A Novel, All-Optical Tool for Controllable and Non-Destructive Poration of Cells with Single-Micron Resolution

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Abstract: We demonstrate controllable poration within ≈1 μm regions of individual cells, mediated by a near-IR laser interacting with thin-layer amorphous silicon substrates. This technique will allow new experiments in single-cell biology, particularly in neuroscience.

As our understanding of the fundamental mechanistic processes underpinning biology expands, so does the need for high-precision tools to allow the dissection of the heterogeneity and stochastic processes that dominate at the single- and sub-cellular level. Here, we demonstrate a highly controllable and reproducible optical technique for inducing poration within specific regions of a target cell's plasma membrane, permitting localized delivery of payloads, depolarization and lysis experiments to be conducted in unprecedented detail. Experiments support a novel mechanism for the process, based upon a thermally-induced change triggered by the interactions of a near-IR laser with a biocompatible thin film substrate at powers substantially below that used in standard optoporation experiments.

OCIS codes: (170.1530) Cell analysis; (240.0310) Thin films.

1. Introduction

The toolkit of techniques that makes up the bulk of biochemical research has achieved many notable successes and has provided us with our first real glimpses into the fundamental workings of the cell, but the miniscule sample volumes presented by individual cell means that the approaches are unsuitable for the analysis of anything less than ensembles of tens of thousands of cells. This is a serious limitation, as stochastic processes have been demonstrated to make a substantial if not the dominant role in any number of cellular responses, even amongst supposedly clonal cell cultures [1]. This leads to a significant loss of resolution as a major percentage of a tissue sample or culture must respond similarly in order to be detected, and meaning that outliers and rare cells (such as potentially dangerous cancer precursor cells damaged through oxidative stress) are lost into the noise [2].

As a result, efforts have focused upon the development of extremely sensitive platforms for the quantitative (or at least semi-quantitative) analysis of single cells, focusing particularly on rare cell types such as the circulating tumor cells thought to be one of the major factors behind cancer metastases [3,4]. These technologies are typically based around microfluidic [5] or optical [6] technologies (and often in combination), to provide the levels of both precision and throughput required to make such single cell investigations feasible. In particular, the experiments seek to quantify the differences in individual cellular responses to stimuli, be they drugs, oxidative environments or nucleic acids. These experiments have the added complexity that a technique must generally be employed to deliver the stimulus to the cell interior, across its plasma membrane, whilst ideally causing minimal perturbation to the rest of the cell so as not to induce artefacts. Previous techniques have focused upon either delivery vectors such as cationic polymers which stimulate endocytosis (but often introducing chemical species which are broken down slowly inside the cell, if at all) [7], or using directly applied forces such as high voltage gradients [8] or extremely high-intensity pulsed lasers [9] which may risk damaging the target cell.

Here we present a novel, optically-mediated cell poration technique which combines a gentle and non-destructive approach with single-micron spatial precision. This technique has demonstrated high reproducibility across cell lines alongside essentially quantitative success rates and post-experimental cell viability, and for the first time allows the direct delivery of material to regions of interest at a sub-cellular level [10]. Quantitative investigations and computer modelling of the structure and dynamics of the pore formation process has suggested a mechanism radically different from those previously identified, based upon rapid and reversible changes within the lipids of the cell's plasma membrane itself.

2. Summary of methods

Human-derived cell lines representing a range of tissues and tumor types were cultured inside a 100 μm-deep spacer on a ≈1 μm-thick layer of amorphous silicon (a-Si) deposited on indium tin oxide-coated coverslips by plasma-assisted chemical vapor deposition. During poration experiments, the spacer was loaded with medium containing the dye or probe of choice and capped with a second coverslip. The resulting chip was inverted and loaded onto the stage of a Nikon TE2000E inverted microscope which had been adapted for holographic optical trapping as described by Lanigan *et al.* [11]. This system was capable of delivering individually addressable laser trapping spots of ≈1 μm diameter from a λ = 1070nm Yb-fibre laser, spots which were focused onto the plane of the a-Si layer from which the cells were now suspended. The a-Si target was irradiated for a period of 10-60s under typical intensities of ≈150 mW. Similar experiments were conducted using a Leica TSC SP5 II inverted confocal microscope, irradiating using a Ti Sapphire 1 MHz pulsed laser (λ = 960 nm), scanning the manually selected region of interest at a rate of 400 Hz and with a power of 200 mW. After irradiation, the extent of poration was monitored *via* fluorescence and cell viability was monitored using calcein release assays.

