Trince's LumiPore™ technology enables efficient delivery of macromolecules in equine and human muscle derived mesenchymal stem cells

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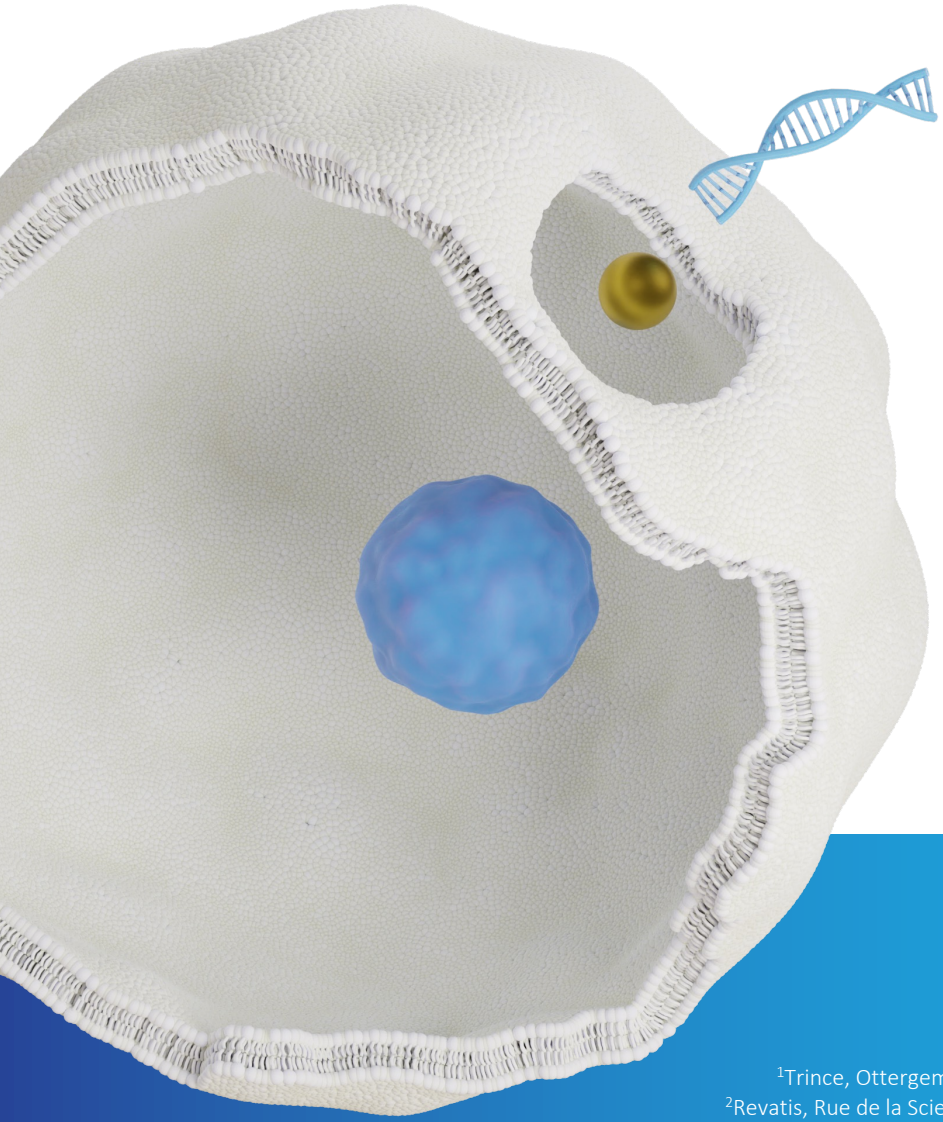

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In partnership with

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The LumiPore™ platform developed by Trince can deliver a broad variety of cargo molecules into virtually any type of cell. By manually tuning the system's parameters, an optimal balance between intracellular delivery efficiency and cell viability can be achieved. The platform was used to successfully deliver macromolecules into equine and human mesenchymal stem cells provided by Revatis. Notably, this white paper demonstrates that human MSCs can be successfully transfected with Enhanced Green Fluorescent Protein (eGFP) mRNA.

