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Cellaxess® HT: high-throughput transfection for genome-wide RNAi

Cellaxess® HT is a fully automated transfection system for reagent-free small interfering RNA (siRNA) delivery to a wide variety of primary and hard-to-transfect cell types with excellent transfection efficiencies and cell viabilities. CellaxessHT transfects cells directly in high-content analysis (HCA)-compatible 384-well microplates at a throughput of up to 50,000 wells per day. Because the system enables genomic screening of biologically relevant cell types, CellaxessHT can have an important role in identifying high-quality drug targets.

The lack of delivery systems is a bottleneck in RNAi screening

RNA interference (RNAi) has revived the field of functional genomics and has huge potential for target discovery and validation. Genome-wide loss-of-function screening will not only enable identification of high-quality drug targets but will also aid in resolving and understanding biological pathways. However, one critical bottleneck limiting implementation of such studies is the lack of suitable transfection methods for cells that are biologically relevant for the therapeutic area of interest.

Chemical delivery methods—for example, those using lipids or polymers—are routinely used for high-throughput transfection[1], but they are limited in terms of the cell types that can be addressed. Another delivery method, electroporation, works on a wide range of cell types[2], but conventional electroporation does not allow scaling into the true high-throughput regime. Consequently, there is a need for a generic delivery method enabling high-throughput transfection of biologically relevant cell types to facilitate large-scale RNAi screening.

Here we use gene knockdown in 3T3-L1 adipocytes to exemplify the applicability of CellaxessHT to high-throughput transfection of hard-to-transfect cells.

Bringing biological relevance to target discovery

The CellaxessHT system is based on a patented capillary electrode array technology for efficient delivery of genetic materials to a wide range of cell types (see **Fig. 1** for a system overview). Cells are transfected directly in HCA-compatible 384-well microplates at a throughput of up to 50,000 wells per working day. The system is self-contained and is designed to automatically handle all steps of the transfection procedure. This includes removal of medium before transfection, addition of electroporation buffer, and transfer of siRNAs from source and control plates. After transfection, the system adds culture medium to the plate, after which the plate is ready for transfer back to an incubator. The CellaxessHT system may be operated in two configurations: either as a stand-alone instrument or as an integrated system in which plate transfer and certain work steps are outsourced to laboratory automation system robots and external liquid handlers.

RNAi in hard-to-transfect cells: 3T3-L1 adipocytes

The 3T3-L1 cell line is a widely used model for the study of adipocyte development. However, delivery of cDNA and siRNA to fully

Figure 1 | Layout of the CellaxessHT system. (**a**) An overview of the system in stand-alone configuration. The HEPA filter unit can be seen on top of the system. (**b**) The deck layout during an experiment, the deck being loaded with the electroporation module, liquid handling tips, trays with electroporation buffer and culture medium, and cell source and control plates. (**c**) A close-up image of the capillary electrodes as they are approaching the CellaxessHT 384-well plate. (**d**) The plate.

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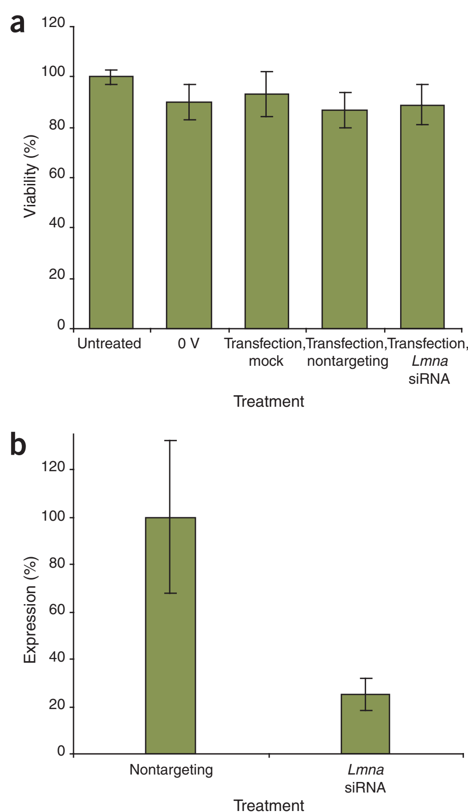

Figure 2 | Viability and knockdown efficiency in 3T3-L1 adipocytes assayed using software analysis–aided fluorescence microscopy. (**a**) Cell viability of untreated cells, un-electroporated cells (0 V), mock-transfected cells, cells transfected with a nontargeting siRNA and cells transfected with *Lmna* siRNA. (**b**) Lamin expression after knockdown with *Lmna* siRNA compared to transfection with nontargeting siRNA. Remaining expression after knockdown was 25 ± 7%. Error bars represent s.d.

