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**A new technique for extracting the contents of living cells**

Alumina nanostraws allow longitudinal intracellular monitoring on live cells

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Being able to monitor cells at different times is key to tracking fundamental cellular processes such as differentiation, cellular senescence, as well as disease progression and the effectiveness of drugs. However, most approaches are destructive and involve lysing the cells to analyse their contents. If you want to study different time points, this might necessitate using parallel cell cultures. This can result in problems due to cell heterogeneity (*1*), where cell-to-cell variation in phenotype and other differences mean that comparisons between different cultures may be unrepresentative (*2*). Hence, there’s a clear need for techniques that allow small quantities of cytosolic contents to be extracted for analysis, to enable the long-term tracking of cellular response.

The challenge is how to sample the contents of a cell without destroying it. Any technique must be capable of manipulating small pL-scale volumes, maintain high cell viability, give an accurate reflection of the cell’s multiple biological components, as well as avoid influencing the ongoing development of the cell (*1*, *3*).

In a recent publication, Cao *et al.* approached this problem by culturing cells on top of a random arrangement of hollow cylinders, which they call nanostraws (*2*). They argue that these 150 nm diameter straws can sample 5-10% of proteins, mRNA and small molecules from the cells, while only reducing cell viability by approximately 5%. Critically, their approach allows intracellular sampling and characterisation at multiple time points from the same cells, allowing changes in the same cells to be tracked with time.

Their technique involves electroporating cells in-situ on top of the nanostraws, before allowing the intercellular contents to diffuse passively through the nanostraws into an adjacent buffer. The buffer is then analysed using either fluorescence (when studying modified cells that express green fluorescent protein), enzymatic assays (ELISA), or PCR to determine the intracellular contents. Their study culminates by comparing the mRNA expression in human-induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) using both their nanostraw extraction method, to an analysis of lysed cells. Of the 44 mRNA sequences they were able to identify, only seven of these were under-detected compared to the control. These mRNA sequences were found to be relatively larger molecules suggesting lower diffusion rates could be responsible. The authors also suggest that subcellular localization of mRNA within the cell may also play a role in detection efficiency. Despite this discrepancy, their research demonstrates a valid and new approach to live intracellular sampling, where only a handful of techniques currently exist. Those alternatives (where a portion of the cell contents is collected, as opposed to the introduction of a target-specific marker) include the use glass nanopipettes (*4*), fluid force microscopy (*1*), and the use of carbon nanotube probes (*5*).

Glass micropipettes are commonplace for manipulating larger cells, however their relatively large dimensions (0.5-5 µm) can result in cellular damage (*3*). An alternative is the nanopipette, which uses a quartz capillary with a 100 nm diameter opening to extract components (*4*, *6*, *7*). Actis *et al.* used nanopipettes in combination with a scanning ion conductance microscope setup to extract femtolitre quantities of intracellular material. In their approach, a nanopipette filled with an organic solvent forms a phase-interface with the aqueous cell contents. By applying a bias across the tip and sample, the position of this interface can be shifted, resulting in extraction. A key advantage of this approach is the precision with which they could target organelles within the cell, enabling them to assess heterogeneous variants in RNA and mitrochondrial DNA expression in cultured HeLa cells (*4*).

Fluid force microscopy uses a hollow atomic force microscopy tip to controllably penetrate the cell membrane, resulting in the selective withdrawal of the intracellular contents by applying a negative pressure to the tip (*1*, *8*). Guillaume-Gentil *et al.* reported successfully withdrawing up to 90% of the cytoplasmic content of HeLa cells without adversely affecting cell viability or behaviour over 5 days in comparison to a control, although this was an extreme case. They could also extract structures from both the cytoplasm and nucleus and image them with TEM, and used enzyme-assays and PCR to determine of enzyme activity and mRNA presence in their samples.

Carbon nanotubes have similarly been used for minimally invasive cell monitoring. Signhal *et al.* developed a “carbon nanotube endoscope” by attaching 50-60 µm long multiwalled carbon nanotubes to the ends of glass pipettes (*5*). They could extract fluorescently labelled Ca2+ from the cytosol, and image this in-situ, although the reported attolitre-volumes limited further extraction and analysis.

Both nanopipettes, fluid force microscopy and carbon nanotube endoscopes offer high levels of control over cell-selection and intracellular extraction volume, but come at the expense of throughput. Nanostraw extraction arguably has the advantage of offering much greater throughput, at the expense of not being able to directly choose which cells are addressed. However, it is feasible that with greater control over the spatial location of the nanostraws, combined with a microfluidic approach for systematically processing the extracted buffer, this limitation could be overcome. This is particularly important for cell screening applications where throughputs of greater than a hundred cells per plate are required.

In addition to intracellular extraction, there are many other approaches to assessing the intracellular environment. The use of fluorescent markers, quantum dots, nanoparticles, fluorescent FRET pairs, thermally sensitive fluorescent markers conjugated to specific antibodies or RNA strands are prevalent throughout the literature (*3*). While these continue to offer the advantage of high specificity, contrast and resolution, any given experiment is limited to identify a small number of pre-specified targets, and care must be taken to avoid introducing materials that adversely affect the cell environment.

While intracellular extraction methods provide clear advantages, there is still a need for further investigations into the impact of introducing high-aspect ratio structures into cells. Whether the membrane wraps around the protruding structure, or is penetrated by it is still a topic of much discussion (*9*). In the case of nanostraws it has previously been reported that membrane penetration is rare, and dependent on the cell adhesion behaviour (*10*). In the case of this nanostraw extraction technique, electroporation was necessary to facilitate any extraction, suggesting that membrane penetration was unlikely. This is not inherently a limitation, but it does suggest that if this approach is to be applied to different cell lines care will need to be taken to ensure that the convoluted plasma membrane surface, and electroporation, don’t adversely influence the cell phenotype.



*Four different approaches to intracellular extraction, including the recent report of Cao et al. reporting the use of 150 nm diameter alumina nanostraws combined with electroporation to extract proteins and mRNA.*

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