDs and CdSe/CdS QDs

The absorption (continuous line) and emission (dashed line) spectra of CdSe QDs and CdSe/CdS QDs is demonstrated in Figure. S5a and S5b, respectively. The CdSe QDs exhibit a first excitonic peak at 565 nm and emission peak at 582 nm (Figure. S5a). The size of the seeds was determined to be 3.7±0.1 nm in diameter by absorption spectroscopy using empirical formulas[3] .

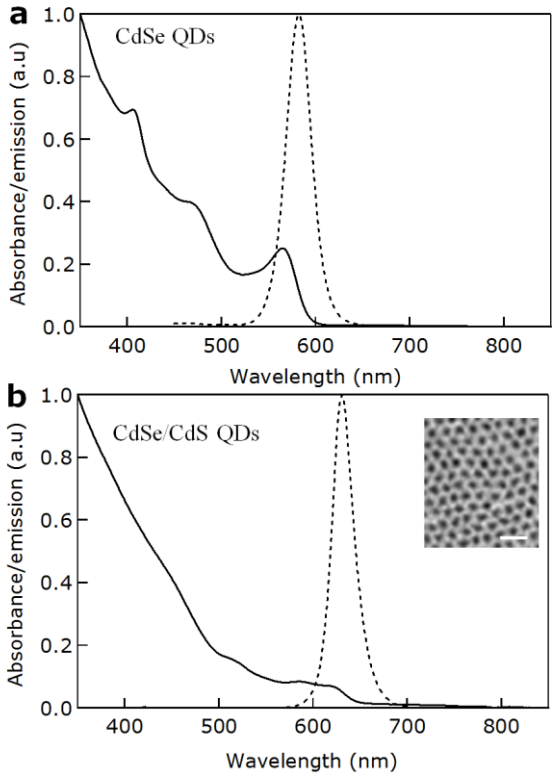

Figure S5. Optical properties of SCNCs. Absorption (continuous line) and emission (dashed line) spectrum of (a) CdSe QDs and (b) CdSe/CdS QDs; Inset: A TEM image of the CdSe/CdS QDs, scale bar is 20 nm.

The CdSe/CdS QDs exhibit a first excitonic peak at 622 nm and an emission peak at 630 nm (Figure. S5b). TEM images of these NPs revealed a homogeneous size distribution of 7.0±0.8 nm in diameter (Figure. S5b, inset).

Loading Yield and Photocurrent Characterization of CdSe/CdS-GSH NR-CNT Films

Next, we examined the conjugation process. CNT films were coated with CdSe/CdS-GSH NRs using the covalent conjugation procedure and through physical adsorption using pristine CNTs without ppAA coating. Covalent conjugation was found to be more effective than physical adsorption in terms of the amount of deposited SCNCs (see Figure. S6, top). Additionally, higher photocurrent was observed using the covalent conjugation compared with the physical adsorption (Figure. S6, bottom).

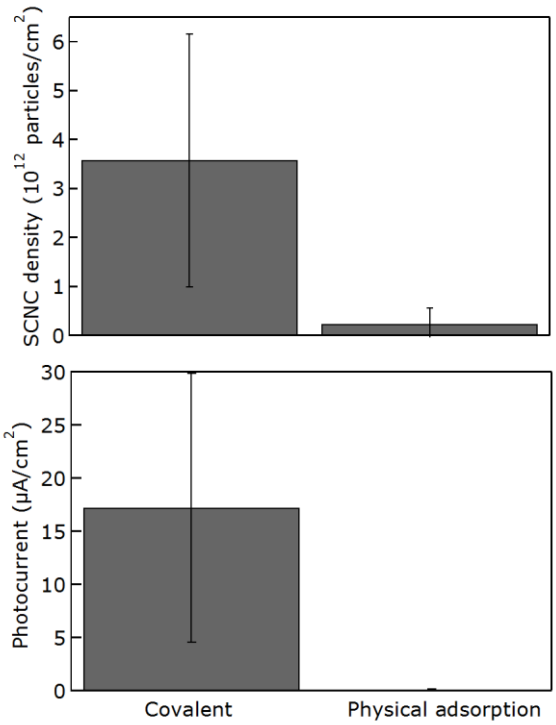

Figure S6. Loading yield of CdSe/CdS-GSH NR-CNT films (top) and average photocurrent of CdSe/CdS-GSH NR-CNT films (bottom) using covalent conjugation and physical adsorption. Higher loading yield and photocurrent were obtained using the covalent conjugation method. Illumination was applied for 100 ms at wavelength and intensity of 405 nm and 30 mW/cm^2. CNT electrode surface area was 1 cm^2 and illumination spot size was 0.46 cm^2.

Supplementary References

1. Wong W. T., Sanes J. R.,Wong R. O. L, *J. Neurosci.* **1998,** 18, 8839-8852.

2. Shein M., Greenbaum A., Gabay T., Sorkin R., David-Pur M., Ben-Jacob E.,Hanein Y., *Biomed. Microdevices* **2009,** 11, 495-501.

3. Yu W. W., Qu L. H., Guo W. Z. , Peng. X. G., *Chem. Mater.* **2003,** 15, 2854-2860.