Plasmon-Coupled Photocapacitor Neuromodulators


ABSTRACT: Efficient transduction of optical energy to bioelectrical stimuli is an important goal for effective communication with biological systems. For that, plasmonics has a significant potential via boosting the light–matter interactions. However, plasmonics has been primarily used for heat-induced cell stimulation due to membrane capacitance change (i.e., optocapacitance). Instead, here, we demonstrate that plasmonic coupling to photocapacitor biointerfaces improves safe and efficacious neuromodulating displacement charges for an average of 185% in the entire visible spectrum while maintaining the faradic currents below 1%. Hot-electron injection dominantly leads the enhancement of displacement current in the blue spectral window, and the nanoantenna effect is mainly responsible for the improvement in the red spectral region. The plasmonic photocapacitor facilitates wireless modulation of single cells at three orders of magnitude below the maximum retinal intensity levels, corresponding to one of the most sensitive optoelectronic neural interfaces. This study introduces a new way of using plasmonics for safe and effective photostimulation of neurons and paves the way toward ultrasensitive plasmon-assisted neurostimulation devices.

KEYWORDS: plasmonics, biointerface, photostimulation, photocapacitor, nanoislands, charge transfer, organic polymers

INTRODUCTION

Extracellular stimulation is the basis for communication with the biological systems to recover lost functionalities,1 understand cellular processes,2 and switch neural networks.3 For that, light offers a noninvasive neuromodulation trigger with high spatial and temporal precision.4−8 Optogenetics introduces light-sensitive opsins into the membrane via a viral transfection, but genetic modification currently raises concerns about their clinical use.9,10 Alternatively, geneless optical stimulation of cells has a high potential to control the neural activity.11,12 For that, optoelectronic biointerfaces can generate photoexcited electrons and holes that can be manipulated for faradic, capacitive, and thermal photostimulation of neurons.

Plasmonics has a high potential to increase the performance of optoelectronic biointerfaces. In principle, plasmonic nanostructures can concentrate the incoming radiation to subwavelength spatial profile due to localized surface plasmon resonance (LSPR)13 and may boost the light–matter interactions for sensitive transduction of optical signals to bioelectrical stimuli. In addition, the light-induced plasmon energy can be transferred to the conduction band of the nanostructure that can produce energetic electrons, known as hot electrons, and these charges may be controlled for photostimulation of neurons. However, despite its high potential for extracellular stimulation, plasmonics has been mainly used for photothermal transmembrane modulation of neurons.14−18 Light is converted to heat energy due to decay of plasmon oscillations, and the resultant temperature variation induces a membrane potential change through a transient membrane capacitance shift.18 Recently, some pieces of evidence showing that metal decoration can support an increase of capacitive and faradic currents were also reported.9

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In this study, we demonstrate that the coupling of plasmons to photocapacitors can improve the charge injection of the neuromodulating displacement currents in the entire visible range. We proved that this broadband enhancement ability mainly stems from hot-electron injection in blue and the local field enhancement in red spectral regions. Finally, the plasmon-assisted capacitive photocurrent modulates transmembrane potential at a single-cell level.

**RESULTS**

**Structure of the Plasmonic Biointerface.** Capacitive charge injection, which is a safe method for stimulation of neurons,8 is based on the electromagnetic attraction and repulsion of the ions in the biological medium due to charge movement in the stimulating electrode. For that, the fabrication of the plasmonic biointerface architecture (Figure 1a) started with the sequential deposition of the ZnO and bulk heterojunction composite PTB7-Th:PC71BM layers on the indium tin oxide (ITO) substrate. According to cross-sectional scanning electron microscopy (SEM) (Figure 1a, left), the thicknesses of the ZnO and PTB7-Th:PC71BM layers correspond to 52 and 104 nm, respectively. Afterward, the gold (Au) thin film is deposited and annealed to form self-assembled nanoislands.19,20 The mechanism of island formation is described by Ostwald ripening of mass transport either by weakly bound individual Au atoms or small clusters combining into larger clusters.21 The surface morphology of the plasmonic biointerface confirms the formation of gold nanoislands (AuNIs) on the photoactive layer (Figure 1a, right), which leads to a plasmonic peak at around 626 nm (Figure 1b, inset). The broad plasmonic band stems from low annealing temperatures,19 which is required to retain optical properties of the organic photoactive layer. The absorption band of the plasmonic biointerface covers all of the visible range, which is useful for the conversion of light within the entire visible range to bioelectrical stimuli (Figure 1b).

