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Designing Nanostructures at the Interface
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Building a Cell-Chip Interface to Sense Response to Drug Leads and Toxins
Focus Group Summary

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Summary:

Imagine being able to take measurements on a single cell, to get accurate readouts that overcome the immense challenges of low signal outputs and interference, with a precision and an effectiveness that allows for early detection of disease and readily gauges the effectiveness of new drug therapies.

In three days of intensive roundtable discussions, an 18-member focus group discussed how to turn that vision into a reality.

With expertise in all of the applied sciences, from cell biology to electrical engineering to biophysics, the group pooled its knowledge and laboratory experience to brainstorm, debate, and mull over a macroscopic answer to a very microscopic problem.

“What if we made it our goal to come up with a laboratory technique that penetrated every biology lab in the country?” asked William King, an assistant professor of mechanical engineering at the Georgia Institute of Technology.

The concept of extracting information from cells is not new. For decades, electrophysiologists have prodded and poked at cells, extracting limited amounts of data on physiological cell states and processes. But the focus group felt that the envelope could be pushed further, that a “nanoprobe” could be created to garner virtually unlimited data from a single cell.

The first task: defining the problem. Because the assignment as presented was intentionally vague, group members had their own interpretations and preconceived notions about how to tackle it. The group drifted into lengthy discussions of existing technologies and how such technologies could fit into a master plan for this project. Microarray technology, which essentially places a human on a chip, was initially viewed as key to building a cell-chip interface, as were standard techniques for measuring protein concentrations and other physiological states inside a cell.

Eventually, the question came back to nanotechnology and how it could be used to find a new solution to an old problem. What more could nanotechnology offer that existing technologies did not already offer?

The group continued to dissect the assignment, next questioning the value and practicality of taking measurements on a single cell. Would interrogating individual cells be possible? Could it be done *in vivo*? Would it be more useful to focus first on an *in vitro* model? Given federal laws governing scientific research on human beings, the group realized that an *in vitro* model would be a more practical approach, at least initially.

But even working with cells in a laboratory comes with its own set of problems, as group members pointed out. The finicky nature of human cells, along with the challenge of artificially inducing sickness or disease, led some to question the necessity of using human cells at all.

“If we can build up an artificial cell, then we won’t have any problems delivering nanoprobes,” said Dan Luo, an assistant professor of biological and environmental engineering at Cornell University.

Edward Sargent, an associate professor of electrical and computer engineering at the University of Toronto, liked the idea, pointing out that one could remove the contents of a cell, then add organelles back one at a time for greater control over an experiment.

But Barbara Baird, director at Cornell University’s Nanobiotechnology Center, pointed out that artificial cells are very different from real cells. “I don’t think anyone would believe an artificial cell would be a good model,” Baird said.

As group members continued to brainstorm and debate, the focus of the discussion gradually began to shift to the next major task: conceptualizing the nanoprobe and its potential – and potential limitations.

Group members agreed that the nanoprobe should act like a camera, taking “snapshots” of the cell at given points in time. Yet what would the nanoprobe measure and report back?

Because the goal of the probe was to detect disease, the group realized that they would first need to know disease expression profiles – that is, the proteins expressed in diseased cells. But scientists do not know expression profiles of all diseases, especially at the intracellular level. Compounding the problem, as some group members pointed out, would be the challenge of designing a probe specific enough to detect only the expression profiles of interest.

Others pointed to the difficulties of interpreting the profiles, and that was assuming such data could be obtained in the first place. The group agreed that a baseline must first be established to gauge changes to a cell in response to single or multiple challenges. However, given cell-to-cell variability, the group acknowledged the enormous challenge of developing accurate baseline readings for a single cell.

Also brought up was the question of scale – that is, how big the nanoprobe would be. The group agreed that the nanoprobe should package the maximum number of parameters into a minimum amount of space. However, figuring out how many parameters could fit into a single probe was not something group members could resolve until they knew what the parameters were going to be.

Then there was the practical question of what the probe would be made of. Would it be encapsulated? If so, how? Resolving these manufacturing questions, the group realized, was not feasible in the time allotted.

Some group members, for the sake of discussion, suggested 10 nanometers as the theoretical diameter of the nanoprobe and asked the group whether a cell could take it up. Their colleagues had the answer: with an average diameter of 10 micrometers, a cell easily could take up a 10-nanometer probe.

With this discussion came the question of whether the probe should enter the cell like a submarine or take all of its readings from the outer surface of the cell membrane. Some group members also asked whether it would be necessary to get the probe back out. Everyone agreed that keeping the probe outside the cell would be easier and pose less of a problem, but the group also realized that far more data could be gathered if the probe also entered the cell.

The answer, then, at least from a theoretical basis, was simple – the probe would almost certainly have to enter the cell. But would it be just one probe? The group discussed the possibility of a number of different probes, some inside the cell and others outside. These probes, supplemented by other cell-targeted techniques, such as dielectric constant spectroscopy, stretching, and compressing, were all viewed as potentially useful in extracting the maximum amount of data from a single cell.

The next logical concern was whether the nanoprobe might have perturbations on normal cellular processes and affect the readouts. A nanoprobe measuring 10 nanometers in diameter would only take up about a billionth of the volume of the average mammalian cell, but because cells have no free space inside, group members pointed out that any volume added to the cytoplasm could have detrimental effects. However, others pointed to the inevitability of invasiveness and advised against dwelling on it.

Also discussed was a viable method of signal detection, given the small size of the nanoprobe and its consequently small signals. Group members pointed out the pros and cons of chemical, electrical, magnetic, and fluorescent signals. They debated using different combinations of these signals and discussed how best to amplify the signals.

Some suggested that detecting tiny signals would be accomplished most effectively by using fluorescent dyes instead of using such instruments as mechanotransducers and magnetic detectors. Others pointed to the efficacy of indicators that track everything from DNA methylation states and gene mutation to cell cycle checkpoints and viscosity.

Throughout the focus group discussions, each issue discussed seemed to raise an entirely different set of questions and challenges. During the first day of talks, group members realized that, because of the size of the group, the discussion was unfocused and not everyone was on the same page or necessarily putting priorities and objectives in the same place. But the group devised an effective solution: each member prioritized his or her goals for the discussion by writing on a sticky note the biggest problem or objective of building the cell-chip interface.

The sticky notes were placed on a whiteboard and grouped into categories. Several members saw the delivery of the nanoprobes as the key issue to resolve. Others saw signal detection as the biggest hurdle. Still others saw resolution, readout, and controlling the cell’s activity as key issues to discuss. Once these priorities had been established, more focused discussions were possible.

By the end of the conference, group members proudly reported that they had developed an outline for interrogating an individual cell using nanotechnology. The theoretical protocol was as follows:

- Position a cell over the nanoprobes.
- Use mechanical force to impale the cell on a microfuidic array.
- Create a cellular activity profile using nanoprobes that act as mechanical and chemical sensors.
- Compare the activity profiles of healthy and diseased cells, to identify diseased states of individual cells.

While the focus group did not generate a detailed plan of attack -- which was not the goal of the conference -- group members learned how their colleagues tackle scientific problems and how to pool knowledge and talents to conduct interdisciplinary research. Perhaps equally important, the group learned to have fun at the same time.

“We decided to build a cell torture device instead,” said Todd Thorsen of the Massachusetts Institute of Technology, who presented the group’s findings to conference attendees.