Intracellular and Extracellular Recording of Spontaneous Action Potentials in Mammalian Neurons and Cardiac Cells with 3D Plasmonic Nanoelectrodes

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Supporting Information

ABSTRACT: Three-dimensional vertical micro- and nanostructures can enhance the signal quality of multielectrode arrays and promise to become the prime methodology for the investigation of large networks of electrogenic cells. So far, access to the intracellular environment has been obtained via spontaneous poration, electroporation, or by surface functionalization of the micro/nanostructures; however, these methods still suffer from some limitations due to their intrinsic characteristics that limit their widespread use. Here, we demonstrate the ability to continuously record both extracellular and intracellular-like action potentials at each electrode site in spontaneously active mammalian neurons and HL-1 cardiac-derived cells via the combination of vertical nanoelectrodes with plasmonic optoporation. We demonstrate long-term and stable recordings with a very good signal-to-noise ratio. Additionally, plasmonic optoporation does not perturb the spontaneous electrical activity; it permits continuous recording even during the poration process and can regulate extracellular and intracellular contributions by means of partial cellular poration.

KEYWORDS: Intracellular recording, multielectrode arrays, plasmonic optoporation, neurons, cardiomyocytes

Three-dimensional micro/nanostructures were recently demonstrated to improve the signal quality of multielectrode arrays (MEA) during the recording of neuronal and cardiac spiking activity in vitro, with the major breakthrough being the ability to measure intracellular-like action potentials of multiple cells.

So far, intimate electrode contact with the intracellular environment has been demonstrated using three different approaches: (i) spontaneous cell-membrane poration induced by the extreme sharpness and small size of nanopillars that can disrupt the cell membrane,1 (ii) electrical poration of the cell membrane caused by bursts of electrical pulses applied through nanopillars,2–4 and (iii) spontaneous tight sealing of the membrane and intimate intracellular contact as obtained by exploiting a specific surface functionalization of mushroom-like microstructures.5–7

In spite of the impact of these pioneering approaches, the above-mentioned techniques suffer from specific drawbacks that limit their experimental use in electrogenic cells, particularly in mammalian neurons. Among these drawbacks, electroporation (i) may perturb spontaneous cell activity, (ii) induces a blind recording time of tens of seconds due to the relatively large electrical pulses that overcharge the MEA amplifiers, (iii) often requires different amplifier gain configurations before and after poration, and (iv) can only be applied to all nanostructures present on the electrode, with no selectivity for different cells or cellular compartments interfaced by different nanostructures on the electrode. In fact, so far, only one study reported the recording of intracellular action potentials from mammalian neurons.4 In this study, only evoked (stimulated) activity was recorded, and the resolution of subthreshold potentials was not clearly evidenced. Alternatively, some of these limitations were addressed and overcome by using functionalized mushroom-like micropillars,5–7, a technique that however presents other drawbacks. First, the micromushrooms that have been presented so far are relatively large (1–2 μm) and, therefore, can only be engulfed by large neurons, such as neurons from invertebrates, or by the neuronal somatic compartment. Thus, intracellular axonal and neuritic signals are much more difficult to collect. Second, since this approach requires the cellular engulfment of the electrode, it leads to the recording of exclusively in-cell action potentials of only one cell for each electrode.
Undoubtedly, intracellular signals provide unique information on the activity of ion channels and synapses of single cells. However, spatially resolved extracellular signals can convey unique information, for instance, the localization of a current’s origin and the sorting of action potentials of multiple cells. Importantly, none of the previous techniques was developed to continuously record both intra- and extracellular signals at each electrode site and to exploit the advantages of both recording modalities. This approach might allow a reduction in the number of individually recording electrodes while maximizing the total number of recorded cells.

