Genetic Materials

and the Means by Which Humans can Systematically Repattern Life

Stanley Bram Literature Seminar October 31, 2019

Stem cell therapy is already being realized for treatment of stroke, traumatic brain injury, neurodegenerative, and even genetic disease.[5] Mesenchymal stem cells (MSCs) are advantageous for cell therapy applications as they lack immune response, have inherent pluripotency, exhibit natural transport to sites of injury, and display reparative properties.[6] The ability to genetically manipulate stem cells using a routine approach that does not elicit cell death offers unprecedented opportunities to extend the therapeutic benefit of a patient's own cells to conquer disease, and potentially, to realize personalized medicine. Of the exogenous stem cells, (MSCs) are ideal candidates for cell therapy applications. Unfortunately, (MSCs) can be difficult to transfect without impacting the pluripotency or cell viability and often result in poor efficiencies.[7] Stem cells are known to be resistant to genetic manipulation with the few successful genetically modified lines produced using inefficient electroporation methods (40% Nucleofector) or very efficient viral vectors (80%), which have led to cases of leukemia during clinical trials albeit their efficiency.[1, 2, 8]

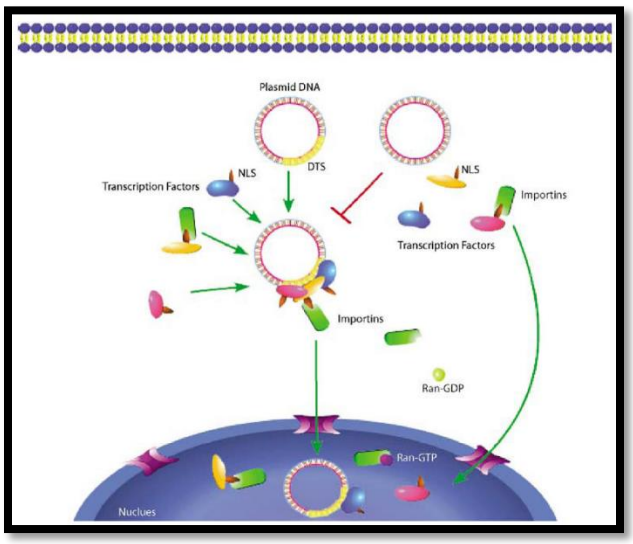

Figure 1. An illustration of the mechanism by which exogenous DNA may be introduced into a cell, and transported into the nucleus

Effective strategies for non-viral transfection of (MSCs) ex vivo typically employ disruption of cell membranes to transfer nucleic acids into cells, or packaging of nucleic acids with nanocarrier materials that facilitates cellular internalization through endocytosis. For both membrane disruption and nanocarrier-mediated delivery, intracellular barriers remain following nucleic acid delivery to the cytoplasm, including lysosomal and nuclease degradation, and for plasmid DNA (pDNA), cytoplasmic transport to and import through the nuclear membrane into the nucleus.[9] Nuclear localization of pDNA is eased in proliferative cell types due to dissolution of the nuclear membrane during mitosis; conversely, nuclear localization of plasmids is challenging in primary cell types such as (MSCs), which proliferate slowly and a hard to transfect.[2,10]

Thus, microinjection of plasmids into (MSCs) is efficient when delivered directly into the nucleus, demonstrated in single or few (MSCs) with nanoneedles 200-275 nm in diameter, with 75% viability retained after injection, and 65-75% reporter-transgene expression efficiency

(percentage of cells expressing transgene). However, microinjection is impractical for transfecting large numbers of cells.[2,11]

Electroporation is a higher throughput alternative to microinjection that applies transient electric fields to cell populations, typically in suspension, inducing pores in cell membranes that allow entry of nucleic acids into the cytoplasm, though induced charge association between nucleic acids and cell membranes --followed by endocytosis-- has been demonstrated as an alternative mechanism.[12] Electroporation is economical (not necessarily requiring additional reagents beyond suspension buffer) and is widely used for DNA and RNA transfection of (MSCs) with high efficiency. Though as with microinjection, nuclear localization is a primary barrier for plasmid delivery via electroporation. A commercial electroporation system known as Nucleofector (Lonza, Germany) employs cell-type specific electric field pulse parameters and proprietary suspension solution formulations to drive plasmid DNA transfer directly to the nucleus, a method termed nucleofection. Nucleofection of (MSCs) has been demonstrated to increase transfection efficiency of plasmid delivery relative to conventional electroporation, with approximately 68% transfection efficiency in (MSCs) electroporated in Nucleofector buffer suspension, and subsequently re-plated.[13,14] However, cell viability after nucleofection was reported as 54%. While effective at transfection, electroporation is limited by cytotoxicity, which is attributed to effect of the pulsed electric fields on biomolecules, including electro-conformation change of lipid membranes, proteins, and DNA, and oxidative damage from generated reactive oxygen species.[2]

The primary alternative to electroporation for nucleic-acid transfer into (MSCs) ex vivo is transfection with nanocarriers: materials that electrostatically condense or encapsulate nucleic acids into nanoparticles or aggregate complexes that favorably associate with cell membranes through charge interaction or surface receptor binding, and are subsequently internalized via micropinocytosis, clathrin-mediated endocytosis, or caveolae-mediated endocytosis, depending primarily on nanoparticle size and charge. Generally, size and charge of nanoparticles can be tuned by varying the ratio of nucleic acid to nanocarrier. A wide variety of carriers have been demonstrated to facilitate transfection of (MSCs), including polymers, lipids, polysaccharides, peptides, and inorganic materials.[2]