Introduction

Delivering macromolecules into cells can benefit and increase the efficiency of many biotechnological and biomedical applications. One well-known example is gene editing machinery, such as the CRISPR technology, which can directly manipulate gene expression in cells. The delivery of molecules directly into cells can also help diagnostics, such as by inserting contrast agents which can be used to image cell migration in vivo. However, these processes are challenging due to the outer cell membrane that presents a protective barrier which prohibits the entry of these molecules. Technology is required to efficiently deliver effector molecules into cells and facilitate applications such as those mentioned previously. The intracellular delivery technology of choice should not only be efficient, but should also have minimal impact on the cell's health and normal function to ensure accurate and relevant results.

At Trince, we wanted to develop technology that could do exactly that. That's why we created the LumiPore™ intracellular delivery platform which delivers molecules into in vitro or ex vivo cells with high efficiency and minimal harm to cell structure and functionality. The LumiPore™ technology combines laser radiation with specially designed LumiSense nanoreagents to gently open up the cell membrane with nanometer precision. By forming pores in the cell membrane, external effector molecules can easily enter the cell's interior and produce their intended effect. As these pores are created with nanoscale precision, the damage done to cells is minimal.

Together with Revatis, we have explored the use of the LumiPore™ platform to deliver macromolecules into equine and human mesenchymal stem cells (MSCs). Revatis holds an exclusive license in human and veterinary applications of a patented, minimally-invasive method to produce large numbers of MSCs from a single muscle microbiopsy in a production unit that is GMP (good manufacturing practice) compliant.

Recently, interest in the mechanisms of action of MSCs has turned towards the analysis of the secretome and the release of microvesicles into the extracellular environment. Secreted bioactive factors modulate the immune response, reduce inflammation, inhibit cell death, and induce and stimulate endogenous regeneration.

As MSCs are attracted to inflammatory and tumor sites and are able to interact with targeted cells, the use of MSCs as drug carriers as well as genetic modification of MSCs will see significant developments in the near future. With this in mind, the minimally invasive approach developed by Revatis is a major asset.

As a proof-of-concept study, the LumiPore™ technology was used to demonstrate delivery of macromolecules in equine and human MSCs. The intracellular delivery parameters of the LumiPore™ system were first optimized by delivering fluorescently-labeled dextran molecules of 10 kDa as a model macromolecule. After that, the LumiPore™ platform was used to successfully transfect human MSCs with mRNA encoding for eGFP.

Materials and Methods

Primary equine mesenchymal stem cells (Revatis) were cultured in DF-20 complete cell culture medium which consisted of DMEM/Ham's F12 culture medium that was further supplemented with 20% heat-inactivated fetal bovine serum (FBS, GIBCO), 1% penicillin (1 000U/ml)-streptomycin (10 000µg/ml) (GIBCO) and 0.5% amphotericin B (250µg/ml) (GIBCO). Cells were passaged using DPBS (-Ca2+, -Mg2+, Gibco-Invitrogen) and trypsin-EDTA (0.25%, Gibco-Invitrogen) for a maximum amount of 5 times after starting the cell culture.

For intracellular delivery of FITC-dextran 10 kDa (FD10) in equine MSCs (eMSCs), cells were first seeded in a 96-well plate at 10 000 cells per well and incubated overnight in the incubator (37°C, 5% CO_2). After 24 hours, the adherent eMSCs were washed once with DPBS (-/-) and were then supplemented with DF-20 medium containing different concentrations of the LumiSense NS-Ia nanosensitizer. After 30 minutes of incubation, the eMSCs were washed once with DPBS medium to remove the unbound nanosensitizers. After washing, DF-20 medium, containing 1 mg/mL FD10, was added to the eMSCs. The cells were then treated in the LumiPore™ instrument using E3 and E7 laser settings. Flow cytometry was used to assess the delivery efficiency of FD10. The cellular viability was assessed after 2 hours with the metabolic Cell Titer Glo assay.