Recently, we showed that, by using 3D plasmonic electrodes, it is possible to open transient nanopores into the cell membrane without compromising the seal between the cell membrane and the nanoelectrode and with no side effects. The process, called plasmonic optoporation, occurs exclusively at the tip of the nanopillars when they are excited by short laser pulses. Such a process, in synergy with other improvements, can be used to achieve major advances in recording technologies and to address the gap in knowledge of acquiring intra- and extra-cellular information about mammalian neurons and cardiomyocytes.

In this work, we demonstrate long-term and stable continuous recordings of both intracellular and extracellular electrical activity in cultures of primary mammalian neurons and of cardiac-derived cells. Intracellular coupling has led to up to 80 min of continuous recordings with a single-shot poration event. Remarkably, the recorded activity is spontaneous, unperturbed, and shows a very good signal-to-noise (SNR) ratio due to the excellent overall sealing. The key point that enables these results relies on the combination of vertical nanoelectrodes with plasmonic optoporation. The former promotes a tight seal with the cell membrane that is essential for a high SNR. The latter is an extremely local process that enables a gentle and well-controlled insertion of the nanopillars in the cell membrane with no effects on the overall sealing. Hence, no passivation of the planar electrode is needed, and it is possible to couple the tip of the nanopillar with the intracellular compartment, whereas the planar electrode remains extracellularly coupled. As a result, both intracellular and extracellular signals can be recorded from each electrode. Importantly, plasmonic optoporation is completely decoupled from electrical processes such as stimulation, recording, and cellular activity; hence it (i) does not perturb spontaneous cell activity, (ii) does not imply any recording blind time, and (iii)
enables a very stable coupling and a long-term observation. We also fabricated plasmonic nanoelectrodes on CMOS-based high density MEAs and on flexible polymeric devices, which makes this technology appealing for high-density intra/extracellular recordings and integration with synthetic scaffolds, either for in vitro or in vivo applications.

The device exploits 3D gold plasmonic nanocylinders (called nanoelectrodes for brevity) that are fabricated on planar electrodes of custom-designed and in-house made standard MEAs using a fast technique compatible with large-area fabrication. The typical size of the cylindrical nanoelectrodes is 150 nm in diameter and 1.8 μm in height, and the metal coating of the nanoelectrodes is connected to the planar metal of the MEA electrodes, as shown in Figure 1a. The 3D scaffold of the nanoelectrodes is made of commercial optical resist S1813 (MICROPOSIT, Philadelphia, PA, USA). For the coating of the 3D nanoelectrodes, we chose gold because of its performance in terms of plasmonic enhancement in the near-infrared range (suitable to avoid cell damage), chemical stability in liquid environment, and ease of use. However, other materials, such as platinum and iridium oxide, are known to offer superior electrical coupling. In perspective, a very thin layer of these materials can be deposited on the top of the plasmonic metal (Au, Ag), thus improving electrical coupling without significantly affecting the plasmonic performance. Throughout our experiments, the electrodes of the MEAs were decorated with different configurations of 3D nanoelectrodes, and the 3D nanoelectrodes varied in terms of total number (1−20) and spacing between them (2−5 μm).

As reported previously, cells cultured on 3D nanoelectrodes may completely engulf the nanostructures, producing a tight contact between the cell membrane and the nanoelectrode tip. Then plasmonic optoporation is used to open nanopores only at the tip of the pillar, as sketched in Figure 1b. The physical mechanism of plasmonic optoporation is described in detail elsewhere and summarized in the Supporting Information (SI). In brief, we used a short-pulse laser (8 ps) with a wavelength of 1064 nm and 80 MHz repetition rate that was focused through an immersion objective (60 ×). Throughout the poration experiments, the short-pulsed laser average power has been in the range of 2−3 mW. No side effects due to laser exposure or heating were apparent in this work, in accordance with previous results. The electromagnetic field, concentrated on the nanometer-size tip of the nanoelectrode (plasmonic hot-spot), presents intensities high enough to generate nanoshockwaves (pure mechanical waves),

