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Optochemical nanosensors for intracellular chemical measurement

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ABSTRACT

The development of a submicron optical fiber “supertip” has provided advantages over previously produced submicron tips, such as facilitating insertion of these sensors into cells while minimizing damage to the cell membrane. Fiber optic ion correlation-based nanosensors for sodium, potassium and chloride employing these “supertips” have been applied to the monitoring of ion concentrations in single mouse oocytes. These sensors have also been used to monitor the effect of an ion channel-blocking agent. In order to address the challenge associated with single-cell simultaneous measurement of multiple analytes, the use of submicron optical fiber multiprobes has been explored.

Keywords: optodes, chemical sensors, fiber optic, multiprobe, intracellular measurements

1. INTRODUCTION

Single cell measurements can provide crucial information about biological processes and biochemical distributions that cannot be obtained from bulk tissue analysis.\textsuperscript{1} Submicrometer sensors\textsuperscript{2-5} provide one method for real-time intracellular measurements. To be effective, these sensors must be highly selective, have fast response times, and cause minimal cellular damage.

Submicrometer fiber optic sensors were first developed\textsuperscript{5-8} using pulled optical fibers, similar to those utilized for near-field scanning optical microscopy,\textsuperscript{9} and a polyacrylamide matrix. Use of these submicrometer fibers was then extended to use with poly(vinyl chloride), allowing decreased effects of photobleaching\textsuperscript{10} and detection of a greater number of analytes.\textsuperscript{11}

Here we present improved submicrometer fiber optic sensors and their application to the detection of various intracellular analytes. The fiber tip shape has been redesigned to be less invasive and easier to insert into a cell. With this new “super tip”, potassium, sodium, and chloride have been measured in viable single cells. In addition, a submicron multiprobe has been developed for simultaneous detection of multiple analytes.

2. EXPERIMENTAL

1. Reagents

Poly(vinyl chloride) (PVC), potassium tetrakis [3,5-bis(trifluoromethyl)phenyl]borate (KTFPB), chromoionophore I (ETH 5294), chromoionophore II (ETH 2439), chromoionophore III (ETH 5350), potassium ionophore III (BME-44), bis(2-ethylhexyl)sebacate (DOS), and 2-nitrophenyloctyl ether (o-NPOE) were all obtained from Fluka. Tetrahydrofuran (THF), acrylamide, N,N-methylenebis[acrylamide], and triethylamine (TEA) were received from Aldrich. Kainic acid, M2 medium and M16 medium and Sigmacote were obtained from Sigma. 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), Calcium Green-1 hexapotassium salt, and Texas Red sulfonyl chloride were received from Molecular Probes (Eugene, OR). Indium(III) octaethylporphyrin chloride, (In(OEP)Cl), was obtained from Midcentury (Posen, IL). The procedure described by Yamamoto and coworkers\textsuperscript{12} was used to synthesize the 1,3-bridged calix[4]crown sodium ionophore.
2. Optode film preparation

All film components were dissolved in freshly distilled THF. The exact film compositions are as follows: The potassium-sensitive film contained 3.2 mg of BME-44, 1.5 mg of KTFPB, 0.9 mg of ETH 5350, 70.4 mg of DOS, and 34.6 mg of PVC. The sodium-sensitive film contained 2.9 mg of 1,3-bridged calix[4]crown, 1.9 mg of KTFPB, 1.1 mg of ETH 5294, 76.1 mg of DOS, and 38.1 mg of PVC. The chloride-sensitive film contained 2.8 mg of In(OEP)Cl, 1.8 mg of KTFPB, 1.5 mg of ETH 2439, 130.4 mg of o-NPOE, 62.1 mg of PVC, and 28 µL of 1.4 mM Dil solution.

3. Optodes

Liquid polymer-based sensors were fabricated with all-silica, single-mode optical fiber with a 3 µm core and an 80 µm cladding diameter (Thor Labs, Newton, NJ). Fibers were pulled to submicron size and aluminum-coated according to a modified procedure for preparation of near-field scanning microscopy light sources. The optodes were prepared by dipping the pulled end of the optical fiber repeatedly (~5 times) into the membrane cocktail solution. The film thickness for optodes prepared in this manner is typically less than 300 nm.

