High throughput microfluidic intracellular delivery platform

We investigate and optimize a recently developed a novel, microfluidic, intracellular delivery device capable of delivery of macromolecules (DNA, RNA, proteins, sugars, peptides) to different cell types by controlled squeezing (Figure 1).[1-3] We are pursuing mechanistic studies of macromolecular delivery to guide development of predictive models, optimization studies and improvement of device design: Based on our initial results, we hypothesize that macromolecular delivery occurs through a pore formation mechanism in an endocytosis-independent mechanism. To validate our hypothesis, we are examine the transport of labeled macromolecules to study the mechanisms of delivery and its dependence on factors such as cell deformability, endocytosis, and operating conditions.

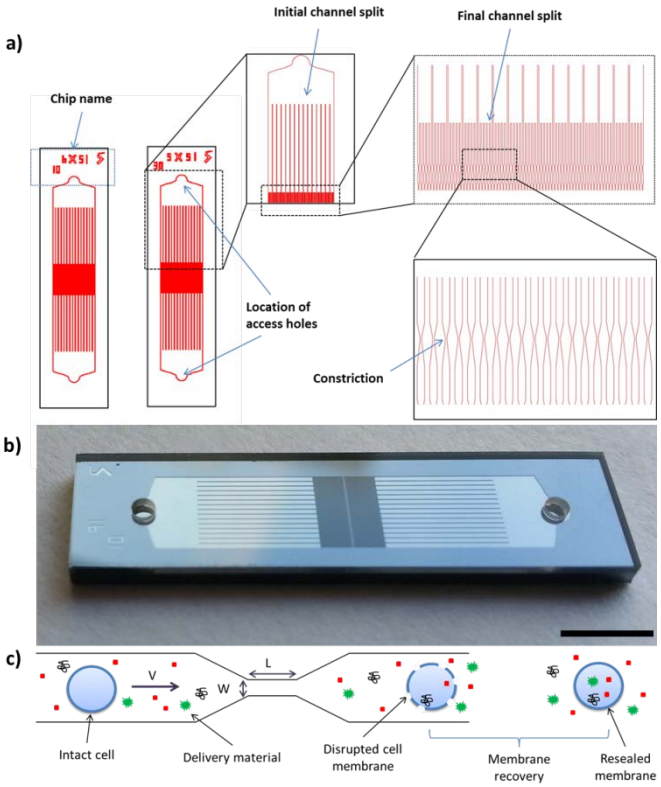

Figure 1. Overview of microfluidic device design operating principle.

To date, we have gained a deeper understanding of the intracellular delivery mechanism, specifically the dependence of membrane recovery on calcium signaling. In a recently published series of studies,[2] we have established that the membrane recovery process after mechanical disruption is mediated by calcium influx into the cytoplasm. This effect was measured by experiments involving the addition or elimination of calcium in the cell suspension during device treatment. This insight enabled us to develop protocols to deliver ~5x more material to live cells. Our results are consistent with other studies that have reported the role of calcium in facilitating membrane closure when the plasma membrane is disrupted. Additional studies also showed that operating the device at refrigerated temperatures (i.e. on ice) resulted in increased delivery efficiency. This finding is contrary to the behavior one would expect of an endocytotic delivery mechanism as endocytosis is inhibited at lower temperatures. These recent findings and our previous studies thus provide significant evidence against an endocytotic delivery mechanism.

COMSOL multiphysics modeling of the original device designs[1] indicated that the fluid flow rate was not uniform across channels. Based on this insight, we developed a new device architecture that has a more uniform flow profile.[2] This was achieved by lengthening the entry region of the channels, thus allowing the flow profile to develop better before being split into the 75 channels containing the constrictions. Future modeling efforts will seek to correlate fluid flow properties with delivery parameters. We are also exploring the device's capabilities by coupling the rapid deformation phenomenon with electroporation. The new device consists of channel flow architecture similar to the current device plus gold interdigitated electrodes to provide an

electric field. These new devices allow us to investigate the effects of electroporating cells before or after cell squeezing. Current experiments aim to identify operating parameter values (electric field strength, frequency, and operating speed) to understand the role of electric fields in the delivery of nucleic acids.

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