For intracellular delivery of FD10 in human MSCs (hMSCs), cells were cultured in MesenCult™-hPL Medium (StemCell Technologies) supplemented with MesenCult™-hPL 10X Supplement containing purified human platelet lysate (hPL) (StemCell Technologies).

The cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C until 80-90% confluency and were then detached using 0.25% trypsin/EDTA (Gibco-Invitrogen). LumiPore™ experiments, analysis of delivery efficiency and cell viability were performed similar as for eMSCs. All experiments on hMSCs were performed with a passage number below 8.

For transfection of hMSCs with mRNA, the cells were seeded at a density of 10 000 cells/well in a 96-well plate and allowed to grow in an incubator at 37°C, 24h prior to transfection. On the day of transfection, hMSCs were incubated for 30 min at 37°C with LumiSense NS-Ib nanosensitizer diluted in DF-20 complete cell culture medium at the specified concentrations, followed by a washing step with DPBS or Opti-MEM (Gibco-Invitrogen) to remove any unbound nanosensitizers. Prior to laser treatment, Enhanced Green Fluorescent Protein (eGFP) mRNA (0.1 mg/mL in DPBS with Ca2+/Mg2+) was added to the hMSCs. Cells were then treated in the LumiPore™ device using the E7 laser setting. The cells were analyzed by flow cytometry and with the Cell Titer Glo assay after 24h.

Results

The LumiPore™ technology was used to successfully deliver FD10 (FITC-dextran 10 kDa) into equine MSCs (eMSCs). The cells were first incubated with different concentrations of the LumiSense NS-Ia nanosensitizer, after which they were washed and supplemented with fresh cell culture medium containing 1 mg/ml FD10. Next, the cells were transferred to the LumiPore™ device for laser irradiation at different energy settings (E3 and E7). After laser treatment, the cells were washed and prepared for flow cytometric analysis. As the results in Figure 1 show, the percentage of FD10+ cells ("Efficiency %") as well as the amount of FD10 per cell (rMFI) increased for higher nanosensitizer concentrations. At the same time, the viability, as measured by a metabolic Cell Titre Glo assay 2h after treatment, gradually decreased. The best balance between delivery efficiency and cell viability was obtained for energy level E7 in combination with a 250x dilution of the nanosensitizer, reaching 83% FD10+ cells with a cell viability of 86%.