4. In vitro application

Mouse oocytes were obtained from the Transgenic Core Facility at the University of Michigan, where they were harvested from superovulated C57BL/6 mice. They were maintained in M16 medium in a 5% CO2, 37°C NuAire incubator (Plymouth, MN). For sensor studies, a single oocyte was transferred by micropipette to a 200 µL drop of M2 medium on a Sigmacote-treated Petri dish. The dish was then filled with mineral oil and placed on the stage of an inverted microscope (see below) mounted on a vibration-isolated optical table. A micropipette manipulated by a three-axis positioner (see below) was used to immobilize the oocyte over the microscope objective. The distal end of a liquid polymer-based optode was attached to a micromanipulator (Newport Model 150) so that it could be inserted into the oocyte in a precise manner. The proximal end of the optode was coupled into an argon ion laser (see below) exciting at 514.5 nm.

5. Photopolymerization solutions

Two distinct photopolymerization solutions were prepared. Solution 1 contained 0.04 mM Calcium Green-1, 36% acrylamide, and 4% N,N-methylenebis[acrylamide] in 0.1 M phosphate buffer, pH 6.5. Solution 2 contained 0.3 mM Texas Red, 36% acrylamide, and 4% N,N-methylenebis[acrylamide] in 0.1 M phosphate buffer, pH 6.5.

6. Multiprobes

Submicron optical fiber multiprobes were provided by Physical Optics Corporation (Torrance, CA). They are comprised of three multimode optical fibers that have been bundled together at their distal ends and pulled down to a submicron tip. This distal tip is silanized for 90 min in a 2% solution of 3-(trimethoxysilyl)propyl methacrylate in pH 3.45 water and then dried in air. The silanized end, supported by a three-axis positioner (Newport 460A Series), was placed in a small Petri dish containing 500 µL of polymerization solution and 20 µL of TEA on the stage of an inverted microscope (see below). The proximal ends of two fibers of a multiprobe were coupled into an argon ion laser (see below) exciting at 488 nm. Polymerization at spatially distinct regions on the multiprobe tip was controlled by allowing the laser beam to enter only the fiber of interest, while physically blocking the beam coupled to the other fiber. Polymer formation was monitored by collecting fluorescence signal originating from the multiprobe tip. The fabricated sensors were dried and stored in air.

7. Experimental configuration

A schematic of the complete optical path and the sample stage has been previously reported. The setup includes the following: Ion Laser Technology (Salt Lake City, UT) argon ion laser and 488 nm or 514.5 nm laser band-pass filter (Newport, Irvine, CA); neutral density filters (Melles Griot); Uniblitz shutter controller (Rochester, NY); fiber coupler (Newport F-915 Series); Olympus inverted fluorescence microscope, IMT-II (Lake Success, NY); Nikon 50 mm f/1.8 camera lenses; Acton 150 mm spectrograph (Acton, MA); and a Princeton Instruments 1024 x 256 LN2-cooled CCD array (Trenton, NJ). A beam-splitter (Melles Griot) and second fiber coupler were added to the setup for the multiprobe experiments.
3. RESULTS AND DISCUSSION

1. Intracellular studies

Several ion-selective fiber optic chemical sensors have been previously developed for use in intracellular applications.11,14,15 The principle of operation of these liquid polymer-based sensors has been detailed elsewhere.16 Previous reports have also investigated the dynamic range, selectivity and response time of each of these sensors. A recent modification of these sensors that has been found to be beneficial to intracellular applications is a change in the shape of the nano-optode’s pulled fiber tip (see Figure 1). The previous tip shape tended to damage the cell upon insertion of the sensor, as it required that a relatively large hole be made in the cell membrane. The new “supertip” shape has a longer taper, which allows the sensor’s insertion to produce a smaller hole in the membrane. In the present report, there are two intracellular studies presented. In the first, sensors selective for potassium, sodium, and chloride have been applied to intracellular ion measurements in a single mouse oocyte. The second monitors the affect of an ion channel-blocking agent on changes in intracellular ion concentrations.

