10.1117/2.1201607.006606

A photothermal nanoblade rescues mitochondria function in human cells

Alexander N. Patananan, Ting-Hsiang Wu, Pei-Yu Chiou, and Michael A. Teitell

A photothermal nanoblade couples a metal-tip-coated, wide-bore micropipette and a pulsed laser to induce a microscopic 'explosion' that cuts cell membranes, enabling the delivery of mitochondria.

Mitochondria are organelles that reside within cells. They are known as the cell's 'powerhouse' because they generate chemical energy in the form of adenosine triphosphate (ATP). Mitochondria are $\sim 2 \times 1 \mu$m in size and contain their own genome, known as mitochondrial DNA (mtDNA), which is independent of the nuclear genome. mtDNA is essential for cell respiration and the production of ATP by a process called oxidative phosphorylation. mtDNA mutations can cause morbidity and mortality in humans, and there are currently no effective treatments or cures available for mtDNA diseases. The ability to transfer isolated mitochondria with a specific mtDNA sequence into target human cells would advance studies on cell metabolism and how mitochondria interact with their host cell, and also could lead to new therapeutic strategies to treat mtDNA-related disorders.

There are few methods for transferring isolated mitochondria into mammalian cells.[1] The most common approach is to fuse a donor cell that contains mitochondria with mtDNA of interest with a recipient cell devoid of mtDNA, also known as a $\rho 0$ (rho-null) cell. The resulting cytoplasmic hybrid (or cybrid) cell contains the mtDNA from the donor cell and the nuclear DNA from the recipient cell. However, the cybrid also has a mixture of other cytosolic components such as mRNAs, proteins, lipids, and other organelles. The 'cleanest' method of transferring isolated mitochondria into cells is by microinjection. However, because tolerated pipette tips have a relatively small diameter, clogging and cargo damage often occur, which reduces efficiency.

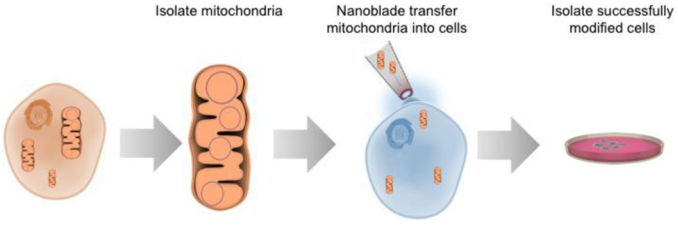

Figure 1. *Mitochondria containing mitochondrial DNA (mtDNA) are isolated from a cell (MDA-MB-453) and transferred into another cell (143 BTK–ρ0) that lacks mtDNA and cannot respire. Cells that receive and replicate transferred mitochondria respire and were cloned from media lacking uridine.*

To transfer large, micrometer-sized cargo into mammalian cells, we have invented the photothermal nanoblade.[2] We took a titanium-coated glass micropipette with a 3μm-inner-tip diameter and loaded it with isolated mitochondria. The pipette was placed adjacent to a cell membrane, and heated with a 532nm-wavelength non-damaging laser pulse. A transient vapor bubble in the surrounding aqueous culture media generated by rapid heat transfer caused a membrane incision by shear stress. This enabled active, pressure-driven cargo delivery of genetic material,[3] conjugated quantum dots,[4] and live intracellular bacterial pathogens[5,6] into mammalian cells with high efficiency and cell viability.

Since bacteria and mitochondria are roughly the same size, we were able to isolate mitochondria from one cell line (MDA-MB-453) and transfer it into a different $\rho 0$ cell line (143BTK$-\rho 0$) using the nanoblade.[7] $\rho 0$ cells cannot survive in culture media deficient in uridine because respiration is required for cells to manufacture this essential nucleic acid building block (see Figure 1). Three cell clones, termed rescue 1–3, received mitochondria by nanoblade transfer and grew on media

Continued on next page

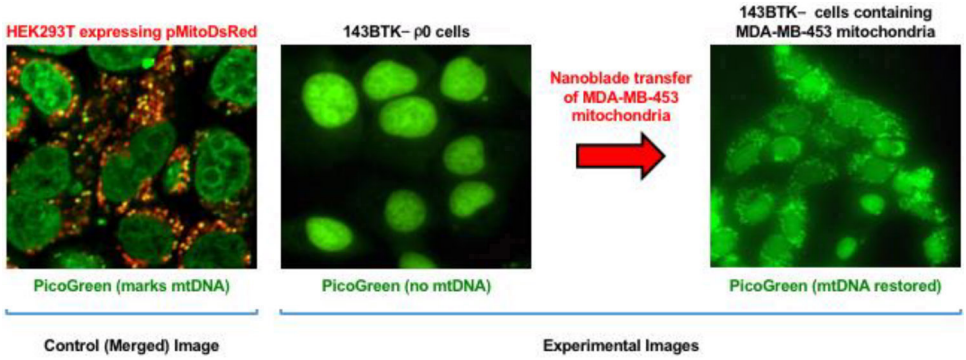

Figure 2. *Cells with functional mitochondria (HEK293T) contain mtDNA (the cells are stained with picogreen and appear yellow where they overlap with MitoDsRed). ρ0 cells (143BTK−ρ0) that lack mtDNA do not stain with picogreen in the cytosol. Delivery of isolated mitochondria (MDA-MB-453) into ρ0 cells by photothermal nanoblade produces rescue cells with speckled picogreen cytosolic staining, which indicates mtDNA replication.*

lacking uridine. The transferred mtDNA was replicated over time by the new host cell clones as they continued to grow (see Figure 2).