Figure 2. Extracellular and intracellular-like firing activity recordings of hippocampal neurons before and after plasmonic optoporation. (a) Spontaneous extracellular activity of neurons at 20 DIV. (b) Intracellular-like spontaneous activity in the same neuron in panel a after optoporation. (c) Amplitude of the positive phase of action potentials after optoporation. (d) Spontaneous extracellular spike taken from the track in panel a where indicated with an asterisk. (e) Spontaneous intracellular-like spike recorded from the same neuron after optoporation. (f) Spontaneous intracellular-like and extracellular spikes recorded from two neurons on the same electrode, taken from panel b where indicated with an asterisk. (g) Experimental spike with 3D nanoelectrode inside the cell superimposed with SPICE simulation. (h, i) Pure intracellular (h) and extracellular (i) spikes extracted from the equivalent circuit obtained by fitting the experimental extra- and intracellular spikes (see the SI).
Neurons were cultured at a density of 10^4 neurons/12 mm^2, and nanoelectrodes are mechanically attached to the neuronal soma (Figure 1e). Our 3D plasmonic nanoelectrodes, due to their small size (<200 nm diameter), show an improving factor for the SNR of our recordings. Normally, during the recording experimental procedure, cells were seeded on MEAs, and the 3D nanoelectrodes showed only negligible deformations that did not influence their optical (plasmonic) or electrical (impedance) performance; higher and noticeable bending angles were observed only after the stronger mechanical stress due to the dehydration step of the fixation process for SEM imaging.

We recorded spontaneous electrophysiological spiking activity from primary neurons cultured on MEAs decorated with 3D plasmonic nanoelectrodes, first without optoporation and then by subjecting neurons to optoporation (Figure 2a−c). The electrophysiological signals were recorded by a small MEA electrode (5 μm diameter) with one 3D nanoelectrode. Neurons were cultured at a density of 10^4 neurons/12 mm^2, and experiments were performed after 20 DIV. First, extracellular spontaneous spiking activity was recorded as shown in Figure 2a. Because the poration method does not involve the planar electrode surface, the MEA electrodes could be designed to obtain high-quality extracellular recordings, with SNR comparable to that of commercial MEAs.

However, immediately after plasmonic optoporation, the spike amplitude and waveform changed dramatically toward a larger positive phase, similar to the shape of the intracellular action potentials. In other words, after the optoporation event on the attached 3D nanoelectrode, the negative spikes of spontaneous activity from the same electrode shown in Figure 2a were inverted to positive spikes, as shown in Figure 2b. Importantly, the spike signals maintained intracellular characteristics for long continuous recording sessions with a very slow reduction in amplitude over time (Figure 2c), presumably due to a tight seal that formed between the cell membrane and the 3D nanoelectrode. We usually recorded for 20−30 min without observing any decrease in the spikes amplitude and with a stability that lasted sometimes even for more than 80 min.

Interestingly, smaller extracellular spike events were still recorded amidst the large, intracellular-like spikes, indicating that the flat electrode surface remained sensitive to the activity of other nearby neurons in the network (Figure 2f). In fact, the plating neuronal density used for these experiments gave rise to more than one neuron sitting on each electrode or in its close proximity (see Figure S8 of the SI). Examples of extracellular (before poration) and intracellular-like (after poration) spikes are shown in Figure 2d and e that highlight the transition of the recorded signals after plasmonic optoporation. The extracellular spike in Figure 2d presents a biphasic shape with a leading positive phase that is often associated with the activity recorded from an axonal site of the cell. In contrast, the shape of the spike in Figure 2e presents the typical shape of intracellular action potentials, with a large transient depolarization followed by a smaller hyperpolarization.