3. Results and discussion

A range of probes were used to observe and measure the extent of cellular poration: propidium iodide was used initially for proof-of-concept work, while characterization of the pore structure and the dynamics of payload delivery was accomplished using CdTe quantum dots (QDs) of a range of diameters, between approximately 3 nm – 7nm [12,13]. Propidium iodide penetration was observed throughout targeted cells almost immediately, but QD experiments revealed far more interesting behavior. QDs up to approximately 4.2 nm diameter were observed to diffuse into HCT 116 cells cell at the site of irradiation only. However, once inside their relatively large hydrodynamic radii coupled with the high viscosity and crowding within the cell led to them retaining the shape of the irradiated region for extended periods, allowing us the ability to tattoo cells with long-lasting shapes as shown in Figure 1. QDs of >4.5 nm diameter, however, were excluded from the cell interior, although became adsorbed to the cell surface allowing their selective bleaching *via* two-photon processes.

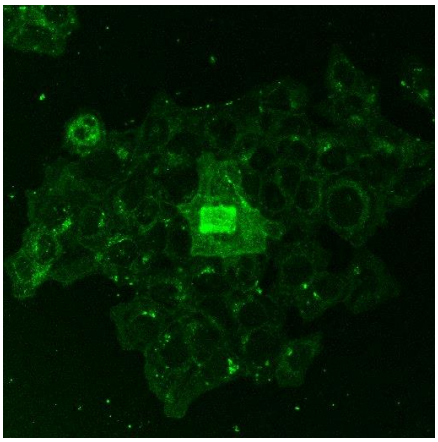

Figure 1: HCT 116 cells after irradiation with λ = 960 nm scanning pulsed laser for 1 minute in the presence of 3.5 nm diameter CdTe quantum dots. Note the bright rectangle in the center of the image where the scanning laser was focused. Image frame is 246 x 246 μm.

These pore diameters remained essentially constant for a given cell line, from the first sign of payload delivery at minimal laser power through to the cells' eventual lysis at much higher irradiation intensities. High intensities could also trigger flash boiling of the medium, centered upon the site of irradiation, and even etch the a-Si surface itself, producing patterns with features of the order of 2 μm (although generally with rough edges). This lack of strong temperature dependence above a critical threshold is indicative of some structure or physical force maintaining the pore size (as opposed to the pore size scaling with irradiation intensity, as seen in classical photoporation experiments [14]).

These results, alongside those control experiments conducted to exclude factors such as electrical field induction or capacitive effects in the membrane, led to the conclusion that the observed phenomena were due to thermally-induced changes in the membrane, which flipped it from its normal state into a structure with defined pores that healed rapidly and reversibly. Subsequent COMSOL simulations have verified that the temperatures generated given both the irradiation times and the spatial localization observed are sufficiently slight as to minimize damage taken by

the cell during the process. The exact structure of the porous region and its mechanism of formation remain a topic of debate, but studies are ongoing to characterize its properties in detail with a view to tailoring it for specific applications.

4. Conclusion

The discovery of this technique is potentially an extremely wide-ranging one: it allows the non-destructive poration of and payload delivery to individual cells of interest in a manner that is both exceptionally precise but also highly amenable to automation, dramatically increasing throughput over many previous technologies. As a result it is likely to find utility in experiments from chemotherapeutic discovery to genetic modification. Furthermore, though, it opens up substantial new fields of study, particularly in areas such as neuroscience where mechanistic study of individual cell contributions to the system has previously been very difficult. This approach will allow the design of experiments to depolarize small regions of membrane within neurons or even dendrons, in effect 'tickling the toes' of individual nerves to assess their function.

5. References

[1] A. Raj and A. van Oudenaarden, "Nature, nurture, or chance: stochastic gene expression and its consequences.," Cell **135**, 216–26 (2008).

