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Programmable macromolecule delivery via engineered trogocytosis

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We use this protocol and it's working

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Abstract

Trogocytosis, the transfer of plasma membrane fragments during cell-cell contact, offers potential for macromolecular delivery. This protocol described a method to engineer donor cells with designed receptors specific to surface ligands on recipient cells, enabling cell-to-cell protein transfer through direct contact. This step-by-step protocol outlines the workflow for producing donor and recipient cells, optimized co-culture conditions and details for measurement of transfer outcomes.

For complete details on the results and validation of this protocol, please refer to our work published in Nature Cell Biology.

Materials

Complete RPMI: RPMI 1640 with HEPES (Thermo Fisher), supplemented with 10% fetal bovine serum (FBS) (Sigma Aldrich) and 100 U/mL of penicillin-streptomycin (Life Technologies).

Complete DMEM: Dulbecco's Modified Eagle Medium (DMEM) with high glucose and GlutaMAX (Thermo Fisher), supplemented with 10% FBS and 100 U/mL penicillin-streptomycin.

Lentivirus production

- 1
 - HEK293T cells (1.5×10^6) were seeded in a 6-well plate well with 2 mL complete DMEM one day before transfection.
 - On the day of transfection, transfection mix was made from 420 μ L of OptiMEM (Thermo Fisher), 1.76 μ g of lentiviral transfer plasmid, 1.26 μ g of psPAX2 vector, and 0.55 μ g of pMD2G plasmid with 10.7 μ L of Mirus TransIT-LT1 (Mirus Bio). The mixture was vortexed and incubated at room temperature for 15 min.
 - 45% of media was removed, and cells were transfected with the mixture dropwise.
 - After 6 hours, media was replaced with 2 mL of complete DMEM containing 1 $\times$ ViralBoost reagent (Alstem).
 - After 18 hours of incubation at 37 °C, lentiviral supernatant was collected, filtered through a 0.45 μ m polyvinylidene fluoride filter (Millipore), mixed with Lentivirus Precipitation Solution (Alstem) at a 4:1 ratio, and refrigerated at 4 °C overnight.
 - Lentivirus was pelleted at 1,500 $\times$ g for 30 min at 4 °C and resuspended in complete RPMI.

Stable cell line generation

- 2
 - Stable Jurkat, K562, NALM6, HEK293T, or HeLa cell lines were generated via lentiviral transduction.
 - Suspension cells (Jurkat, K562, NALM6) were seeded at 1×10^5 cells per well in 400 μ L complete RPMI in 48-well plates on the day of transduction.
 - Adherent HEK293T or HeLa cells were seeded at 4×10^4 cells per well in 400 μ L complete DMEM in 24-well plates one day before transduction.
 - Cells were treated with 50–100 μ L of 10 $\times$ concentrated lentivirus for 24–72 hours before media change.
 - For selection, cells expressing the puromycin resistance gene were cultured in 2 μ g/mL puromycin for at least 2 days, followed by a media change.
 - Cells expressing the zeocin resistance gene were cultured with 400 μ g/mL zeocin for 3–4 days.
 - Transduced cells were used for experiments at least 3 days after transduction.
 - Viral titers were optimized for each construct to ensure comparable expression levels across experimental groups, as measured by fluorescence intensity.

Transfection of VSVg expression plasmid

- 3
 - For non-viral packaging transfection, HEK293T cells were seeded at 5×10^5 per well in 6-well plates one day prior to transfection.
 - On the day of transfection, 2.5 μ g DNA was mixed with 250 μ L of OptiMEM and 7.5 μ L of Mirus TransIT-LT1 reagent, then incubated at room temperature for 15–30 minutes.
 - The transfection mixture was added dropwise to the cells.

- Experiments were performed one day post-transfection.

Co-culture assay

- 4
 - Unless otherwise stated, donor and recipient cells were co-cultured at a 1:1 ratio with a total cell number of 10^5 per well in 96-well plates for 24 hours. Constructs were stably transduced into both donor and recipient cells, except for VSVg and VSVg* or otherwise described. Recipient cells were transduced with nuclear BFP to be distinguished from donor cells. In all co-culture experiments, each cell types were well mixed before seeding into different groups.
 - For Jurkat and K562 co-culture, donor and recipient cells with the indicated constructs were counted and seeded at 10^5 total cells per well in 100 μ L of fresh complete RPMI in 96-well plates.
 - For co-cultures involving adherent cells, experiments were conducted in 100 μ L of fresh complete DMEM.
 - U-bottom 96-well plates were used for co-culture durations under 24 hours, and flat-bottom plates were used for co-cultures exceeding 24 hours. Cells were evenly mixed by pipetting before incubation at 37°C for specified durations.
 - For co-cultures less than 2 hours, plates were centrifuged at 115 x g for 30 seconds to settle the cells. For co-cultures over several days, 100 μ L of fresh media was added to the wells 2 days post-seeding to maintain cell viability.
 - For co-culture involving primary T cells as donor cells, the co-culture period was shortened to 2-6 hours to minimize cytotoxicity on recipient cells. Live/dead staining was performed to gate live recipient cells during analysis.

Flow cytometry and analysis

- 5
 - CytExpert 2.3.1.22 was used for flow cytometry data acquisition.
 - For flow cytometry measurement of co-cultured suspension cells without antibody staining, cells were centrifuged at 234 x g for 5 minutes, washed with 150 μ L per well of ice-cold FACS buffer (PBS + 2% FBS) containing 5 mM EDTA (FACS-EDTA), and resuspended in 100 μ L per well of FACS-EDTA for measurement using a Beckman-Coulter Cytotflex S flow cytometer.
 - For co-cultures involving adherent cells, cells were first centrifuged at 234 x g for 5 minutes and digested with 100 μ L 0.05% Trypsin for 5 minutes. After digestion, cells were centrifuged at 234 x g for 5 minutes, washed with 150 μ L per well of ice-cold FACS-EDTA, filtered using a Multiscreen 96-well plate with a Nylon membrane (Millipore), and resuspended in 100 μ L per well of FACS-EDTA for measurement.
 - For flow cytometry measurements with antibody staining, after the initial wash with 150 μ L FACS-EDTA, cells were incubated with antibodies diluted in 100 μ L FACS-EDTA for 30 minutes on ice in dark. This was followed by another wash with 150 μ L FACS-EDTA and resuspension in 100 μ L FACS-EDTA before flow cytometry measurement.

- At least 10,000 single cells were collected for analysis. Data were analyzed using FlowJo v.10.8.1 (BD Biosciences).

Quantification of cell-to-cell transfer efficiency

- 6 Co-culture experiments were analyzed by first gating single cells and BFP+ recipient cells. Transfer efficiency was quantified using two metrics:
 - (1) The percentage of mCherry⁺ cells among all recipient cells (% mCherry⁺ cells).
 - (2) The median fluorescence intensity (MFI) of mCherry in all recipient cells.For each experiment, matched recipient cells grown in monoculture served as negative controls, with separate controls established for each cell type to account for their distinct baseline fluorescence levels. The mCherry⁺ threshold was defined to classify no more than 0.5% of control cells as positive.