Because the planar metal at the base of the 3D nanoelectrode is not passivated, the recorded signal after optoporation is an average of the intracellular-like action potential detected by the 3D nanoelectrode and of the extracellular signal detected by the planar metal. To investigate how the extracellular and the intracellular components contribute to the final recorded signal, we performed SPICE simulations on a constructed equivalent circuit based on RC (resistance-capacitance parallel) elements that could fit our recorded signals (circuit details in SI, section S3). For these simulations, we considered the planar electrode RC in parallel with the RC of the 3D plasmonic nanoelectrode. Before plasmonic optoporation, both the planar electrode and the 3D nanoelectrode are extracellular and contribute together to the recorded extracellular signal (as shown in Figure S3 in the SI).

After plasmonic optoporation is performed, the 3D nanoelectrode penetrates the cell membrane and bypasses the membrane RC, recording the intracellular signal. However, the planar electrode RC is still connected to the membrane RC and collects the extracellular signal, which is mediated together with the intracellular one by the amplifier; the resulting experimental and simulated spikes are shown in Figure 2g. To match the amplitude of the spike in panel g, a second sealing resistance is inserted to define the intracellular sealing between the 3D nanoelectrode and the porated membrane. In the equivalent circuit, we can then imagine an insulating passivation of the planar substrate by substituting the electrode RC with the RC of a thin insulating passivation (SiO_2, Si_3N_4); the resulting simulated pure intracellular spike is shown in Figure 2h and can have an amplitude as high as 2.5 mV. Similarly, we can extract only the extracellular signal of the planar electrode (without hypothetical passivation) by excluding the contribution of the 3D nanoelectrode inside the cell; such a curve is shown in Figure 2i. In summary, due to the parallel nanopillar−planar electrode configuration, the spikes detected after plasmonic optoporation can effectively provide information about both the intracellular and extracellular signals. Compared to other intracellular or intracellular-like recordings on MEAs found in the literature, the spikes recorded with our 3D nanoelectrodes show a somewhat lower amplitude. It should be considered that our 3D nanoelectrodes are much smaller than micromushrooms, with consequently higher impedance with respect to the intracellular compartment, resulting in recorded signals with lower amplitude. However, the SNR of the recorded intracellular spikes is comparable to that of spikes shown in the literature. Moreover, the smaller size of the nanoelectrodes is advantageous because they can interface intracellularly with all parts of the neurons, including neurites and axons.

Next, we examined in more detail the recorded signals after plasmonic optoporation (Figure 3). It is known that conven-
extracellular signals have similar shapes and amplitudes. They do not have axons or neuritis, and therefore, the recorded signals do not highlight any temporal discontinuity; in fact, such regular extracellular spikes possess regular and periodic electrical activity that can easily be represented a simpler model to study than neurons. First, they can be carried out in cultures of the cardiac muscle cell line HL-1. These cells have also been used in plasmonic optoporation experiments with transfection capabilities. To better evidence those properties, we recorded signals for the same HL-1 cell line. On the extracellular recordings, the peak-to-peak noise ranged from 20 to 40 μVpp according to the size of the electrodes, whereas the recorded extracellular spikes had a negative phase from 100 to 400 μV. After poration, the noise level remained relatively unaltered, whereas the intracellular-like spike amplitudes ranged from 0.6 to 1 mV. Successful poration experiments on primary neurons were obtained from four different cell culture preparations. For these experiments, we used a total of 14 different MEAs decorated with 3D nanoelectrodes; to confirm the robustness of our devices, we performed successful poration experiments in three different HL-1 preparations and reutilized the same MEA with 3D nanoelectrodes. Indeed, we are able to clean the devices without damaging the majority of 3D nanoelectrodes in order to use them multiple times.

