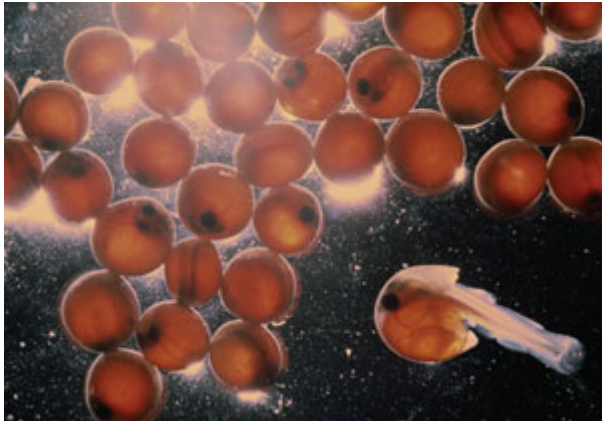


## TECHNOLOGY

### Fish Eggs Spawn a DNA Delivery Revolution

*Caviar inspires a Berkeley bioengineer to take electroporation to the single-cell level*

By **Laura Lane**



Atlantic salmon seems an unlikely source of inspiration for a research gadget. Yet thanks to the fish – and the efforts of University of California, Berkeley researcher Boris Rubinsky – scientists have greater and more precise control over the delivery of nucleic acids to individual cells.

For years, Rubinsky watched technicians spend weeks at a time injecting individual salmon eggs with gene constructs to create transgenic fish that would grow year round and not only during the summer. The only alternative was to place all the eggs in one vessel between two electrodes and "hope for the best," says Rubinsky. But only about 20% of the eggs actually ended up with the gene constructs using this batch electroporation technique. Electroporation is a process in which a high-voltage current is used to open pores in the cell membrane in order to introduce nucleic acids, proteins, or other membrane-impermeable molecules. "The difficulty with these salmon was the inspiration to develop something with 100% success."

Unthinkable with bulk methods, such a lofty goal is now attainable thanks to new instruments that transform the electroporation of single cells from complexity to simplicity. The technology provides an extraordinary level of access to the cell's interior. And that spells big relief for the many researchers trying to insert everything from small oligonucleotides, to large proteins, to therapeutic genes into the cell.

"What we now have is the ability to manipulate individual cells under real-time control," says Rubinsky, distinguished professor of bioengineering at Berkeley. "We can adjust the electrical parameters to open and close the membrane at will with our fingertips. That's a powerful tool for studying this whole field of cell manipulation."

## PERMEATING FINDINGS

Scientists have known since the late 1960s that electric fields can induce the cell membrane to break down. They then learned that a prescribed set of electric pulses could restore the membrane's integrity and stop intracellular components from hemorrhaging. But it wasn't until the early 1980s that Eberhard Neumann, now of the University of Bielefeld in Germany, applied the phenomenon to genetic engineering.<sup>1</sup>

Researchers still don't know exactly how electrical pulses induce the cell to become permeable to substances that would otherwise not be able to infiltrate the membrane.<sup>2</sup> Nor does anyone know precisely how its structure rearranges to form so-called "pores" that are big enough for bulky plasmids to pass through and hospitable enough for charged molecules to enter. What researchers do know is that the electric pulses must be strong enough to cause permeabilization but not so intense that the membrane fails to reseal and the cell dies.

"The major problem with electroporation is the stress on the cell," says Justin Teissie, director of research at the Institute of Pharmacology and Structural Biology at CNRS in France. "With all the electric pulses, cell contents leak out, reactive oxygen species form. We must protect the cell from electroporation, which is really toxic for cells."

