

Remote switching of cellular activity and cell signaling using light in conjunction with quantum dots

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Abstract: Stimulating cells by using light is a non-invasive technique that provides flexibility in probing different locations while minimizing unintended effects on the system. We propose a new way to make cells photosensitive without using genetic or chemical manipulation, which alters natural cells, in conjunction with Quantum Dots (QDs). Remote switching of cellular activity by optical QD excitation is demonstrated by integrating QDs with cells: CdTe QD films with prostate cancer (LnCap) cells, and CdSe QD films and probes with cortical neurons. Changes in membrane potential and ionic currents are recorded by using the patch-clamp method. Upon excitation, the ion channels in the cell membrane were activated, resulting in hyperpolarization or depolarization of the cell.

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1. Introduction

Electrical signals in the brain govern the complexity of the human body and mind. Being able to switch and control these signals externally represents an important tool to answer questions about sensory, motor and behavioral events, which fundamentally control our health. For example, it can help us understand the circuits involved in specific disorders, finding alternative paths for treating brain diseases such as Parkinson's and Alzheimer's. The study of electrical signaling and communication among neurons has benefited from tools capable of exciting neurons in a spatially and temporally controllable manner in order to help us understand the correlation of neuronal firing with specific sensory, motor or behavioral events [1].

Various methods, more recently involving the use of nanomaterials, have been explored to stimulate neural cells electrically [2–4]. Compared to these methods, using light to remotely control the events of cell signaling is attractive because of its non-invasiveness and flexibility in probing different locations [5,6]. Different photostimulation techniques have been used to evoke or stimulate neuronal firing and hence control activity in neural circuits. Photoactivatable caged compounds, such as the neurotransmitter glutamate, become active upon exposure to ultraviolet light. The temporal resolution of this approach can be limited as many caged compounds act for long periods once released [7] and selectivity is often poor since common transmitters act on many neuronal cell types [1]. Another photostimulation technique is to introduce natural light sensitive channels, found in single-cell algae and archaeobacterium, in neurons [5,8]; this approach has been very successful, but requires genetic manipulation of mammalian cells. Photoconductivity where light induces local electric fields has also been used for stimulating the cell membrane but requires a conductive substrate [9], which makes it unsuitable for *in vivo* applications.

Quantum dots offer an alternative. They have unique optoelectronic properties such as spectrally sharp emission peaks when excited and high quantum efficiency. Furthermore, their surface chemistry can be modified for selective attachment to biological particles. QDs have been integrated with cells and used in various applications such as fluorescent bio-labels for targeted imaging. Here, we investigate a new method of using QDs to control cellular activity and cell signaling through light excitation of ion channels. Photoexcitation of CdTe and CdSe QD films and probes integrated with cells caused activation of ion channels, leading to membrane hyperpolarization or depolarization.

2. Light in conjunction with QDs to stimulate cells

Introducing optically excited QDs near the cell membrane can perturb the electrochemical equilibrium between the inside and outside of the cell. Voltage-gated ion channels, protein channels which control the flow of ions between the exterior and interior of the cell, often modulate their conductance when the cell membrane potential changes. In other words, ion channels facilitate the diffusion of ions across the biological membrane, and in doing so control many basic cellular behaviors such as electrical excitability and synaptic transmission. Different classes of ion channels can produce action potentials when sufficient depolarization occurs (i.e. for positive changes in transmembrane voltage) or inhibit action potentials by hyperpolarizing the cell membrane.

Using light in conjunction with QDs to stimulate cells has advantages over other approaches. QDs are semiconductor nanoparticles with a 3-D confinement. This results in discrete energy levels and spectrally sharp emission when they are excited, and high quantum efficiency which leads to high sensitivity and absorption. Their surface chemistry can be modified for selective attachment to biological particles such as antibodies or peptides that recognize particular proteins. They can be injected to biological systems suitable for *in vivo* applications. In addition, QDs' physical size is comparable to the thickness of the cell membrane, which is about 3-4 nm, and the size of typical proteins.