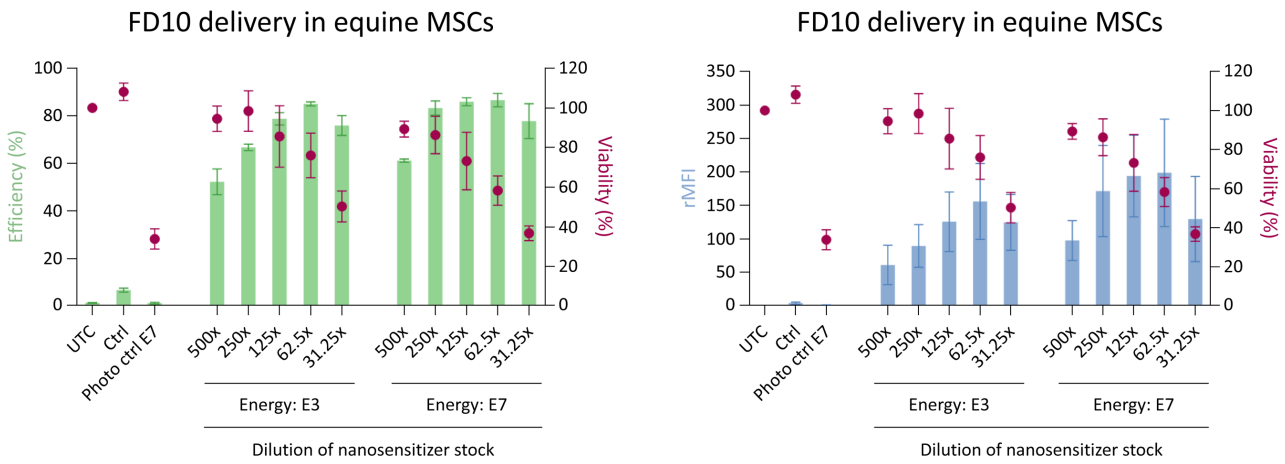

Figure 1. (Left) The percentage of FD10+ eMSCs (% Efficiency) for increasing nanosensitizer concentrations (expressed as dilutions of the stock solution) was measured by flow cytometry. **(Right)** The corresponding amount of FD10 fluorescence per cell is shown relative to non-treated cells (rMFI). The cell viability measured after 2h with the Cell Titer Glo assay is added to both graphs. UTC: untreated control, Ctrl: mock FD10 control (cells only exposed to FD10), Photo ctrl E7: mock photoporation control (cells treated with the LumiPore™ technology in the absence of FD10).

Similar experiments were performed on human MSCs (hMSCs) using energy level E7, of which the results are shown in Figure 2. In this case the best trade-off between delivery efficiency and viability was obtained for the highest nanosensitizer dilution of 160x, reaching 87% FD10+ cells for 90% viable cells.

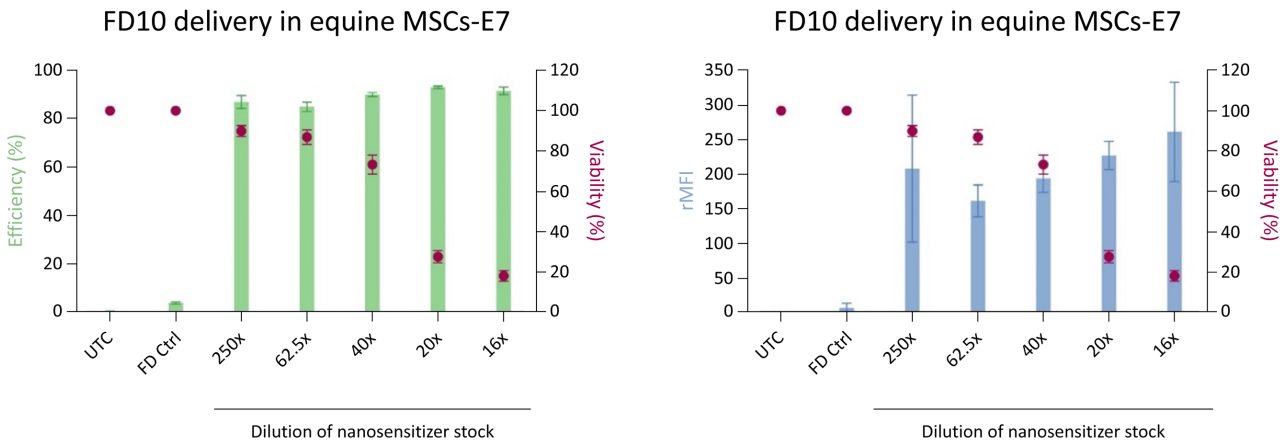

Figure 2. (Left) The percentage of FD10+ hMSCs ("% Efficiency") was measured by flow cytometry for increasing nanosensitizer concentrations (expressed as dilutions of the stock solution). **(Right)** The corresponding amount of FD10 fluorescence per cell is shown relative to non-treated cells ("rMFI"). The cell viability measured after 2h with the Cell Titer Glo assay is added to both graphs. UTC: untreated control, Ctrl: mock FD10 control (cells only exposed to FD10), Photo ctrl E7: mock photoporation control (cells treated with the LumiPore™ technology in the absence of FD10).

Finally, we performed transfection of hMSCs with 0.1 mg/mL eGFP-mRNA. In this case, the LumiSense NS-Ib nanosensitizer was used which is optimized for the intracellular delivery of large macromolecules. Based on the fluorescence signal measured by flow cytometry, an increasing percentage of transfected cells was again found for an increasing nanosensitizer concentration at the expense of cell viability (Figure 3). It shows that the user can finetune the conditions to find the desired balance between transfection efficiency and cell viability.

