Podcast Interview: Nick Melosh

PNAS: Welcome again to Science Sessions. I'm Brian Doctrow.

Cells can undergo numerous changes over their lifetimes. Stem cells transform into neurons, or muscle cells, or other specialized cell types as part of normal development. And some cellular transformations can lead to disease. Scientists can now transform human cells from one type, such as skin cells, into a different type, such as neurons. These transformations are accompanied by changes in the levels of various proteins and messenger RNA, or mRNA, within cells, and monitoring the levels of these molecules over time can help better understand and control cellular transformations. However, with most conventional methods, cells have to be destroyed to obtain their contents, which prevents any given cell from being measured more than once. Nick Melosh, a materials scientist at Stanford University, along with his colleagues, developed a technique for sampling proteins and mRNA from cells without destroying the cells in the process. This technique enables multiple measurements to be made over time from the same cells. A PNAS article describing this technique earned Melosh and his co-authors the 2017 Cozzarelli Prize for excellence and originality in Engineering and Applied Sciences from PNAS. I recently spoke with Melosh about the technique, which is based on "nanostraws."

Melosh: It's this array of tiny straws, about 100 nm in diameter; that's about 1000 times smaller than a single hair on your head. These tiny little straws can form tight interfaces and even penetrate the cell, and they're small enough they don't tend to hurt the cell. Then you can apply a little bit of electric field to pull some of those molecules out of the cell and then analyze them using more traditional methods. It's kind of like a blood draw for a cell. We can just take a little bit out without harming it and then analyze that, and then come back the next day and do it again.

PNAS: Melosh explained how the nanostraws are created.

Melosh: We start with this water-filtration membrane, similar to a high-quality water purification system would have, and in it you have very small pores, about 100 nm in diameter, that go through the entire plastic material. We can use a newly developed technique to deposit inorganic material—in this case, glass—all over a substrate from the gas phase. That will go and penetrate all the tiny little pores. Then we remove some of the host matrix, revealing these sharp little straws underneath. You can control the height, the diameter, the density of the straw matrix, which may be important for different cell types or different cargoes. We were able to pattern the extraction region so that we could narrow it down to just a single cell. Or many cells.

PNAS: One of the benefits of the nanostraw extraction technique is that it allows cells to remain in contact with their neighbors during sampling.

Melosh: Cells are inherently social creatures—they want to talk to each other, and they need that feedback stimulus from other cells. And so, when you take them and isolate them by themselves, they miss that mechanical and chemical environment. And so, is it

really representative of the kind of tissue that you're interested in? We wanted an analysis system that was able to extract cells in their native state, as they were.

PNAS: Melosh and his colleagues demonstrated that cells subjected to nanostraw extraction continued to live and function normally, even after repeated extractions over a period of days or weeks.

Melosh: The longest that we did was 21 days, on neurons—normally very finicky cells. And those were fine. That's sampling once a day which, for a developmental process for a cell, that's about the right frequency that you'd want to see.

PNAS: Melosh further demonstrated that the protein and mRNA levels measured by nanostraw extraction quantitatively matched those obtained by conventional methods.

Melosh: We could always just sample something and say, "hey, this material is present." But what we really want to know is how much of it is present and in what ratio, and so it's important to establish that there's a quantitative relationship between what we extract and what was there. We did that by doing an extraction, measuring the distribution, and then taking that group of cells and grinding them up and doing a traditional assay on them. Then we could compare one-to-one, and show that, in fact, our technique was quantitatively accurate as well. Certain mRNA—very large ones, and particularly ones that were associated with membranes—didn't appear to come out very well, and so we had a deficiency relative to what they natively were. That's really not surprising if these were membrane-associated, because they're not freely floating in the cell, and so they're not going to be extracted during this process.

PNAS: Human cells can be reprogrammed from one specialized type into another. Melosh thinks that his nanostraw extraction process could help illuminate this reprogramming process and apply it in the clinic.

Melosh: There's a major need to be able to do, say, repair of the heart after a heart attack. And it requires a lot of cells; estimates are around a billion cells. And so, you actually have to transform these cells efficiently and in large numbers, and you want to know that what you're reimplanting is fully mature and isn't going to generate tumors or other kinds of malignant tissue. And so, we're going to be applying this technique to try to look at that transformation and use it to really validate the cells that you have.

PNAS: I asked Melosh what his reaction was upon learning that his paper had won the Cozzarelli prize.

Melosh: Wow, I mean, we were really surprised, I think, and excited. It's a unique honor and we're really excited about the technology and what we've been able to develop, so it was fantastic to see it recognized.

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