The good recording performance in both extracellular and intracellular configurations is exemplified by the HL-1 signals in Figure 4a and b. Before plasmonic optoporation, the noise level is ~20 μVpp, and the spike negative phase reaches an amplitude of 0.4 mV (Figure 4a); this SNR compares well with commercial passive MEAs. Moreover, the shape and amplitude of the extracellular spikes closely resemble previously recorded signals for the same HL-1 cell line. On the electrodes where plasmonic optoporation is performed (panel b), the noise level is maintained at the same level of approximately 20 μVpp, while the positive phase of the signal reaches an amplitude of 1.8 mV; the SNR has therefore

Figure 3. Characteristics of intracellular-like firing activity recordings in hippocampal neurons. (a) Primary neuron spontaneous activity with the presence of small positive peaks before the action potential. (b) Superimposition of small peaks that might be related to subthreshold synaptic potentials arising from single cells, while our optoporation method might enable sensing these signals. Thus, we examined whether after plasmonic optoporation the recorded intracellular-like signals presented any signal that could be ascribed as a subthreshold event that was not present before optoporation. We found that, in a few recorded traces, small amplitude signals appeared after poration, either in addition to the already present spontaneous activity or as bumps at the beginning of the intracellular-like spikes. Given the amplitude, duration, and shape of these small-amplitude signals and because these signals appeared only after poration, this observation more likely indicates the potential of our method to sense subthreshold synaptic potentials.

The recordings exhibit high SNR both before and after plasmonic optoporation. Before poration, throughout the experiments, the peak-to-peak noise ranged from 20 to 40 μVpp according to the size of the electrodes, whereas the intracellular-like spikes had a negative phase from 100 to 400 μV. After poration, the noise level remained relatively unaltered, whereas the intracellular-like spike amplitudes ranged from 0.6 to 1 mV. Successful poration experiments on primary neurons were obtained from four different MEA biosensors and three different cell culture preparations. Figure 3d shows an example of multiple intracellular-like activity recording from two electrodes on the same MEA after plasmonic optoporation has been performed.

The complete decoupling of plasmonic optoporation from the electrical recording leads to a series of new very interesting and unique observations that are intrinsically beyond electroporation capabilities. To better evidence those properties, we also carried out plasmonic optoporation experiments with engineered transfection capabilities. The cells represent a simpler model to study than neurons. First, they possess regular and periodic electrical activity that can easily highlight any temporal discontinuity; in fact, such regular activity enables a time lapse of the electrical recording. Second, they do not have axons or neuritis, and therefore, the recorded extracellular signals have similar shapes and amplitudes independently of the part of the cell where they are detected. Successful poration experiments on HL-1 cardiac cells were obtained on 17 HL-1 cultures distributed in seven distinguished preparations. For these experiments, we used a total of 14 different MEAs decorated with 3D nanoelectrodes; to confirm the robustness of our devices, we performed successful poration experiments in three different HL-1 preparations and reutilized the same MEA with 3D nanoelectrodes. Indeed, we are able to clean the devices without damaging the majority of 3D nanoelectrodes in order to use them multiple times.

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improved by a factor of 4.5. Figure 4c depicts the activity of the same HL-1 cell shown in Figure 4a and b after membrane reformation, which occurred approximately 90 min after optoporation.

The ability to monitor spontaneous cell activity during and immediately after the moment of poration reveals an interesting effect. In Figure 4d, the noisy recording patch on the left side represents the moment at which an HL-1 cell is porated by the laser excitation of a 3D nanoelectrode; in the following 2 s, the amplitude of the intracellular signals gradually increases before settling to a more or less constant value of approximately 1.5 mV. We observed this type of behavior in most plasmonic optoporation experiments on HL-1 cells. The time required for the settling of the signal amplitude after poration was $\sim 1.5 - 2.5$ s, with an average rate of change of $0.58 \pm 0.09$ mV/s. The gradual increase in signal amplitude after plasmonic optoporation may be attributed to the progressive development of the membrane/nanoelectrode interface after the pores are produced. Thus, plasmonic optoporation offers a unique point of view for investigating the dynamics of membrane behavior. The chemical and physical mechanisms of membrane rupture and reforming are still not fully explored. Therefore, the possibility of performing these types of studies can be of great importance. In contrast, after electroporation, a blind-time window of tens of seconds is typically present, due to a charge that accumulates on the MEA electrodes during the electrical pulses. Until now, the direct observation of the transition from extracellular to intracellular recording could only be observed by means of extremely sharp nanowires that were functionalized with phospholipid bilayers.$^{1,25}$