[2] G. T. Roman, Y. Chen, P. Viberg, A. H. Culbertson, and C. T. Culbertson, "Single-cell manipulation and analysis using microfluidic devices.," Anal. Bioanal. Chem. **387**, 9–12 (2007).

[3] S. K. Arya, B. Lim, and A. R. A. Rahman, "Enrichment, detection and clinical significance of circulating tumor cells," Lab Chip **13**, 1995–2027 (2013).

[4] A. A. Powell, A. H. Talasaz, H. Zhang, M. A. Coram, A. Reddy, G. Deng, M. L. Telli, R. H. Advani, R. W. Carlson, J. A. Mollick, S. Sheth, A. W. Kurian, J. M. Ford, F. E. Stockdale, S. R. Quake, R. F. Pease, M. N. Mindrinos, G. Bhanot, S. H. Dairkee, R. W. Davis, and S. S. Jeffrey, "Single cell profiling of circulating tumor cells: transcriptional heterogeneity and diversity from breast cancer cell lines.," PLoS One **7**, e33788 (2012).

[5] I. Cima, C. Wen Yee, F. S. Iliescu, W. Min Phyo, K. Hon Lim, C. Iliescu, and M. Han Tan, "Label-free isolation of circulating tumor cells in microfluidic devices: Current research and perspectives," Biomicrofluidics **7**, 011810 (2013).

[6] A. Salehi-Reyhani, J. Kaplinsky, E. Burgin, M. Novakova, A. J. DeMello, R. H. Templer, P. Parker, M. A. A. Neil, O. Ces, P. French, K. R. Willison, and D. Klug, "A first step towards practical single cell proteomics: a microfluidic antibody capture chip with TIRF detection," Lab Chip **11**, 1256–1261 (2011).

[7] L. Wasungu and D. Hoekstra, "Cationic lipids, lipoplexes and intracellular delivery of genes.," J. Control. Release **116**, 255–64 (2006).

[8] W. Kang, F. Yavari, M. Minary-Jolandan, J. P. Giraldo-Vela, A. Safi, R. L. McNaughton, V. Parpoil, and H. D. Espinosa, "Nanofountain probe electroporation (NFP-E) of single cells.," Nano Lett. **13**, 2448–57 (2013).

[9] M. Waleed, S.-U. Hwang, J.-D. Kim, I. Shabbir, S.-M. Shin, and Y.-G. Lee, "Single-cell optoporation and transfection using femtosecond laser and optical tweezers.," Biomed. Opt. Express **4**, 1533–47 (2013).

[10] D. R. Casey, D. Wylie, J. Gallo, M. R. Dent, A. Salehi-Reyhani, R. Wilson, N. J. Brooks, N. J. Long, K. R. Willison, D. R. Klug, M. A. A. Neil, S. L. Neale, J. M. Cooper, and O. Ces, "Single-cell control of membrane poration and lysis via spatially localised optically-induced phase transitions of the plasma membrane," Chem. Sci. (2015).

[11] P. M. P. Lanigan, I. Munro, E. J. Grace, D. Casey, J. Phillips, D. R. Klug, O. Ces, and M. A. A. Neil, "Dynamical hologram generation for high speed optical trapping of smart droplet microtools," Biomed. Opt. Express **3**, 1609–1619 (2012).

[12] L. Li, H. Qian, N. Fang, and J. Ren, "Significant enhancement of the quantum yield of CdTe nanocrystals synthesized in aqueous phase by controlling the pH and concentrations of precursor solutions," J. Lumin. **116**, 59–66 (2006).

[13] J. Gallo, I. García, N. Genicio, and S. Penadés, "CdTe-Based QDs: Preparation, Cytotoxicity, and Tumor Cell Death by Targeting Transferrin Receptor," Part. Part. Syst. Charact. **31**, 126–133 (2014).

[14] M. Tsukakoshi, S. Kurata, Y. Nomiya, Y. Ikawa, and T. Kasuya, "A novel method of DNA transfection by laser microbeam cell surgery," Appl. Phys. B Photophysics Laser Chem. **35**, 135–140 (1984).