**Photocurrent Analysis.** We investigate the photocurrent response of the plasmonic biointerface in an artificial cerebrospinal fluid (aCSF) solution. We use a patch-clamp system (HEKA, EPC 800) in voltage-clamp mode (with ~6 MΩ patch pipette tips) under the free-standing condition (Figure 1c). Since the optoelectronic processes are quantal, the biointerfaces were pumped under illumination of blue, green, and red lights by keeping the photon counts at the same level (Figures S2 and S3), which correspond to 8.8 ± 0.2 × 10^{16} photons s^{-1} cm^{-2} with 10 ms pulse widths. The plasmonic biointerface generates several nanoamperes of photocurrent in aCSF in the entire visible spectrum (Figure S4). Among different excitation wavelengths, the green spectral window shows the highest photocurrent for both biointerfaces due to
higher absorption strength and external quantum efficiency of the photoactive blend in comparison with blue and red illumination (Figure S4). Two spikes were observed at the onset and offset of light illumination. These two spikes (i.e., onset and offset peaks) show that the charge-transfer mechanism is based on the capacitive charge transfer.

Here, the current generation mechanism is controlled by the energy band alignment of the layers at the biointerface (Figure 1d). Photogenerated charge carriers initially dissociate in the photoactive blend, and electron transport toward the ZnO layer takes place. Holes are mainly localized in the proximity of the electrolyte interface that induces a displacement current. Hence, the initial hole accumulation near the electrolyte interface generates onset capacitive photocurrent from the electrolyte (Figure 1e). After the light is turned off, the decrease of the hole concentration due to recombination leads to another offset capacitive spike in the opposite direction.

We compared the light-induced current and charge generation characteristics of the biointerface with those of the control biointerface. For the analysis, we identified faradic and capacitive contributions in blue, green, and red spectral windows under the same photon counts (Figure S5 and eqs S1–S4). The total capacitive charge transfer to the electrolyte is improved by 185% in the entire visible spectrum (Figure 2a and Tables S1 and S2). Furthermore, the plasmonic biointerface enhances the peak photocurrents to the levels of 185.6, 163.2, and 207.9% in blue, green, and red spectral windows, respectively, which correspond to an average increase of 185% in the entire visible spectrum (Figure 2a and Tables S1 and S2).