Teissie, a pioneer in electroporation methods, says transfection efficiency and cell viability depend on several parameters. In addition to the intensity of the electric field, the frequency and pattern of the electric pulses must be optimized to allow enough time for plasmids or other molecules to enter, and yet also be short enough to prevent the cell from losing its contents and absorbing too much buffer, which can cause irreversible damage to the membrane. Buffer chemistry, temperature, and other factors must also be tested with each cell type and size.<sup>2</sup>

It's a frustration that researchers are willing to face when they've ruled out all other transfection methods. The two other physical methods for single cell transfection – injection, and the biolistic approach, or the so-called "gene gun" – offer high rates of transfection but also long hours at the bench.<sup>1</sup> Biological strategies, such as liposomal and viral vectors, suffer from delivery issues and ensuring that these reach the target cells.

## SINGLES SCENE

Last year Axon Instruments of Union City, Calif., introduced to the market its Axoporator 800A (\$5,800 US), an electrical stimulator designed specifically to electroporate single cells (Axon was acquired by Sunnyvale, Calif.-based Molecular Devices in July 2004).

The Axoporator's electrode placement makes it unique, says Owe Orwar, chief scientific officer at Celectricron of Goteborg, Sweden, who licensed the technology to Axon. Hooked up to the device, the electrode is housed in a glass micropipette. This configuration keeps the electrode from getting too close to the cell and prevents electrochemical byproducts from harming the cell. While one micropipette can remain fixed anywhere in the solution, the other must be manually positioned in close proximity to each cell on the stage of a microscope, which guides the pipette's placement.<sup>3</sup> "The yield is extremely high and the methodology is efficient," Orwar says. "But it's one cell at a time."

Once positioned against a select portion of the cell, the micropipette produces an electric current with a flow of ions and the molecules intended for delivery. "This is a big advantage," Orwar said. "The consumption of reagents is much lower because you only need enough to put into the one cell."

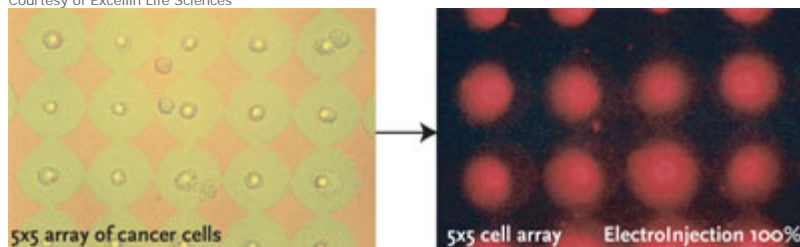
"The Axoporator offers great functionality," says neuro-scientist Kurt Haas, an expert in single-cell electroporation (SCE) and professor of neuroscience at the University of British Columbia in Vancouver, who uses the Axoporator in his research. "It's as easy as you can get."

Nevertheless, Celectricron is developing a higher-throughput variant of the instrument, says Orwar. Slated to launch this December at the American Society for Cell Biology's annual meeting, the Cellaxess simultaneously electroporates cells in a 96-well plate. Though each well contains tens of thousands of cells, the device functions on the same principles as the Axoporator. The cells are electroporated in an adherent state, such as on a plate, so the cells retain their natural morphology and position in relation to each other. Celectricron has yet to set a price for the device.

## GROWING FAMILY

Excellin Life Sciences of Menlo Park, Calif., plans to release its own SCE device, the CellArray, in late 2006. Based on Rubinsky's research, this chip-based instrument lets researchers verify transfection, in addition to enabling downstream assays and in situ detection of their results.

Courtesy of Excellin Life Sciences



**ZZZAP!**

A 5 x 5 array of cells shows 100% uptake following electroinjection with the reporter, EthD-2. Excellin Life Sciences is now developing a high-throughput version of the technology that will deliver nucleic acids to millions of cells at once.

"It's perfect for the situations when you have precious few cells," says CEO Laura Mazzola, pointing to the increasing use of stem cells in research and the need, in clinical diagnostics, to make the most of the limited number of patient cells.

