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Cell-specific transmembrane injection of molecular cargo with gold nanoparticle-generated transient plasmonic nanobubbles

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ABSTRACT

Optimal cell therapies require efficient, selective and rapid delivery of molecular cargo into target cells without compromising their viability. Achieving these goals *ex vivo* in bulk heterogeneous multi-cell systems such as human grafts is impeded by low selectivity and speed of cargo delivery and by significant damage to target and non-target cells. We have developed a cell level approach for selective and guided transmembrane injection of extracellular cargo into specific target cells using transient plasmonic nanobubbles (PNB) as cell-specific nano-injectors. As a technical platform for this method we developed a laser flow cell processing system. The PNB injection method and flow system were tested in heterogeneous cell suspensions of target and non-target cells for delivery of Dextran-FITC dye into squamous cell carcinoma HN31 cells and transfection of human T-cells with a green fluorescent protein-encoding plasmid. In both models the method demonstrated single cell type selectivity, high efficacy of delivery (96% both for HN31 cells T-cells), speed of delivery (nanoseconds) and viability of treated target cells (96% for HN31 cells and 75% for T-cells). The PNB injection method may therefore be beneficial for real time processing of human grafts without removal of physiologically important cells.

1. Introduction

Selective and efficient intracellular delivery of molecular cargo such as drugs, diagnostic and genetic agents into specific target cells determines the success of cell-based therapeutics. However, this function is impeded by cell defense systems and by limited efficacy, speed, selectivity and safety of various delivery technologies, especially in heterogeneous tissues where target cells are mixed with non-target cells and tissues. There are two major approaches to intracellular delivery: specific targeting of the vehicles that carry the linked or encapsulated cargo, and modification of the permeability of cellular membranes during their exposure to extracellular cargo.

The greatest selectivity and efficacy of delivery has been achieved using nanoparticles (NP) as carriers and as mediators of the cargo release [1–5]. However, in heterogeneous cell systems the selectivity of NP targeting is limited by the unavoidable coupling of NPs to non-target cells and dependence upon a release mechanism whose selectivity and activation are also limited. NP-based delivery (like any delivery platform) requires linking or encapsulating the cargo, which may fail to isolate it from the environment, potentially leading to bystander-cell toxicity. The encapsulation of toxic cargo may reduce non-specific toxicity [6], but at the same time limits cargo release at the destination to rates determined by slow diffusion and does not address the problem of selective intracellular release of cargo. Several methods have been developed for on-demand release of encapsulated cargo with laser radiation [2,3,6–8] but all require the use of complex liposome-like NPs with cargo and gold NPs, which are difficult to synthesize, must be engineered for each application, and can be unstable *in vivo*. Moreover, their unavoidable non-specific uptake by normal cells reduces the selectivity of the delivery.

Extracellular molecular cargo can also be delivered following non-specific permeabilization of cell membranes with mechanical, electrical or thermal impacts [9–12] but these methods cannot discriminate between target and bystander cells in a heterogeneous population. Furthermore, these methods do not actively deliver the cargo into cells, they just allow passive delivery due to the gradient of the cargo concentration outside and inside the cell. Hence, delivery is slow and the amount of cargo delivered cannot be

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