We propose using QDs as effective photostimulators for cells. QDs have shown their ability in biological targeting and fluorescence imaging [10–12]. It has been possible to synthesize and achieve surface modification of QDs for *in vivo* cancer imaging applications using nanoparticle-based near-infrared fluorescence (NIRF) probes. QDs have overcome some of the limitations of other conventional NIRF techniques, such as organic dyes, for *in vivo* implementations. Insufficient stability in biological systems, low quantum yield, low detection sensitivity, poor hydrophilicity and photostability have been improved using QDs [11]. For photostimulation, QD fluorescence is not as significant as their other benefits found in sensitivity, stability, biocompatibility, surface-chemistry control, physical size comparable to the cell membrane and optical enhancement. Such benefits have been employed for exploring QD-Neuron interfaces for stimulation and voltage sensing [13–16]. The creation of an electric dipole moment from optically excited QDs and its associated electric field can perturb the cell membrane potential by changing the voltage immediately outside the cell. Cell stimulation will occur if the perturbation is strong enough to modulate a substantial complement of ion channels. QDs can be activated and deactivated by turning on and off the excitation light, and their surface chemistry can be modified to selectively bind them to specific biological particles to control spatio-temporal resolution in this photostimulation technique. The work reported in this paper provides additional evidence of QDs as photostimulators for cells by using different cells, QDs and integration methods than in past work [16].

3. Theoretical calculation of fields

Ion channels in the cell membrane control ion flux and can be turned on and off by changes in the membrane potential. These voltage-gated ion channels will open and permit ions such as Na^+ , K^+ or Cl^- to enter or leave the cell given sufficient membrane depolarization. Here we propose to modulate voltage-sensitive ion channels using photo-excited QDs placed close to the cell membrane, as shown in Fig. 1. The excited QD experiences electron-hole separation, which exhibits an electric dipole moment, and perturbs the cell membrane potential through a dipole-induced electric field. The photo-generated dipoles are aligned by the field from the cell membrane.

The strength of the electric field at the cell membrane will depend strongly on the proximity of the QDs to the membrane. The effect of QD stimulation on the membrane potential will also depend on the identity of ion channels near the QDs. A net depolarization

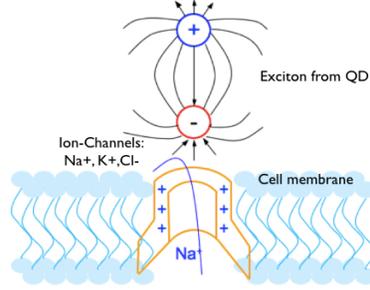


Fig. 1. Interaction of a QD with a cell membrane.

can occur if the electric field generated from each excited QD results in opening of Na^+ channels and inward movement of Na^+ ions. This will further depolarize the cell and can lead to action potential generation. Alternatively, hyperpolarization can occur if negatively charged ions such as Cl^- enter the cell or positive ions such as K^+ leave the cell.

Theoretically, the QD-induced electric field is related to membrane proximity as Eq. (1) described below, where ϵ is the permittivity of the medium, k is the wave number, \hat{r} is the unit vector between the dipole and where the field is measured, and \vec{p} is the vector dipole moment [17]:

$$\vec{E} = \frac{1}{4\pi\epsilon} \left\{ \frac{k^2}{r} \hat{r} \times \vec{p} \times \hat{r} + \left(\frac{1}{r^3} - \frac{ik}{r^2} \right) [3\hat{r}(\hat{r} \cdot \vec{p}) - \vec{p}] \right\} e^{ikr} \quad (1)$$

At optical frequencies, this relation can be considered as a superposition of electrostatic potentials from each electron-hole pair generated. Each charge q will generate an electric potential at a distance r from the cell membrane. Considering the total electric potential of point charges is the superposition of their potential, Eq. (2), where $q = 1.6 \times 10^{-19} \text{ C}$, r_1 is the distance between the closer point charge to the cell membrane, r_2 corresponds to the nanoparticle diameter plus r_1 , and ϵ is the permittivity of the medium; see Fig. 2(a). In free space without taking into account the losses of the cell medium, Fig. 2(b) shows potentials generated from an electric dipole versus the distance of the charge closer to the cell membrane, r_1 , and for different quantum efficiency. The potentials at $r_1 = 20$ and 50 nm , with 7 EHp (Electron-hole pair) generated per QD, are $V_t(r_1 = 20 \text{ nm}) \cdot 7 \text{ (EHp/QD)} = 0.876 \text{ V}$ and $V_t(r_1 = 50 \text{ nm}) \cdot 7 \text{ (EHp/QD)} = 0.378 \text{ V}$. The potential will drop exponentially by including the Debye length of normal saline $k_0 = 0.8 \text{ nm}$, Eq. (3). Potentials at 10 nm and 20 nm distances from the cell membrane will be screened from free-ions to 5.74 uV and 12.17 pV respectively. The coating on the cell and QD surface may result in further screening effect:

$$V_t = \frac{1}{4\pi\epsilon} \sum_i \frac{q_i}{r_i} \quad (2)$$

$$V = V_t e^{-r_1/k_0} \quad (3)$$

To evoke an action potential, the cell membrane requires a depolarization of $5\text{-}10 \text{ mV}$ from a resting potential of -40 mV to -70 mV . The negative sign comes from the convention to subtract the outer cell membrane potential from the inner cell membrane potential. Since at resting potential more positive ions are found outside the cell membrane, the result is a negative resting potential for the cell. As shown in the experimental results below, in some cases of using multi-layer QD films, sufficient depolarization can be achieved to reach action potential threshold.