As a result of the simulations, we did not observe any nanoantenna effect in green and blue spectral regions (Figure 1f). For example, when photons are absorbed by AuNI, electrons gain kinetic energy, which can be calculated as follows: KE_{electron} = E_{photo} - E_{barrier} - E_{deep\ trap} where E_{barrier} = 1.4 eV and E_{deep\ trap} = [0.5–0.8] eV. Assuming the highest energy deep trap states, under blue excitation, the kinetic energy of the electron is KE_{electron at 450 nm} = 2.75–1.4–0.8 = +0.55 eV. Hence, hot-electron injection is possible. When the plasmonic biointerface is illuminated with a green light-emitting diode (LED) at 530 nm, the kinetic energy of the electron KE_{electron at 530 nm} = +0.14 eV is enough to transport it to the photoactive layer. Hence, under green excitation, hot-electron injection is also probable. Finally, under red LED excitation at 630 nm, the kinetic energy of the electrons is
The Bode phase angle (to the double layer formed at the photoelectrode/electrolyte interface is depressed semicircle with a linear extension in the low-frequency region in the complex plane (Figure 3b and a). The Impedance responses of the control and plasmonic biointerfaces are semicircle in the high- to mid-frequency region. The inset of Figure 3c shows the Nyquist plots of the control and plasmonic biointerfaces under blue light excitation. The Nyquist plots are fitted by an equivalent circuit model (Figure 3d). The Rrec is assigned to the recombination resistance (Rrec) in parallel with the constant phase element (CPE1). The recombination resistance (Rrec) is similar for both biointerfaces under blue and green light illumination, and it is interestingly observed higher (~62°) for the plasmonic biointerface under red light illumination (Figure 3e,f). To correlate with the physical mechanisms in the plasmonic biointerface, we analyzed the frequency response and fitted the equivalent circuit model (χ² = 0.01–0.03) comprising a metal–insulator–metal structure (Figure 3g). Cg represents the geometrical capacitance of the device, and Rf is assigned to the electrical resistance that is present at metal electrodes and electrode/contact layer/active layer interfaces. The recombination resistance (Rrec) in parallel with the constant phase element (CPE1) corresponds to the charge dynamics in the photoactive bulk heterojunction. The random characteristics of the blend active layer in the organic bulk heterojunction can be attributed to the distribution of relaxation times and are related to the impedance as $Z = Y_0^{-1}(j\omega)^n$, where $Y_0$ is the CPE coefficient and the ideality factor n = 0 for pure resistive and 1 for pure capacitive behavior—is the characteristic of the distribution of relaxation times. The plasmonic photoelectrode/electrolyte interface is defined by the parallel combination of the Warburg element (W) with the double-layer capacitance ($C_{dl}$). Using the fitted macroelectrical parameters (Table S5), the optoelectronic parameters of the biointerfaces such as the effective lifetime ($\tau_n$) and mobility ($\mu_n$) of charge carriers are determined (Table S6). We observed that while the recombination resistance ($R_{rec}$) is similar for both control and plasmonic biointerfaces under blue and green light illumination, it increases approximately by two times ($R_{rec} = 1.6 \, \text{k} \Omega$) for the plasmonic biointerface under red light illumination in comparison with that for the control.
This leads to a rise of effective recombination lifetime and indicates the generation of a large number of photoactivated charge carriers. Since the peak plasmonic absorption by the gold nanoislands is in the red spectral range, the presence of gold nanoislands at the biointerface can increase the electron concentration by near-field enhancement of the incoming electromagnetic wave, which is observed by an increase in the corresponding recombination lifetime (Table S6).

In the blue spectral region, while the recombination resistance ($R_{rec} = 0.66 \text{ k}\Omega$) and effective lifetime ($\tau_n = 0.3 \text{ ms}$) remain at a similar level to those of the control biointerface, there is an increase in electron mobility for blue excitation compared to red excitation of the plasmonic biointerface. This means that electron injection becomes more probable in the blue spectral range. According to the band energy diagram, the energy of blue photons can lead to the excitation of the electrons to an energy level that is higher than the barrier between gold nanoislands and the highest occupied molecular orbital (HOMO) level of donor molecules (PTB7-Th). Hence, the hot electrons with high kinetic energy have a higher probability to reach the ITO/ZnO interface.26

Biocompatibility. To test the biological and electrophysiological activity, we used SHSY-5Y cells. These kinds of nonspiking cells such as N2A and oocytes, are already used to prove the neuromodulation ability.27,28 Initially, we tested the mitochondrial activity with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays to determine the toxicity of the plasmonic biointerfaces on the SHSY-5Y cell line. In these assays, we used the ITO substrate as the control group. Plasmonic biointerfaces did not exhibit any toxicity on the cells by either assay (Figure 4a,b). We also evaluated the effect of plasmonic biointerfaces on cell growth by investigating the morphology of the SH-SY5Y cells via fluorescence microscopy. Cells grown on the samples were fixed with 4% paraformaldehyde (PFA). Nuclei of the cells were stained with 4′,6-diamidino-2-phenylindole (DAPI), and the cytoplasm was visualized by anti-βIII tubulin immunolabeling. As can be seen in Figure 4c, cells grown on biointerfaces exhibited comparable morphology with the cells grown on the ITO control. All of these results suggested that plasmonic biointerfaces are suitable for biological experiments with SHSY-5Y cells.