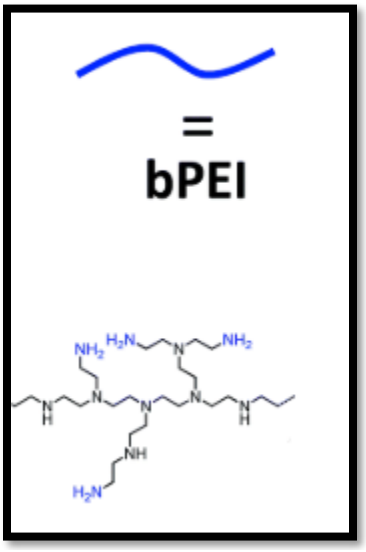

Figure 2. The structure of branched polyethylene imine –the cheapest and most used cationic transfection polymer.

Cationic lipids are, by far, the most commonly used for transfection of (MSCs). As an example, Hoare et al. transfected (MSCs) with pDNA encoding for enhanced green fluorescent protein (eGFP), complexed with the commercially available cationic lipid transfection reagent, Lipofectamine (LF2000). Transfection efficiency increased from 20 to 40% and viability decreased from 80 to 50%, as the lipid/pDNA (v/w) ratio increased from 5 to 20, respectively.[16] A newer version of Lipofectamine, LF-LTX, was used by Kelly et al. and achieved 2-6% transfection efficiency after 48 hours, in (MSCs), with significantly decreased

metabolic activity compared to untransfected cells. Meanwhile, the latest Lipofectamine iteration, LF3000, achieves up to 26% transfection efficiency in (MSCs), according to de Carvalho et al., though viability was not reported. [2, 17, 18]

For comparison of several other types of nanocarriers, Gonzalez-Fernandez et al. tested biocompatible mineral nan-hydroxyapatite (nHA), the ubiquitous cationic polymer transfection reagent 25 kDa branched polyethyleneimine (bPEI), and repeating arginine-alanine-leucine-alanine (RALA) amphipathic peptide, for porcine (MSCs) transfection with pDNA encoding GFP. All three nanocarriers exhibited transfection efficiency between 15 and 20% and metabolic activity was not significantly decreased after 3 days, except for PEI, which exhibited a 30% decrease in metabolic activity, relative to a non-transfected control.[2, 19]

The highest transfection efficiency reported in the literature for transfection of (MSCs) via nanocarriers has been achieved by biocompatible and degradable poly(β-aminoesters) (pβ-AE). In Mangraviti et al., a library of (pβ-AEs) were screened in high-throughput to determine which polymers could mediate high transfection without decreased viability in (MSCs). The highest-performing polymer, had a molecular weight of 8.5 kDa, and when mixed with DNA in a 40:1 ratio, by mass, achieved 75% transfection efficiency and 71% viability. These aminoester carriers achieve transfection efficiency similar to, and viability higher than, optimized electroporation methods, and are therefore promising candidates for scaling non-viral gene delivery to (MSCs) for clinical applications.[2, 20]

Interestingly enough, the ability to form geometric nanostructures from DNA itself (termed DNA nanotechnology) has also been explored as a method to introduce genetic material. DNA nanotechnology leverages the programmability of DNA base-pairing to assemble DNA-nanostructures into custom, pre-designed shapes via sequence-specific hybridization of template and staple DNA strands. Zhang et al. explored DNA-nanotechnology as a biomolecule-delivery platform. The group designed DNA-nanostructures of controllable size, shape, stiffness, and compactness with attachment loci, onto which DNA/RNA/Protein-cargoes may be conjugated. By hybridizing fluorophore-conjugated-DNA strands onto the loci of the DNA-nanostructures, the possibility emerged to track nanostructure internalization into the cell cytoplasm, concluding the stiffness and size to be important design elements for nanostructure internalization.[3]

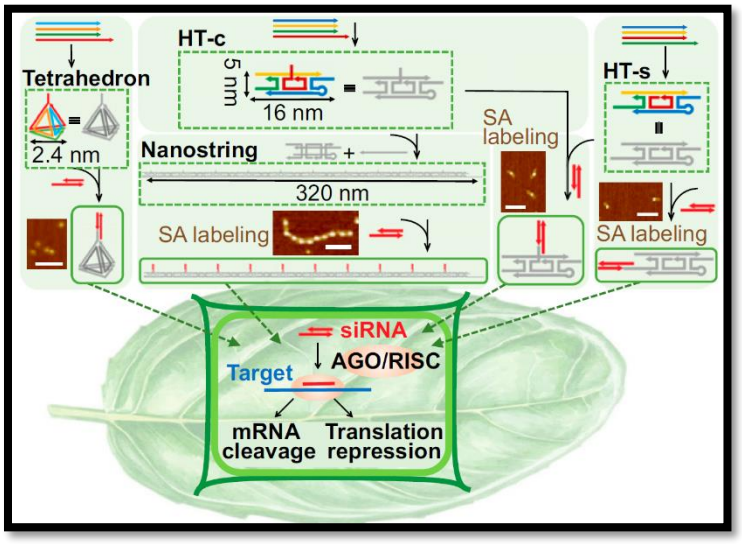

Figure 3. An illustration of the different geometries of DNA nanostructures that may be synthesized, and subsequently internalized, into plant cells.

It may be worth noting, the contents described above (pDNA, mRNA, etc…) are mere examples, and these delivery models may be extended to emerging technology such as the CRISPR/Cas 9 system, as well.[4]

References

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