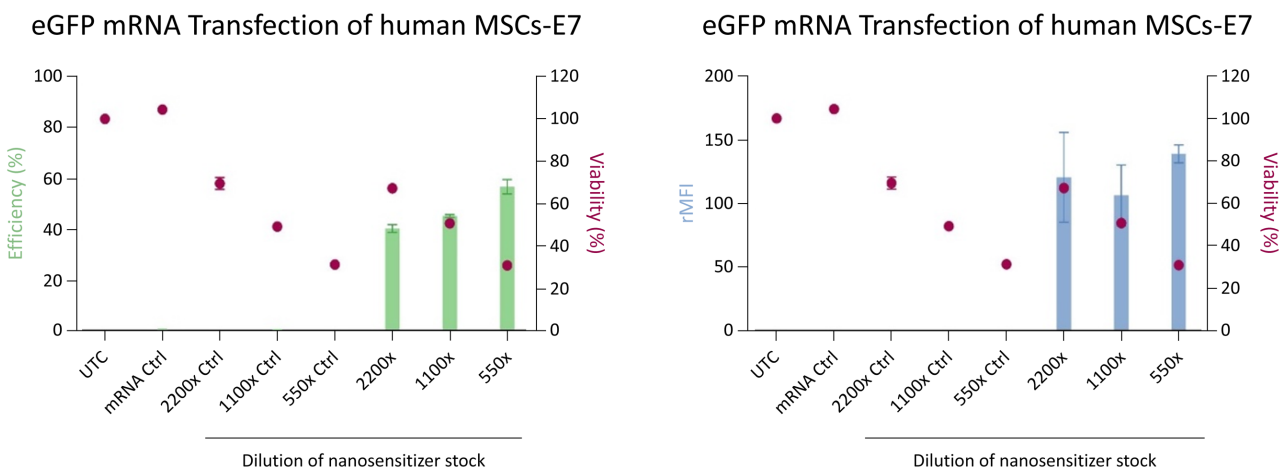

Figure 3. (Left) The percentage of eGFP+ hMSCs ("% Efficiency") was measured by flow cytometry for increasing nanosensitizer concentrations (expressed as dilutions of the stock solution). **(Right)** The corresponding amount of eGFP fluorescence per cell is shown relative to non-treated cells ("rMFI"). The cell viability measured after 24h with the Cell Titer Glo assay is shown in both graphs as well. UTC: untreated control, Photoporation Controls: 2200x Ctrl, 110x Ctrl and 550x Ctrl.

Conclusions

In this proof-of-concept study we have demonstrated that the LumiPore™ platform is able to efficiently deliver macromolecules in equine and human mesenchymal stem cells. By varying the nanosensitizer concentration and laser energy levels, an optimal balance between intracellular delivery efficiency and cell viability can be achieved. The results and data presented in this white paper demonstrate that the LumiPore™ technology is compatible with a broad variety of cell types and effector molecules.

About RevaTis

RevaTis, a spin-off from Liege University in Belgium, was founded in 2013 and is dedicated to advanced regenerative medicine and cell therapy. RevaTis has developed an innovative and patented technique to obtain pluripotent mesenchymal stem cells through a minimally invasive muscle micro-biopsy. RevaTis' technique involves the use of an aseptic isolator in a "turnkey" system, which meets GMP standards.

www.revatis.com

About Trince

Trince is a spin-off company from Ghent University dedicated to advancing cell-based science and therapeutics by facilitating the delivery of molecules into cells, both in vitro and ex vivo. The company's LumiPore transfection platform, which is covered by a portfolio of pending patents, combines laser exposure with photothermal nanoparticles to convert light energy into heat to transiently permeabilize the cellular plasma membrane. It can be used to deliver a wide variety of payloads into virtually any cell type, including hard-to-transfect cells. The technology is very gentle to cells, maximizing the therapeutic quality of the final product.

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