Photostimulation of Neurons. To investigate the membrane potential variation, we perform patch clamping in a whole-cell configuration (Figure 5a). The $I$–$V$ characteristics of SHSY-5Y cells grown on both control and plasmonic biointerfaces under the dark condition shows that the SHSYSY cells exhibit typical resting membrane potential in the range between −20 and −40 mV (Figure 5b). The SHSY-5Y cells exhibit a quasi-linear response around the resting membrane...
potential for both biointerfaces. Under 10 ms green light (1–40 mW cm⁻²), an initial depolarization of a single-cell membrane depolarization is followed by a hyperpolarization (Figure 5c). Since the current direction of the first capacitive peak current is from the biointerface toward the substrate in the attached membrane part, the current leads to a depolarization of the free membrane. In contrast, the second peak of the capacitive current leads to a hyperpolarization of the free membrane (see Figure S10). Similar membrane behavior is also observed under green and red illumination as well (Figure S11). Moreover, under repetitive excitation, the peak membrane potential change is also stable under different illumination colors (Figures 5e and S12 and Table S8).

In comparison with the control biointerface, we observed a 37.3% enhancement in the peak depolarization of the transmembrane potential of the SHSY-5Y cells under blue excitation (Figure 5d). When the excitation is shifted to red color, we observed a lower peak enhancement of 7.7% (Figure 5e,f, eqs S5 and S6, and Table S7). This shows that the strength of the stimuli due to the nanoantenna effect shows a reduction in comparison with the hot-electron injection. We attribute that since the wave vector orthogonality to the plane of the biointerface is important for the nanoantenna effect (Figures S13 and S14), the refractive index profile change due to the cell membrane and intracellular environment surrounding the metallic nanoislands possibly disturbs the wave vector of the biointerface and decreases the enhancement in the red spectral window.

**DISCUSSION**

Hot-electron injection is the dominant electronic mechanism for the enhancement of capacitive stimulation. The photons in both the blue and red spectral windows have sufficient energy to generate hot electrons. Since the excited-state energy of hot electrons under blue excitation is higher than that under red excitation, the hot-electron injection to the biointerface pumped under blue illumination is more probable. However, the decrease in the hot-electron injection probability in the red spectral region is compensated by the near-field enhancement of the optical energy in the bulk heterojunction (BHJ) composite.

To capture the hot electrons efficiently, we formed a Schottky barrier with the PTB7-Th:PC71BM bulk heterojunction (BHJ) composite. Since the generated hot electrons with energies higher than the Schottky barrier energy experience lower interfacial resistance toward the BHJ in comparison with the electrode–electrolyte interface, they can be efficiently transferred to the photoactive layer, instead of donating the electron to the electrolyte for faradic reactions.
In this study, both hot-electron injection and near-collisions that are converted into heat energy was generally relaxation through electron–electron and electron–phonon collisions that are converted into heat energy generally used as the main mechanism for cell stimulation, differently in this study, both hot-electron injection and near-field enhancement are used to improve the displacement currents.

The intensity levels that are applied here for cell stimulation can be safely used for all of the tissue or cell types. For example, the most light-sensitive part of the human body is the retina, and the design of the biointerfaces needs to satisfy maximum permissible exposures for ocular safety limits allowed for ophthalmic applications. In this regard, 1 mW·cm⁻² light sensitivity of the plasmonic biointerface corresponds to more than three orders of magnitude smaller than ocular safety limits (see the Materials and Methods section).

Although the transmembrane potential variation in the free membrane seems low, the variation in the attached membrane is significantly larger; moreover, the current levels (over 100 pA) that are observed using similar electrophysiological measurements are enough to elicit action potentials. Therefore, these plasmonic biointerfaces are potentially applicable to recover the vision due to photoreceptor loss in the retina. This kind of sight loss is frequent in the clinics due to age-macular degeneration, retinitis pigmentosa, and Stargardt’s disease.

Gold nanoislands have a high potential for biological applications due to their biocompatibility, easy fabrication, and control over optical properties. They have strong absorption from the visible to the NIR region; therefore, plasmonic-coupled biointerfaces can lead to a new solution for photomedicine.