In the first study, measurements were made with each of the three distinct sensors, first in the culture medium, then inside the cytoplasmic space of the mouse oocyte. The data from these measurements are shown in Figures 2 and 3. The response of ion-correlation optodes is a function of the activity of the analyte ion and pH. For cation selective sensors, the response is a function of the ratio of the analyte cation activity to the hydrogen ion activity (i.e. $K^+/H^+$). For anion selective sensors, this response is governed by the product of these activities (i.e. $Cl^-H^+$). Intracellular levels of sodium are reported to be lower than those in the medium while intracellular potassium and chloride levels are reportedly higher.17 Nanosensor measurements indicated a decrease in the $Na^+/H^+$ when the sensor was inserted into the oocyte and an increase in the $K^+/H^+$ and the $Cl^-H^+$. The system was then perturbed using a stimulant, kainic acid, which is known to open ion channels and depolarize the cell. Indeed, a decrease in the $K^+/H^+$ was seen upon addition of kainic acid. An increase in the $Na^+/H^+$ and the $Cl^-H^+$ were also observed in the presence of kainic acid. Even though each ion measurement is dependent on pH, when the data were compared to one another, they did not follow a trend indicative of a simple pH change.
In the second set of experiments, potassium channels of some mouse oocytes were selectively blocked using TEA. Potassium fiber sensors were inserted into mouse oocytes treated with TEA or into untreated oocytes, and the cells were then stimulated using kainic acid in the presence of sodium chloride, calcium chloride or potassium chloride. The influx of ions from the extracellular space results in rapid depolarization of the membranes of excitable cells. Figure 4 shows intracellular responses to sodium chloride, calcium chloride and potassium chloride monitored by the potassium fiber optode in the cytoplasm of a mouse oocyte in the absence of potassium channel blockers. The changes in $K^+/H^+$ following addition of sodium chloride, calcium chloride and potassium chloride are real-time measurements of ion flux following depolarization of the cell. The large signal change in response to low levels of $K^+$ with respect to smaller signal changes induced by high levels of $Na^+$ and $Ca^{2+}$ indicates that the potassium nano-optodes were highly selective for potassium at physiologically relevant concentrations of sodium and calcium. In the future, relationships between $K^+$ and $H^+$ need to be determined.
Figure 4. Measurement of potassium/hydrogen in the cytoplasm of a mouse oocyte without a potassium-channel blocker (TEA). Values represent the ratiometric measurement of ion activities in the cell culture medium, inside the oocyte, then inside the stimulated oocyte in the presence of NaCl, CaCl$_2$, and KCl, respectively.

Figure 5 shows intracellular responses monitored by the potassium sensor in the presence of TEA. Note that depolarization of the cell and the influx of sodium and calcium ions did not yield a change in the response of the potassium sensor. In addition, treatment of the cell with potassium chloride did not produce the intracellular response observed in the absence of TEA. The lack of response after treatment with potassium chloride confirms cell viability and successful blockage of potassium channels in the oocyte.

Figure 5. Measurement of potassium/hydrogen in the cytoplasm of a mouse oocyte treated with 6 mM TEA. Values represent the ratiometric measurement of ion activities in the cell culture medium, inside the oocyte, then inside the stimulated oocyte in the presence of NaCl, CaCl$_2$, and KCl, respectively.
In summary, we have observed three distinct analytes with fiber optic probes, inserted one at a time. It is straightforward to generalize this to many additional analytes, such as nitrite and pH. However, it gets increasingly challenging to insert more than two fiber sensors at a time into an oocyte (see Figure 6). A better solution presents itself through the use of optical fiber multiprobe sensors.

![Figure 6. Two fiber optic nano-optodes inserted into a single mouse oocyte.](image)

2. Multiprobe experiments

In an effort to work toward simultaneous multianalyte measurement, preliminary investigations have begun employing optical fiber multiprobes. The design of these multiprobes allows each fiber to deliver light signal to a corresponding location at the multiprobe’s submicron tip (see Figure 7). Photopolymerization at different tip locations was accomplished by sequentially coupling different fibers of one multiprobe to an argon ion laser. Dyes having different emission spectra were entrapped within the polymer at different tip locations in order to determine the extent of cross-talk in the completed sensor. Early data reveals only minimal cross-talk between two fibers of a three fiber multiprobe (see Figure 8). However, further studies have suggested wicking (via capillary action) of the dye-containing photopolymerization solution into cavities of some multiprobes, which could contribute to the delocalized fluorescence signal that has been observed. These problems must be addressed so that fluorescence signal from various ion-sensitive dyes can be selectively excited. This selective excitation is prerequisite for intracellular multianalyte detection to be possible.
Figure 7. SEM of optical fiber multiprobe submicron tip. Scale bar represents 1 μm.

Figure 8. Multiprobe sensor with sensors polymerized through two distinct fibers of the three fiber bundle. Fiber 1 corresponds to the region of the multiprobe tip where Calcium Green-I was entrapped, while Fiber 2 corresponds to the region containing Texas Red. The two regions are < 500 nm apart.
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5. REFERENCES