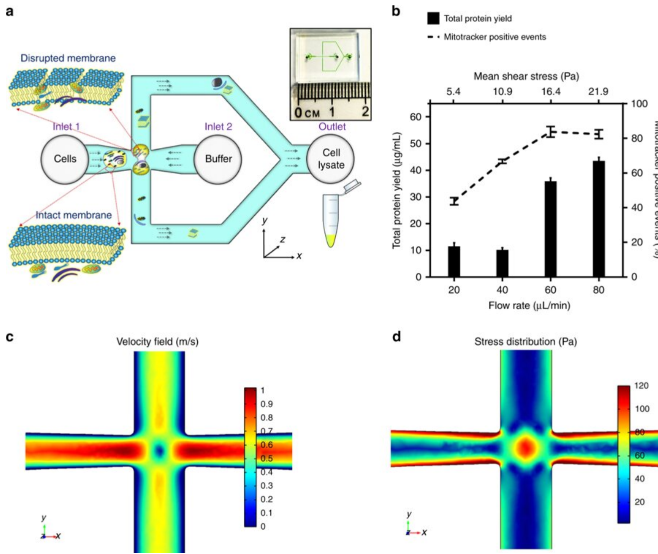
Extracting functional mitochondria using microfluidics devices

16 January 2019, by Thamarasee Jeewandara

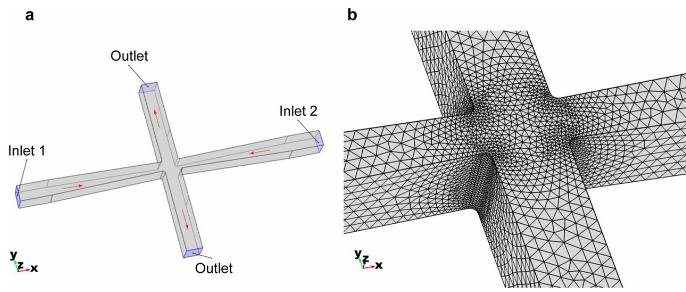


a) Cells are introduced into the cross-junction of the microchannel. The stress applied on the cell is optimized to disrupt the cell membrane and release subcellular components, while maintaining the integrity of mitochondria. The overview of the microfluidics chip is shown in the inset. b) The applied mean stress, modulated by controlling the volumetric flow rate for a given channel geometry, has been optimized by the maximal protein yield (an indication of quantity of the extracted subcellular contents) and the maximal mitotracker positive events (a hallmark of functional mitochondria). Results were obtained by shredding HEK293 cells (embryonic kidney cells, 106 cells/mL) by a range of shear stress and plotted as mean ± SD (n = 3 independent experiments). A finite element simulation model was established by COMSOL Multiphysics® to illustrate the fluidic flow at the cross-junction. Give a volumetric flow rate at 60 ?l/min, c) illustrates the velocity profile and the stagnation point at the center (where the flow velocity is zero), and d) illustrates the stress distribution and the extensional flow fields around the stagnation point, which contributes significantly to the cell deformation and disruption. Credit: Microsystems & Nanoengineering, doi: https://doi.org/10.1038/s41378-018-0037-y.

Mitochondria are dynamic, bioenergetic intracellular organelles, responsible for energy production via ATP production during respiration. They are involved in key cellular metabolic tasks that regulate vital physiological responses of cells, including cell signaling, cell differentiation and cell death. Defective mitochondria are linked to several critical human genetic diseases, including neurodegenerative disorders, cancer and cardiovascular disease.

The detailed characterization of functional mitochondria remains relatively unexplored due to a lack of effective organelle extraction methods. For instance, the extraction process must sustain sufficient functionality of the organelle ex vivo to illuminate their cytosolic functions in the presence of cytoskeleton and other subcellular organelles. Since mitochondria grow in a complex reticular network within cells to undergo structural alternations, their intracellular characterization is further complicated. As a result, in vitro analysis of mitochondria remains the mainstream method, to separately extract and understand the intrinsic properties of mitochondria, without the interference of other subcellular organelles.

In a recent study, now published in *Microsystems & Nanoengineering*, Habibur Rahman and colleagues at the Department of Biomedical Engineering explored the possibility of controlling hydrodynamic stress for efficient mitochondrial extraction. For this, they used cross-junction microfluidic geometry at the microscale to selectively disrupt the cellular membrane while securing the mitochondrial membrane's integrity.

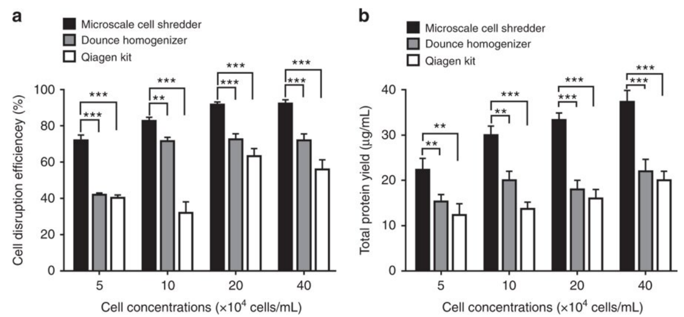


3D geometry of the cross-slot microfluidics channel. (a) Overall geometry and the boundary conditions of the model. (b) Meshing of the elements as zoomed in the cross-slot region. Credit: Microsystems & Nanoengineering, doi: https://doi.org/10.1038/s41378-018-0037-y

Advances in microfluidics have demonstrated the advantages of on-chip laboratory procedures with significantly reduced sample size and increased experimental reproducibility. Hydrodynamic stress produced in microfluidic chips can be used to open cellular or nuclear membranes transiently during intracellular gene delivery. The potential of such techniques has rarely been examined for extracting subcellular organelles since the constrained geometries of microchannels can cause subcellular component clogging in the micromachines.

The authors optimized the experimental conditions of operation based on previous studies to effectively shred cell membranes while retaining intact mitochondria in model mammalian cell lines. The model cell lines of interest were human embryonic kidney cells (HEK293), mouse muscle cells (C2C12) and neuroblastoma cells (SH-SY5Y).

In the working principle of the proposed microscale cell shredder, the scientists measured the difference in elastic modulus between the mitochondrial membrane and the cellular membrane to disrupt the cell while retaining the mitochondrial membrane. An increased stress level in the system could disrupt cell membranes with higher elastic moduli (as seen with the neuroblastoma cell line). The study compared the protein yield and the concentration of extracted functional mitochondria using the proposed method vs. commercially available kits for a range of cell concentrations.



Cell disruption and protein extraction efficiency using the microscale cell shredder, the Dounce Homogenizer and Qiagen Mitochondria Isolation Kit. a) Cell disruption efficiency, determined by the fraction of disrupted cells against total number of intact cells, was quantified at different cell concentrations. b) After the centrifugation steps, total protein yield was determined accordingly. These experiments were conducted using HEK293 cells. Results were plotted as mean ± SD (n = 3 independent experiments, **P

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