The laser excitation needed for optoporation is also not deleterious to cells. We tested this by defocusing the laser slightly from the 3D nanoelectrode tip (to avoid activating the hot-spot and producing further pores in the cell membrane) while irradiating the cell and the underlying gold nanoelectrode. Figure 4e shows five intracellular action potentials from a porated HL-1 cell during a 350 ms train of slightly defocused laser pulses. Before and after the second spike, there are two artifacts that represent the beginning and end of the train of laser-excitation pulses. The action potential recorded in the midst of the laser pulses was identical to the previous or to the following spike, and the firing frequency was also unaltered. Such behavior could not be investigated using electroporation, because the electrodes cannot be used simultaneously for
recording and electroporation. The onset and the ending of the laser-pulse train are detected by the electrode as sharp voltage peaks that are observable before and after the second action potential; in fact the laser-excitation induces currents that are recorded by the MEA acquisition system. However, the photoinduced current “during” the laser-pulse train is rejected by the DC filters integrated in the MEA amplifiers, enabling recording capabilities during the plasmonic optoporation event.

A unique feature of plasmonic optoporation is that each 3D nanoelectrode on the same planar electrode can be independently optically addressed for plasmonic poration, thus allowing intracellular recording at specific user-selected cellular compartments. Figure 5a shows an example of cardiac cell activity in a continuous recording from one electrode while optoporation was performed on 3D nanoelectrodes one at a time. The spike shape changed during the experiment according to how many 3D nanoelectrodes entered the cell, but the other recording characteristics, such as the noise level, did not change. Moreover, because no changes were made in the recording capabilities of the MEA (i.e., gain), the other electrodes continued to record extracellular activity while plasmonic optoporation was performed. This feature allows extracellular recording from a complete network while one cell is porated and its intracellular activity recorded.

The analysis of the spike shape during subsequent poration of single 3D nanoelectrodes also permits the study of all of the specific contributions to the resulting recorded spike: the intracellular 3D nanoelectrodes (that have been used for optoporation and penetrated the membrane), the extracellular 3D nanoelectrodes (that have not been used for optoporation and remained outside the cell), and the extracellular planar electrode. In Figure 5b we show the proposed equivalent circuit for an HL-1 cell lying on four 3D nanoelectrodes that are used progressively for cell poration. Figure 5c–f show the recorded and simulated spikes according to how many 3D nanoelectrodes penetrated the cell membrane. The 3D nanoelectrodes not used for opto-porating the cells remain extracellularly coupled to the cell and are taken into account in all simulations; their contribution to the recorded signal remains unchanged. In addition, since we used MEA electrodes with size up to 30 μm, in several cases some of the 3D nanoelectrodes were not covered by cell bodies or processes and were therefore directly exposed to the cell culture media. These exposed 3D nanoelectrodes contributed to reduce the sealing resistance \(R_{\text{cell}}\) of the cell/electrode interface as did the exposed planar MEA electrode; in our simulations we took this into account by defining an \(R_{\text{seal}}\) resistance that could fit the amplitude of the recorded signals. This experiment clearly presents the unique capability of plasmonic optoporation to selectively porate individual regions of a cell lying on one electrode and to record the extracellular and intracellular-like components. In section S1 of the SI, we present the circuit analysis used to obtain the simulated spikes in Figure 5.