We characterized the function of transferred mitochondria in the rescue cells. ATP concentrations were comparable with the mitochondrial donor cell and 143BTK parent cell (from which the $\rho 0$ cell was generated). We also recovered respiration in rescue clones 1–3 to a level comparable with the mitochondrial donor and parent cell lines, and determined the expression of 33 nuclear-encoded metabolism-regulating genes, as well as levels of ∼100 small metabolites. Principal component analysis showed that rescue lines 1 and 3 were similar to the parent cell line in these key features, whereas rescue line 2 was most similar to the $\rho 0$ recipient cells and not fully rescued. In other words, the transfer of mitochondria reset the metabolic profile of a $\rho 0$ cell to that of the parent cell in most, but not all cases. This phenomenon left open questions related to the mechanism(s) of metabolic rescue.

In summary, to enable studies of mitochondrial processes and to potentially provide a futuristic pathway for addressing mtDNA diseases, we outfitted a photothermal nanoblade to transfer isolated mitochondria into $\rho 0$ cells to rescue their metabolic defects. Because the nanoblade transfers mitochondria to one cell at a time with an output of ∼100 cells/hour, we are developing a higher throughout method called a biophotonic laser-assisted surgery tool (BLAST) to transfer mitochondria into 100,000 cells/minute.[8] A commercial prototype combining high-throughput delivery with ease-of-use features is now under development by the biotech start-up company NanoCav, LLC. With BLAST, we aim to improve our understanding of fundamental mitochondrial biology and also come closer to developing potential approaches to address mtDNA disorders.

The authors acknowledge UC Discovery Biotechnology Award 178517, Air Force Office of Scientific Research FA9550-15-1-0406, NIH grants GM007185, GM114188, GM073981, GM061721, EB014456, CA009056, CA90571, CA009120, CA156674, CA185189, and CA168585, NSF grant CBET-1404080, CIRM grants RB1-01397 and RT3-07678, a Prostate Cancer Foundation Challenge Award, a Broad Stem Cell Research Center Training Grant and Innovator Award, and support from NanoCav, LLC. The authors thank K. Niazi and S. Rabizadeh (NantWorks, LLC). NantWorks, LLC has licensed the photothermal nanoblade and BLAST from the Regents of the University of California. Pei-Yu Chiou and Michael A. Teitell received sponsored research funding from NantWorks, LLC.

Author Information

Alexander N. Patananan, Pei-Yu Chiou, and Michael A. Teitell
University of California, Los Angeles
Los Angeles, CA

Alexander Patananan is a postdoctoral fellow in the Department of Pathology and Laboratory Medicine. His research interests are in large cargo delivery technologies and the influence of mitochondria and mtDNA on human metabolism, physiology, and aging.

Continued on next page

Pei-Yu Chiou is a professor in the Departments of Mechanical and Aerospace Engineering and Bioengineering. His research interests include optofluidics, laser surgery, biophotonics, nanophotonics, and lab-on-chip systems.

Michael Teitell is a professor in the Departments of Pathology and Bioengineering. His research interests are in developing advanced instrumentation to quantify live cell responses and cell manipulation for studies in stem and cancer cells, development, and metabolism.

Ting-Hsiang Wu
NantBioscience, Inc.
Culver City, CA

Ting-Hsiang Wu is a principal scientist who heads commercialization efforts of cell engineering and cell diagnostics platforms for applications in immunotherapy and regenerative medicine.

References

1. A. N. Patananan, T. H. Wu, P. Y. Chiou, and M. A. Teitell, *Modifying the mitochondrial genome*, **Cell Metabol. 23**, pp. 785–796, 2016.
2. T. H. Wu, T. Teslaa, M. A. Teitell, and P. Y. Chiou, *Photothermal nanoblade for patterned cell membrane cutting*, **Opt. Express 18**, pp. 23153–23160, 2010.
3. T. H. Wu, Y. C. Wu, E. Sagullo, M. A. Teitell, and P. Y. Chiou, *Direct nuclear delivery of DNA by photothermal nanoblade*, **J. Lab. Autom. 20**, pp. 659–662, 2015.
4. J. Xu, T. Teslaa, T. H. Wu, P. Y. Chiou, M. A. Teitell, and S. Weiss, *Nanoblade delivery and incorporation of quantum dot conjugates into tubulin networks in live cells*, **Nano Lett. 12**, pp. 5669–5672, 2012.
5. C. T. French, I. J. Toesca, T. H. Wu, T. Teslaa, S. M. Beaty, W. Wong, M. Liu, *et al.*, *Dissection of the Burkholderia intracellular life cycle using a photothermal nanoblade*, **Proc. Nat'l Acad. Sci. USA 108**, pp. 12095–12100, 2011.
6. B. E. Teh, C. T. French, Y. Chen, I. G. Chen, T. H. Wu, E. Sagullo, P. Y. Chiou, M. A. Teitell, J. F. Miller, and Y. H. Gan, *Type three secretion system-mediated escape of Burkholderia pseudomallei into the host cytosol is critical for the activation of NFkappaB*, **BMC Microbiol. 14**, p. 115, 2014. doi:10.1186/1471-2180-14-115
7. T. H. Wu, E. Sagullo, D. Case, X. Zheng, Y. Li, J. S. Hong, T. TeSlaa, *et al.*, *Mitochondrial transfer by photothermal nanoblade restores metabolite profile in mammalian cells*, **Cell Metabol. 23**, pp. 921–929, 2016.
8. Y. C. Wu, T. H. Wu, D. L. Clemens, B. Y. Lee, X. Wen, M. A. Horwitz, M. A. Teitell, and P. Y. Chiou, *Massively parallel delivery of large cargo into mammalian cells with light pulses*, **Nat. Methods 12**, pp. 439–444, 2015.