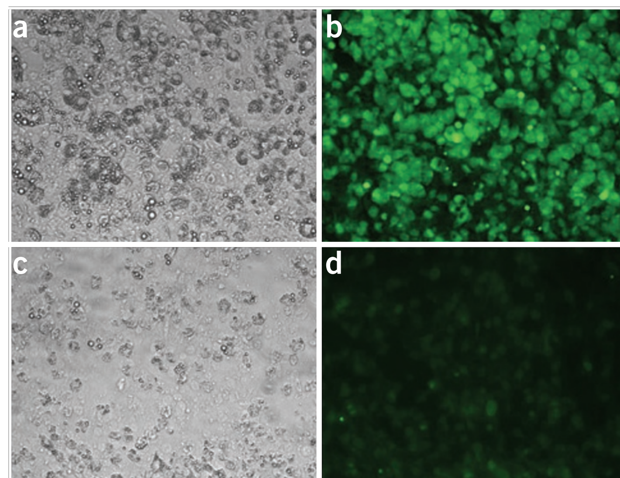

Figure 3 | Analysis of lamin knockdown in 3T3-L1 adipocytes. (**a**–**d**) Representative bright-field (left) and fluorescence (right) images of cell morphology and lamin expression in cells transfected with nontargeting siRNA (**a**,**b**) and with siRNA targeting *Lmna* (**c**,**d**). Fluorescence images show cells stained with an antibody to lamin. Note the distinct difference in fluorescence intensity between **b** and **d**.

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differentiated 3T3-L1 adipocytes is notoriously difficult, limiting the research that can be conducted on this cell type. To exemplify the capability of the CellaxessHT system, we demonstrate gene silencing in differentiated 3T3-L1 adipocytes cultured in the 384-well format. The method results in excellent efficiency and cell viability, and it opens up the possibility of genome-wide RNAi screening using 3T3-L1 adipocytes.

We cultured and differentiated mouse 3T3-L1 fibroblasts (American Type Culture Collection (ATCC); CL-173) according to standard methods. Gene silencing was accomplished by delivery of an siRNA against lamin A/C (*Lmna*)[3] with CellaxessHT 6–7 days after differentiation. Then, 48 hours after transfection, we fixed the cells and stained them for lamin A/C using standard immunohistochemistry methods. We quantified lamin A/C knockdown in two ways: using a plate reader (**Fig. 2**) or a fluorescence microscope (**Fig. 3**). The lamin knockdown was 75% compared to a negative control, based on quantitation of lamin protein amounts. We obtained equivalent results regardless of the evaluation method. Cell viability was stable and virtually independent of treatment; in the case of *Lmna* siRNA transfection, viability was 89 ± 8% compared to untreated cells measured on control plates run in parallel.

Conclusions

The CellaxessHT system is a revolutionary new concept in transfection, enabling the transition from 'cooperative' cell lines to more biologically relevant model systems in RNAi and cDNA screening. The system is based on a patented capillary electrode array technology and can efficiently transfect cell types that are considered very hard to transfect, without the known drawbacks of using chemical transfection reagents or classical electroporation. Accordingly, CellaxessHT opens the door for using biologically relevant cell types previously inaccessible for large-scale RNAi or cDNA screening in target discovery and pathway analysis.

1. Echeverri, C.J. & Perrimon, N. High-throughput RNAi screening in cultured cells: a user's guide. *Nat. Rev. Genet.* **7**, 373–384 (2006).
2. Hamm, A., Krott, N., Breibach, I., Blindt, R. & Bosserhoff, A.K. Efficient transfection method for primary cells. *Tissue Eng.* **8**, 235–245 (2002).
3. Jiang, Z.Y. *et al.* Insulin signaling through Akt/protein kinase B analyzed by small interfering RNA-mediated gene silencing. *Proc. Natl. Acad. Sci. USA* **100**, 7569–7574 (2003).

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