Materials. Poly-[4,8-bis(5-(2-ethylhexyl)thiophen-2-yl)benzo[1,2-b:4,5-b']dithiophene-2,6-diyl-alt-(4-(2-ethylhexyl)-3-fluorothieno-[3,4-b]thiophene)-2-carboxylate-6-diyil)] (PTB7-Th) with a molecular weight of 57 467 g mol⁻¹ and [6,6]-phenyl-C71-butyric acid methyl ester (PC71BM) with a molecular weight of 1031 g mol⁻¹ were purchased from Ossila Ltd. and used as received. Commercial gold (Au) with 99.95% purity was used for plasmonic gold nanoisland fabrication. Other materials and processing chemicals such as zinc acetate dihydrate (Zn(CH₃CO₂)₂·2H₂O) with a molecular weight of 183.48 g mol⁻¹, ethanolamine (HOCH₂CH₂NH₂) with a molecular weight of 61.08 g mol⁻¹, and 1,2-dichlorobenzene were purchased from Sigma-Aldrich and used without any purification.

Biointerface Preparation. Two types of biointerfaces with and without gold nanoislands (AuNIs) were fabricated. Indium tin oxide (ITO) on glass substrates (side length, 20 mm; side length, 15 mm; thickness, 1.1 mm; resistance, 14–16 Ω·cm⁻²) was cleaned with 10 wt % sodium hydroxide solution for 5 min, 2% (by volume) of specific tension-active agent in a water solution (HELLMANEX II, 3%) for 15 min at 55 °C, DI water for 15 min, acetone for 15 min, and isopropyl alcohol for 15 min using an ultrasonic bath. The cleaned ITO on glass-coated substrates was dried in an oven at 50 °C and treated with UV–ozone for 15 min. The ZnO precursor sol–gel solution (0.45 M) was prepared by mixing 219.3 mg of zinc acetate dihydrate (Zn(CH₃CO₂)₂·2H₂O), 2 mL of 2-methoxyethanol (C₃H₇O₂), and 73 mg of ethanolamine (HOCH₂CH₂NH₂) and ultrasonicated at 55 °C for 15 min.24 The donor copolymer PTB7-Th (14.4 mg mL⁻¹) and 21.6 mg mL⁻¹ acceptor molecule PC71BM solution were prepared in o-dichlorobenzene and stirred overnight at 70 °C on a hot plate. Then, 14.4 mg mL⁻¹ PTB7-Th and 21.6 mg mL⁻¹ PC71BM (1:1.5 wt ratio) in o-dichlorobenzene and 3% (by volume) of 1,8-diiodoanetane (C₆H₄I₂) were added and stirred for 3 h on a hot plate at 70 °C. The control biointerface was fabricated by spinning the ZnO precursor sol–gel solution at 2000 rpm for 60 s on a cleaned ITO coated on the glass substrate and annealed on a hot plate at 290 °C for 15 min.23 Further, on the ITO/ZnO substrate, the photoactive blend of the PTB7-Th:PC71BM (1:1.5 wt ratio) solution in o-dichlorobenzene was spin-coated at 600 rpm for 200 s, then dried under nitrogen flow for 15 min, and heated at 100 °C for 5 min. The plasmonic biointerface was fabricated on a control biointerface by coating 5 nm of Au using a thermal evaporator (Brucker, MB Evap) at the rate of 0.02–0.03 nm s⁻¹ under 6.0 × 10⁻¹⁰ mbar vacuum pressure. Due to the thin layer of gold forming nanoislands even at room temperature, it is annealed at 100 °C, which is the maximum temperature that the photoactive layer can withstand before its optical properties start deteriorating.

Optical and Surface Characterizations. UV–vis transmittance (Edinburgh) was used to measure the absorbances of the control and plasmonic biointerfaces. Scanning electron microscopy (SEM, Zeiss) was used for imaging the surface profiles and the cross-sectional structures of the control and plasmonic biointerfaces. For better visualization of the surface, an additional thin gold coating is deposited.