We demonstrated the performance of 3D plasmonic nanostructures on multielectrode arrays for long-term and stable recordings of both intracellular and extracellular electrical spiking activity in primary mammalian neurons and cardiac derived HL-1 cells. Remarkably, the efficacy of the proposed method was demonstrated with the recording of spontaneous and unperturbed electrical activity with high SNR. The key point enabling these results is the combination of vertical nanoelectrodes structured on planar microelectrodes with plasmonic optoporation. The former promotes a tight seal with the cell membrane that is essential for achieving a high signal-to-noise ratio in extracellular recordings. The latter enables an extremely local membrane poration process to gently penetrate the intracellular compartment with only the tip of the nanoelectrode, without affecting the tight seal. Because the optical excitation used to induce the plasmonic cell-membrane poration is completely decoupled from the electrical recording circuit, hybrid MEA electrodes could be designed to optimize both extracellular and intracellular signals, and continuous recordings could be made throughout the poration event, without needing to adjust recording parameters such as amplifier gain. This complete decoupling of plasmonic optoporation from electrical recording also allows recording electrical activity instantaneously after poration, revealing cellular events related to cell–electrode coupling when the membrane is locally porated. Interestingly, in the presence of external forces, the 3D nanoelectrodes tend to bend rather than break or detach from the substrate; this flexibility might be an important factor to improve cellular adhesion to the nanoelectrodes and, consequently, the recording performance.

Although further optimizations will be needed to promote plasmonic optoporation toward the signal quality of a standard intracellular recording technique for primary neurons, our results demonstrate the vast potential of our approach and several appealing features to advance the quality of multisite electrophysiological recording technologies. The poration process can be scaled up to thousands of electrodes per minute, and both the fabrication process and the poration mechanism are compatible with high-density CMOS-MEAs (see the SI). Additionally, 3D plasmonic nanoelectrodes are very efficient surface-enhanced Raman spectroscopy (SERS) probes; in the near future, electrical information may be combined with spectroscopic investigations of biochemical processes occurring at the cell membrane and/or in the cytoplasm. This technology can be used for the selective and controlled intracellular delivery of nonmembrane-permeable molecules, thus potentially enabling radical new experiments in which biomolecules are selectively delivered into neurons while the intracellular and the extracellular electrical activity are monitored on the large scale. Finally, for in vivo implantable probes, this approach might be combined with the recent advancements in integrated optical probes, allowing the recording of intracellular signals from specific neurons and of the extracellular spikes and low-frequency signals of surrounding neuronal populations while minimizing the total number of individually routed electrodes.

### ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.7b01523.

(1) Plasmonic optoporation of cardiomyocytes and the equivalent circuit. (2) Sealing formation after plasmonic optoporation. (3) Neuron/3D nanoelectrode equivalent circuit. (4) Integration on CMOS-MEA. (5) Long-term recording. (6) Plasmonic optoporation. (7) 3D nanoelectrodes on flexible substrates. (8) Experimental setup. (9) SEM imaging of primary neurons on MEA. (10) MEA fabrication. (11) Preparation and surface coating of nanostructured MEAs. (12) Preparation of rat hippocampal primary neurons and scanning electron micros-
copy. (13) Culture of HL-1 cardiac cells. (14) Acquisition system for electrical recording. (15) Optical setup for plasmonic optoporation (PDF)

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Author Contributions

L.B. and F.D.A. conceived and supervised the work. M.D., G.C.M., F.T., and F.M. designed the optical-electrical setup and the optical measurements. M.D., F.M., and V.C. designed and fabricated the MEA devices. L.L. optimized and cultured the HL-1 cells. H.A. optimized and cultured the primary rat hippocampal neurons. M.D. and L.L. performed experiments on HL-1 cells. M.D. and H.A. performed experiments on neurons. M.D. and V.C. made the SEM images of neurons. F.D.A. supervised the whole project. All authors contributed to the manuscript preparation.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Micha Spira, Fabio Benfenati, John Assad, and Alessandro Maccione for the very useful discussions. The research leading to these results has received funding from the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement no. [616213], and CoG: Neuro-Plasmonics. H.A. was partially supported by the European Commission for Research within the Seventh Framework Programme for the NAMASEN (FP7-264872) Marie-Curie Initial Training Network.

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