Rubinsky's work created a system that releases pulses of increasing electrical intensity into a chamber containing a single cell. The pulses level off when the system detects current flowing through the cell, indicating that the membrane is permeable. Instead of the 1,000 or so volts delivered via traditional bulk electroporation, the CellArray requires only a few hundred millivolts. As the membrane reseals, the system monitors the cell for current flow, providing direct feedback that the cell has survived the invasive procedure.

Mazzola says each CellArray chip will ultimately contain either 25 cells in a five-by-five array or 100 cells in a 10-by-10 format. "When we scale up from 25 cells to 25 million cells, our efficacy and viability remains in the high 90s," says Mazzola, referring to the company's high-volume electroporation system slated for release in early 2006. The company calls the process electroinjection, and "its sensitivity hinges on the scalable, tunable Rubinsky technology." (No price has been set yet for the CellArray chips.)

In the laboratory of Luke P. Lee, assistant professor of bio-engineering at Berkeley, graduate student Michelle Khine recently developed an SCE chip as part of her doctoral studies. Hooked up to syringe pumps to push cells through, the chip consists of multiple lateral channels, each of which can trap a small portion of one cell, Khine says. This small, trapped area can then be made permeable with a few hundred millivolts.

The cells can also be readily monitored both optically and electrically, she says. Formed of transparent polydimethylsiloxane, the chip allows fluorescent or other probes inside the cell to be visually detected, while the cells' electrical resistance can be monitored to observe when the pores open and close.

Khine, who will graduate this summer, continues to refine the chip design while also making plans for an automated chip-processing instrument. She is optimistic about this technology's promise to dramatically improve the efficacy of transfection and drug discovery, and is keeping the market in mind as she designs.

## IN VIVO FUTURE

Other companies have also considered transfection's value in the clinic. San Diego-based Inovio Biomedical Corp. and Gaithersburg, Md.-based MaxCyte, for instance, have developed flow-through systems that electroporate cells by the hundreds. Based on Teissie's work, among others, these systems provide a high-throughput way to transfect cells with therapeutic genes at rates that rival SCE, company executives say.

### Selected Suppliers

**Axon Instruments**[<http://www.axon.com>]

**Cellectricon**[<http://www.cellectricon.com>]

**Excellin Life Sciences**[<http://www.excellin.com>]

**Inovio Biomedical Corp.**[<http://www.inovio.com>]

**MaxCyte**[<http://www.maxcyte.com>]

"We've been able to develop a very large-scale clinical process that can match a few of the performance characteristics of SCE and apply it to cell volumes that are relevant for human therapeutic purposes," says Doug Doerfler, CEO of MaxCyte, which is currently involved in several clinical trials in oncology and cardiopulmonary disease.

Currently focused on developing in-house therapeutics, MaxCyte is not now distributing its electroporation system broadly, Doerfler says. But further out, MaxCyte intends to first market the device to leading cell therapy centers at teaching hospitals and commercial laboratories, he says. The company also licenses its technology to other companies who are working on ex vivo cell-based therapies, but the financial terms of these arrangements are confidential.

Inovio and its rivals are taking their cues from the work of Luis Mir, director of research at CNRS of France's Institut G. Roussy, who first introduced in vivo electroporation for drug therapy. Currently in Phase III clinical trials for head and neck tumors, Inovio's MedPulser system inserts needle electrodes directly into tumors to make cancer cells more permeable to the chemotherapeutic agent Bleomycin.

The company is also focusing on making muscles more permeable to genes that treat infectious diseases and cancer DNA vaccines, says Dietmar Rabussay, Inovio's vice president of research and development.

Currently at the precommercial stage of development, Med-Pulser does not yet have a set price, says Bernie Hertel, Inovio's director of corporate communications. But use of the MedPulser will require purchase of a single-use applicator that contains the electrodes; for most cancers, that applicator will cost \$2,000.

### References

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2. M Golzio et al, "In vitro and in vivo electric field-mediated permeabilization, gene transfer, and expression," *Methods* 2004, 33: 126-35. [[PubMed Abstract](#)][[Publisher Full Text](#)]
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