Photocurrent Measurements. Further, the photocurrent measurements were performed on a setup including an Olympus T2 upright microscope. An extracellular patch-clamp (EPC) 800 patch-clamp amplifier (HEKA Elektronik) is used for measuring photocapacitive currents. The experiment was carried out at room temperature in an extracellular aCSF aqueous medium as the supporting electrolyte solution. The extracellular medium (aCSF) was prepared by mixing 10 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 mM glucose, 2 mM CaCl₂, 140 mM NaCl, 1 mM MgCl₂, and 3 mM KCl in distilled water, and the pH was calibrated to 7.4 using 1 M NaOH. For light stimulation, a Thorlabs blue LED (M505L1P1) with a nominal wavelength of 445 nm was varied between 165 μW·cm⁻² and 169 μW·cm⁻², a green LED (M503L3) with a nominal wavelength of 530 nm was varied between 89 μW·cm⁻² and 41 μW·cm⁻², and a red LED with a nominal wavelength of 630 nm was varied between 64 μW·cm⁻² and 85 μW-
cm$^{-2}$ (Figures S2 and S3). All LEDs were driven by a DC2200 High-Power 1-Channel LED Driver with Pulse Modulation (Thorlabs). A power meter (Newport 843-R) was used to measure the exact power of light reaching the surface of the biointerface. The illumination was focused on the water immersion objective holder from the photoactive layer side of the biointerface. The illumination area was 1 cm.$^2$. The biointerface corners were cleaned with toluene to stabilize the photocurrent measurements.

**Electrochemical Measurements.** Electrochemical impedance spectroscopy (EIS) in frequency response analysis (FRA) potential scan mode was performed on an Autolab Potentiostat Galvanostat PGSTAT (Metrohm, Netherlands). A three-electrode setup consisting of Ag/AgCl as the reference electrode, a platinum wire as the counter electrode, and the biointerface as the working electrode was used. The experiment was carried out at room temperature in an extracellular aCSF aqueous medium as the supporting electrolyte solution. The biointerfaces were excited by blue, green, and red LEDs with an optical power output of 25 mW cm$^{-2}$. In this mode, a sinusoidal alternating voltage perturbation with a root-mean-square (RMS) frequency of 100 kHz was applied across the biointerface. The resulting small signal current was amplified and recorded using NOVA software.

Using the macroelectrochemical parameters obtained by fitting, the driving electrical parameters in the bulk of the biointerface such as the effective lifetime ($\tau_e$) and mobility ($\mu_e$) of charge carriers can be estimated using the following equations:

$$C_p = \frac{(YR_{ac})^{1/2}}{R_{sec}}$$  \hspace{1cm} (1)

$$\tau_e = R_{ac}C_p$$  \hspace{1cm} (2)

$$\mu_e = \frac{1}{\tau_e}$$  \hspace{1cm} (3)

$$D_n = \frac{L^2}{\tau_e}$$  \hspace{1cm} (4)

$$\mu_e = \frac{dD_n}{k_BT}$$  \hspace{1cm} (5)

where $C_p$ is the chemical capacitance corresponding to carrier accumulation in the bulk, $\tau_e$ is the electron diffusion time, $D_n$ is the diffusion coefficient of electrons, $L$ is the thickness of the photoactive thin film, and $k_BT$ is the thermal energy.

**MITT Assay.** The cytotoxic effect of biointerfaces on SHSY-5Y cells was assessed by measuring the mitochondrial activity with the MITT assay. The MITT assay was performed as described by Bahmani Jalali et al. Briefly, biointerfaces and the ITO control were sterilized with 70% (by volume) ethanol solution for 30 min. Sterilized samples were placed in a six-well cell culture plate. Then, SH-SY5Y cells were seeded into each well in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cell attachment and growth were allowed by 48 h incubation at 37 °C and 5% CO$_2$. After incubation, the growth medium was discarded, and samples were transferred to a new six-well plate. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 1 mg mL$^{-1}$) was added to the samples in serum-free DMEM and incubated for 4 h at 37 °C to allow formazan formation. The MITT solution was removed, and formazan salts were dissolved in 50:50 (v/v) ethanol/dimethyl sulfoxide mixture (EtOH/DMSO). Absorances of each sample and ITO control were estimated using the following equation:

$$A = 6443 \text{ mW} \times \frac{1}{10^{-2}} \text{ m}^{-2} \times \frac{1}{10^3} \text{ m}^{-2}$$

and the relative absorbance was calculated to determine the relative biocompatibility.

