"Intracellular Electric Field Sensing using Nano-sized Voltmeters"

Intracellular electric fields are of great interest because of the important role they play in the biological processes of a cell. It is known that intracellular electric fields play a major role in sub-cell processes like cell division and DNA uptake, as well as on the macroscopic level of neural signaling and cardiac rhythm. The ability to study these electric fields, until recently, was limited to the use of voltage-sensitive dyes, voltage/patch clamps, fluorescent proteins, and fluorescent energy transfer methods. These methods are membrane-dependent and are limited to only detecting electric fields in/near organelles in intact cells (which account for less than .1% of entire cell volume). Electric fields permeate the entire cell, not just near and in the organelles, and in order to study and model certain biological processes more effectively, the electric field throughout the entire cell volume must be mapped. Also, more and more studies are currently underway investigating the effects of an externally applied electric field to a cell's biology. It has been proven that external electric fields can be used to modify certain cell behaviors. However, with the aforementioned methods, detection of the intracellular electric field's response to an external field is limited both spatially and temporally.

The invention and implementation of E-PEBBLES solves many of the issues associated with conventional cellular voltage measuring techniques. E-PEBBLES are nanosized voltmeters that can diffuse throughout the entire cell volume. The E-PEBBLES contain a voltage sensitive dye whose optical spectrum shifts according to the electric field present. The response time of the optical shift to a change in the surrounding electric field is instantaneous and thereby gives a real-time measurement of biological processes going on within the cell. The conventional voltage sensitive dyes measure voltage slightly differently. Once in the cell, the positively charged dye molecules diffuse across the cell along the electric field gradient toward the area of higher negative charge (usually to mitochondria). However, if the system is perturbed (i.e. by adding a toxin) resulting in the decrease of the electric field, the diffusion process reverses, but very slowly. Thus, this class of sensing dyes is classified as "slow response" and is virtually ineffective in measuring a cell's real time response to stress.. Furthermore, voltage dyes tend to be sequestered selectively by cell compartments such as membranes. The E-PEBBLES on the other hand, are not only "fast-response" voltage sensors, but they can also be adapted to measure other physical parameters within the cell including temperature, pH, and viscosity.

Another major advantage of E-PEBBLES is that they don't require recalibration once inside the cell. Calibration of sensors used in intercellular voltage measurements is a major issue for all of other methods. Once the sensor is introduced to the cell's environment, there is some variation from cell to cell and in depth calibration schemes are needed to extract viable data or rid it of significant inaccuracies. The voltage sensitive dye used in the E-PEBBLES is encapsulated in a silane-capped micelle which provides a universal environment for the sensing dye, no matter what type of cell, cellular structure, or non-cellular environment the particle may be exposed to. This environment, along with only one required calibration, allows for a universal baseline for all measurements, no matter the nature of the specific experiment.

The initial experiments completed with E-PEBBLES are very successful on every front; however, the calibration scheme needs to be improved to allow for even higher electric field measurements. Currently, in order to calibrate the E-PEBBLES they are put in an aqueous suspension or gel, and then loaded into a glass capillary. The capillary is attached to a cover slip and a pair of wires connected to a voltage supply using clear varnish and silver paint. Various voltages are applied to the E-PEBBLES and the emission images are collected and analyzed. Only voltages up to 1250V are able to be applied to the E-PEBBLES because once this voltage is surpassed the calibration device can short or arch, or the capillary can melt due to heating. Thus, the calibration curves used in the analysis of the cell data necessarily omits electric fields of more that $1.5(10^6)$ V/m. Also, because of the very nature of the calibration setup, there are significant variations in the slopes of the individual calibration curves, although overall the curves are all similar. The dye and the E-PEBBLES will most certainly work in the higher electric fields (up to 10^7 V/m), thus the need for a better calibration method is apparent.

Better calibration techniques are currently being investigated, and the calibration curves used previously are being checked for their validity; thus, the goal now is to follow changes in the intracellular electric field as a cell undergoes natural processes or is exposed to physical and chemical stress with the use of confocal microscopy. The intracellular electric field's role in cell reproduction is not well understood, and of particular interest is the behavior of the electric field during cell division. The E-PEBBLES will allow the mapping of the intracellular electric field throughout the entire cell volume for the duration of the life cycle of a cell; mouse glioma or other cancer cells will be the first candidates for this type of study. Likewise, E-PEBBLES can be introduced into a cell and then through the use of chemical toxins and temperature, the immediate effects of these chemical and/or physical perturbations to the cell's biology can be studied in real time and for many types of biological cells. From these types of studies invaluable information will be gained about a cell's ability to readjust to its biological equilibrium and will be instructive in understanding what happens when a cell's biological functions are perturbed.

The experimental promise of E-PEBBLES lies in the fact that they are long-lasting and have been shown to be insensitive to pH, oxygen gradients, reactive oxygen species, and temperature. With the implementation of a more reliable calibration scheme for the E-PEBBLES, their adaptation into selective targeting sensors and/or sensors that can track temporal changes in electric field, temperature, and viscosity in both biological and non-biological systems is more realizable. The next step in E-PEBBLES sensing could be experiments that follow the changes in the intracellular electric field as the cell undergoes natural processes or when the cell is exposed to internal stress, such as due to cell division. Changes in a cell's electric field can also be an indication of changes in a cell's normal functioning and has been observed in Alzheimer's disease or as a precursor to cell death. E-PEBBLES hold the promise of not only being experimental tools for sensing biological processes within a cell, but may eventually become medical diagnostic tools.