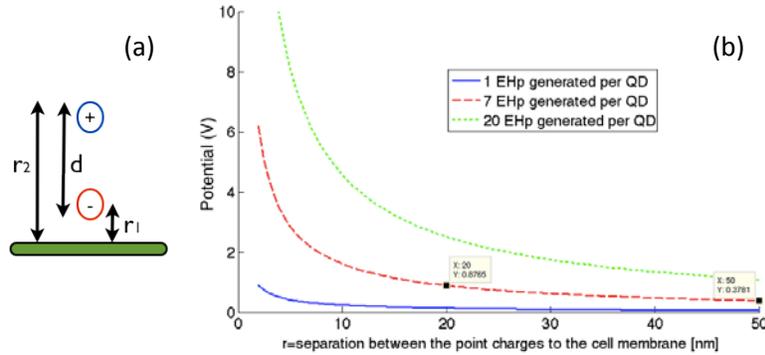


Fig. 2. (a) Definition of distances between charges and cell membrane. (b) Potential generated by electron-hole pairs in a quantum dot versus distance.

4. Experimental results

The general experimental approach is to illuminate the QD-cell system with excitable and non-excitable wavelengths to QDs and to record cells' voltage and current changes using the patch-clamp technique. We have proposed two approaches for integrating QDs with cells to monitor how QDs excitation affects electrical signaling. Using first cultured prostate cancer (LnCap) cells on CdTe QD films and secondly employing cultured neurons on CdSe QD films and CdSe QD probes.

LnCap cells were cultured in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum in a humidified 5% CO₂ atmosphere at 37°C. The QD film was sterilized and transferred to 12-well culture dishes. The QD film surface was coated with poly-lysine to enhance cell attachment, and cells were plated with 5×10^4 cells/well and incubated for 24-48 hours.

Cortical neurons from neonatal (postnatal day 0-2) mice were plated onto a bed of confluent astrocytes at a density of ~30,000 per well in 12-well plates, and cultured in Minimal Essential Medium (MEM) with B27 and 10% horse serum, as described previously for hippocampal cultures [18]. To prevent proliferation of astrocytes, mitotic inhibitor (5 mM 5-fluoro-2'-deoxyuridine and 12.5 mM uridine) was added to the neuronal cultures at 2 days in vitro. Experiments were performed according to the guidelines for the care and use of animals approved by the Institutional Animal Care and Use Committee at the University of Washington.

We tested a range of incident light intensities. In general we started to see the excitation effects for lights around 10^7 photons/um²/sec. Assuming an average QD cross-sectional area of 10 nm² [19] and quantum efficiency of 50% [20], the rate of EHp generation would be around 50 EHp/sec.

4.1. CdTe QD film and LnCap cells

CdTe QD films were fabricated using electrostatic layer-by-layer self-assembly [19]. A similar process has also been reported in [16]. QDs with opposite-polarity surface charges are required for this process. This is achieved by capping CdTe QDs with short ligands, 2-mercaptoethylamine (MA) for positive charges and thioglycolic acid (TGA) for negative charges. The synthesis process, described in [19], is performed in aqueous solution using cadmium perchlorate hydrate and Al₂Te₃. The two types of QDs have emission peaks at 620 nm and 580 nm respectively, with absorbance below 610 nm (Fig. 3(a)). Figure 3(b) shows a cultured LnCap cell on the QD film and a patch-clamp probe coming from the right to measure the cell membrane potential. Prostate cancer cells survived when cultured on the QD film.

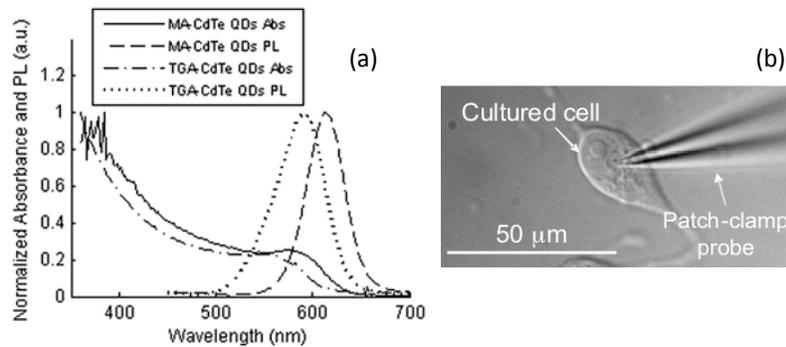


Fig. 3. (a) UV-visible absorbance and photoluminescence (PL) spectra of CdTe QDs [19]. (b) LnCap cell cultured on the CdTe QD film.