**LDH Leakage Assay.** The LDH leakage assay (CytoSelect LDH cytotoxicity assay kit, CBA-241, Cell Biolabs) was performed to assess the membrane integrity of the cells grown on the substrates. The experiment was performed according to the manufacturer’s manual. Briefly, 3 × 10$^5$ SHSY-5Y cells were seeded onto sterilized biointerfaces and control groups as explained in the MITT Assay section. Two sets of the ITO control were included as positive and negative control groups. Samples were incubated at 37 °C and 5% CO$_2$ in a time-dependent manner (24, 48, and 72 h). Negative and positive ITO controls were treated with 1% Triton X-100 and dH$_2$O, respectively. The medium (90 µL) from each sample was transferred to a 96-well plate and mixed with 10 µL of LDH cytotoxicity assay reagent. The plate was incubated at 37 °C for 30 min. The presence of LDH in the medium was measured by absorbance at 450 nm with a Synergy H1 microplate reader (BioTek).

**Immunolabeling and Fluorescence Microscopy.** Substrates were sterilized, and SHSY-5Y cells were seeded on substrates into a six-well plate as explained in the cytotoxicity assessment section. The six-well plate was incubated at 37 °C and 5% CO$_2$ for 48 h. Cells grown on the substrates were washed three times with phosphate-buffered saline containing 0.1% Triton X-100 (PBS). Cell fixation was performed by 30 min incubation at 37 °C in 4% paraformaldehyde (PFA). Fixed cells were washed three times with PBS and blocked with PBS containing 5% bovine serum albumin (BSA) for 2 h at RT. The anti-β III tubulin (ab78078, Abcam) primary antibody was used for cytoskeleton labeling, and 4′,6-diamidino-2-phenylindole (DAPI) staining was performed for nucleus visualization. Samples were incubated with 1:250 diluted anti-β III tubulin primary antibody for 2 h and washed three times with PBS. The Alexa-Fluor-conjugated goat antimouse IgG & H&L (ab150113) secondary antibody (1:1000) and DAPI (1:10 000) were diluted in PBS containing 5% BSA, and samples were incubated in the mixture for 1 h. Substrates were washed three times with PBS and mounted onto microscope slides with Mowiol. Imaging was performed with a fluorescence microscope (Zeiss Observer Z1).

**Electrophysiology.** Electrophysiology measurements were performed with an EPC 800 patch-clamp amplifier (HEKA Elektronik). The biointerfaces were cleaned with 70% (by volume) ethanol solution and incubated for 2 days in water. The pulled patch pipettes were performed using 1 M KOH, and patch pipettes were filled with an intracellular aCSF aqueous medium as the supporting electrolyte solution containing 140 mM KC1, 2 mM MgCl$_2$, 10 mM HEPES, 10 mM ethylene glycol-bis(β-aminoethoxy)ether)-N,N′,N′,N′-tetraacetic acid (EGTA), and 2 mM Mg-ATP in water and its pH was calibrated to 7.2–7.3 using 1 M KOH, and patch pipettes were filled with the intracellular solution. A digital camera integrated with an Olympus T2 upright microscope was used to monitor the cells. The whole-cell patched cells were investigated up to 30 min to avoid damage done by patched pipettes.

**Optical Safety Considerations.** According to ocular safety standards for ophthalmic applications, the maximum permissible radiant power (MPF) that could enter the pupil chronically or in a single short exposure is calculated. For single-pulse exposures (pulse width of 10 ms) at 400–700 nm, the peak limit is governed by the following equation:

$$MPF = 6.93 \times 10^{-4} C Psalm^2 - 0.25,$$

where $C$ is the chemical capacitance corresponding to carrier accumulation in the bulk, $\tau_e$ is the electron diffusion time, $D_n$ is the diffusion coefficient of electrons, $L$ is the thickness of the photoactive thin film, and $k_BT$ is the thermal energy. For retinal spot sizes greater than 1.7 mm, $\alpha$ is the chemical capacitance corresponding to carrier accumulation in the bulk, $\tau_e$ is the electron diffusion time, $D_n$ is the diffusion coefficient of electrons, $L$ is the thickness of the photoactive thin film, and $k_BT$ is the thermal energy.

**Supporting Information.** The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.0c09455.

**SEM Images.** SEM images of control and plasmonic biointerface, AuNI size distribution, LED’s spectrum, optical setup, figure and table of whole photocurrent measured data, thermal investigation, figure and table of electrochemical measurements, figure and table of whole electrophysiology measured data, Lumerical FDTD simulations for different $k$ vectors, and AuNI and control biointerface temperature treatment data (PDF).
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