Illuminating the QD film with short-wavelength (430 nm) light from a mercury source hyperpolarized LnCap cells (Fig. 4). The membrane hyperpolarization likely was produced by activation of potassium channels, which are present at high density in prostate cancer cells [21]. The effect of QD stimulation depended on the proximity of the cells to the QD film; when the cells were lifted 20-30 μm above the film no changes in voltage with QD excitation were observed. Once the cells were attached solidly to the patch-clamp electrode, this could be achieved simply by moving the electrode. Furthermore, using a 740 nm light source (longer than the QD absorption cutoff wavelength), no cellular response was observed, confirming the role of QDs in stimulating the cell.

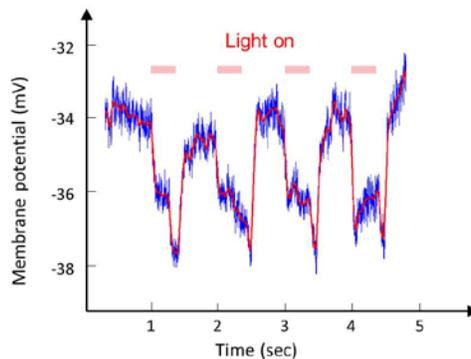


Fig. 4. Patch-clamp recording: effect of CdTe QDs excitation on membrane potential.

4.2. CdSe QD film and cortical neurons

We also tested the ability of QD activation to alter electrical activity of cultured cortical neurons. CdSe QD films and probes with an emission peak at 640 nm and absorption at wavelengths below 630 nm were used, see Fig. 5.

CdSe QDs from NN-Labs were drop-cast on an ITO substrate. Electrostatically-charged QDs are not required in this process. Cortical neurons were then cultured on the QD films. The QDs bind to each other through Van der Waals force, and the film is able to hold its integrity through the cell culture process. Excitation of the QD films by 550 nm wavelength light could cause the cell membrane to depolarize, evoking multiple action potentials (Fig. 6(a)). The cell membrane potential goes to its initial and resting state of about -60 mV after some delay during the process of repolarization before evoking another action potential. This is due to a delayed opening of K^+ channels triggered by membrane depolarization [22].

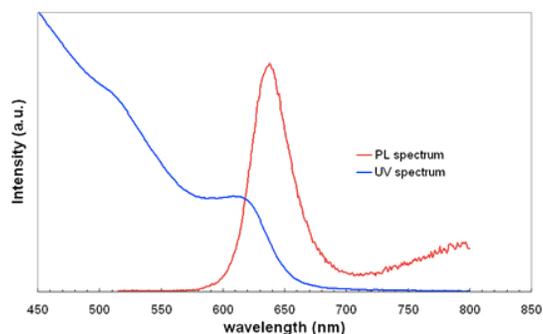


Fig. 5. UV-visible absorbance and PL spectra of CdSe QDs. (Figure source, www.nanocotechnologies.com.)

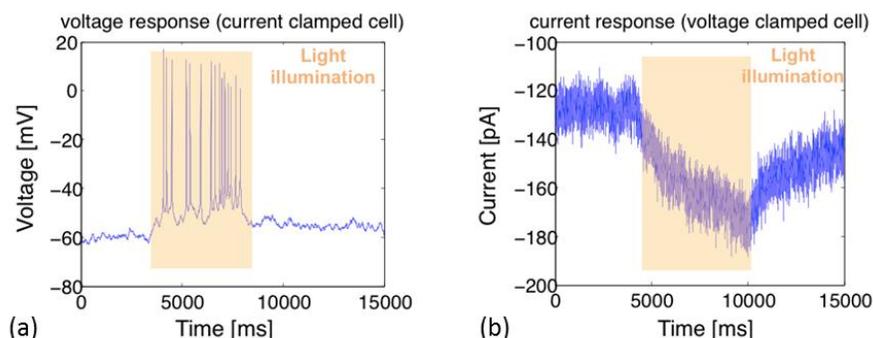


Fig. 6. (a) Current-clamped cortical neuron recording on CdSe QD film. (b) Voltage-clamped cortical neuron recording on CdSe QD film.

Figure 6(b) shows the change on current when the membrane voltage is held fixed during QD excitation. By convention, current flowing into a cell is inward (negative) current. Corresponding to the depolarization in Fig. 6(a), QD excitation produced an increase in inward current (Fig. 6(b)).

The effect of stimulation of QD films on electrical signaling of cortical neurons was quite variable, with some cells depolarizing and producing action potentials, as in Fig. 6, and others hyperpolarizing. Generally the health of neurons that were in direct contact with the QD film appeared compromised, which may contribute to variability in the responses to QD stimulation. We saw responses overall in about 10% of the cultured neurons we recorded from; of those showing a clear response 30% were depolarizing. We would like to note that there were days in which none of the cells responded, and days in which the majority responded. Most of the variability might be associated with how close the cells were to the substrate, and how healthy they were; these two placed competing demands on the culture conditions.

4.3. CdSe QD probe and cortical neurons

We also attempted to stimulate cultured cortical neurons using glass micropipettes with 5-10 μm diameter tips coated with CdSe QDs. This approach eliminated the need to culture on the QD film, and instead cells could be cultured in their conventional environment. Figure 7 shows the fluorescence image of a QD-coated micropipette under mercury-xenon light excitation. In the patch-clamp experiment, one probe coated with CdSe QDs was placed on the cell for light excitation while another one, without QDs, was used for electric recording.

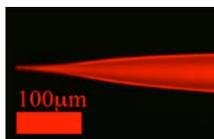


Fig. 7. Fluorescence image of a micropipette coated with CdSe QDs.

Figure 8(a) shows an example trace from one such experiment. The cell was voltage clamped to measure current changes produced by QD activation. With the voltage allowed to change, the effect of such a reduction in inward current would be to hyperpolarize the cell. Figure 8(a) shows the perturbation of the current on the neuron when the system is illuminated and excited QDs interact with the cell membrane potential. Illumination produced a decrease in inward current. To show the QD effects, we illuminate the cell-QD probe system with two wavelengths (one excitable and one non-excitable to QDs). An excitable wavelength ($\lambda = 550$ nm) produced a decrease in current (Fig. 8(b)), while a non-excitable wavelength ($\lambda = 720$ nm) longer than the QD absorption cutoff did not.

As for cells cultured on QD films (Fig. 6), we observed a good deal of variability in the responses produced by stimulation using QD probes. Stimulation of QD probes often produced little or no response. Variations in the distribution of different ion channels on the cell membrane, such as K^+ and Na^+ , and the conditions of the cells, could cause the variability in the effect of QD stimulation. Although data is only shown up to 5 seconds in Fig. 4, 15 seconds in Fig. 6 and 25 seconds in Fig. 8, responsive cells continued to respond to QD activation for periods up to 4-5 min. We did not record from cells for more prolonged periods.

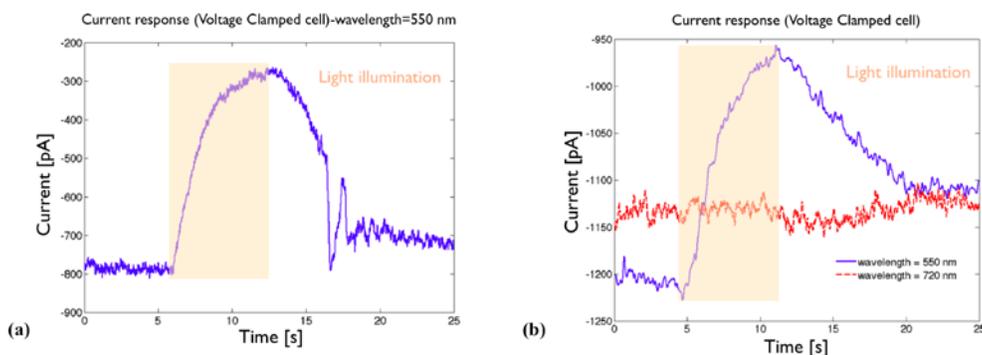


Fig. 8. Patch-clamp recording of CdSe QD-cell system on ionic currents. (a) Light excitation 550 nm and (b) light excitation 550 nm and 720 nm.

5. Conclusion

Using CdTe QD films with LnCap cells, CdSe QD films and CdSe QD probes with cortical neurons, we have demonstrated activation of voltage-gated ion channels through light. Hyperpolarization and depolarization have been achieved and observed with patch-clamp recording as a primarily result of activating K^+ and Na^+ channels. The fields produced by the photo-generated dipoles from the QDs perturb the cell membrane potential, which can generate action potentials that govern communication and signaling among cells. This non-invasive method for switching cell activity can target specific cells through protein binding by changing the QD surface chemistry.

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