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Perspective

A New Tool for Oligonulceotide Import into Cells

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The field of gene therapy potentially offers physicians an entirely new set of armaments with which to battle disease. The approach, radical in concept but powerful in its potential effect, generically aims to correct defective genes responsible for disease development by inserting the normal gene into a nonspecific location in the genome or by swapping out the abnormal gene through homologous recombination. Perhaps more tangible, near-term approaches are those that look to either repair the abnormal gene via selective reverse mutation or "knockdown" the expression of the mutated gene. Delivery of oligonucleotides, whether as entire genes or shorter antisense strands, remains one of the most significant challenges in the field. The linchpin to success is the ability to efficiently and selectively insert oligonucleotides into the desired cells. Current efforts in the application of oligonucleotide delivery in this and other arenas are restricted by the relatively low efficiency of methods used for oligonucleotide transfer. In addition, potential hazards exist with any method that introduces foreign biological material into the body—toxicity issues as well as the potential for mounting an immune response against a specific gene delivery vehicle complicate the challenge.

It is against this backdrop that a series of papers out of the Mirkin group at Northwestern University is significant *(1–4)*. Few disagree with the power that oligonucleotide probes offer as detectors of nucleic acids and regulators of gene expression in living cells. However, the utility of such probes is hampered by difficulties in cellular uptake and degradation once inside the cell. Coupling these probes to gold nanoparticles is one approach that may have measurable utility. The use of biomolecule-modified nanoparticles by Mirkin and colleagues has established that these entities may present a new arsenal of versatile tools for intracellular reporting and gene expression control. These tools will facilitate research in living, functioning cells, possibly leading to novel therapeutic agents and perhaps laying the groundwork for a new generation of molecular vehicles for oligonucleotide delivery.

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Cellular Uptake

Gold nanoparticle–oligionucleotide complexes can regulate protein expression in cells *(1)*. The advantages over other methods are that the complexes do not require a separate transfection agent to enter the cell in substantial quantities and once there are less susceptible to DNase digestion. More than 60% of the oligionucleotides remain bound to the gold nanoparticles after 48 h inside C166 cells, a mouse endothelial cell line. Further experiments showed that bound oligonucleotides were less susceptible to enzymatic degradation, opening the possibility for longer knockdown than oligonucleotides alone. The nanoparticles outperformed commercially available transfection agents (lipoplexes) in percentage knockdown in expression of enhanced green fluorescent protein, total antisense oligonucleotide delivery, and observed nontoxicity. The expression was decreased by 6%–8% with the commercial kits, but the nanoparticles decreased expression by up to 20% *(1)*. Further studies confirmed that the uptake of nanoparticles is high across multiple cell lines *(2)*, more than 3 orders of magnitude higher than the uptake of unmodified nanoparticles alone. The modified nanoparticles were shown to change markedly in both diameter and surface potential on exposure to cell culture media. This effect is attributed to the binding of positively charged serum proteins to the nanoparticles, a process that is key to cellular uptake. The nontoxic cellular uptake of large masses of stable oligonucleotides opens the possibility of delivering reporters and controllers of gene activity in unprecedented ways.

Nano-flares

Probes to detect intracellular levels of RNA are often difficult to transfect and susceptible to degradation. Recently, Seferos et al. showed that oligonucleotide-modified nanoparticles could be used as intracellular reporters of gene expression in living cells *(3)*. Nano-flares combine transfecting and reporting RNA concentrations in living cells by hybridizing a fluorescently labeled oligonucleotide with its bound complement. When hybridized, the fluorophore is quenched by the gold nanoparticle. The bound oligonucleotides are complementary to the target mRNA sequences and, in the presence of the correct mRNA, the fluorescent reporter is released from the nanoparticle as the mRNA binds to the bound complement. Fluorescence data from

a population of nano-flare–treated cells was obtained, with a 2.5-fold increase in fluorescence in the surviving recognition sequence compared to a population treated with noncomplementary nano-flares. The background fluorescence levels of noncomplementary molecular beacons in a population of cells from the SKBR3 human breast-cancer cell line were almost twice those of the background of noncomplementary nano-flare–treated cells. Ultimately, the nano-flares compared favorably to RT-PCR quantification of survivin expression in cells exposed to small interfering RNA.

Peptide Antisense Nanoparticles

Patel et al. demonstrated the synthesis of gold nanoparticles modified with both synthetic peptides and oligonucleotides designed to regulate gene expression, a technique that may lead to novel therapies *(4)*. Building on their previous work with proteins facilitating intracellular trafficking, the peptides bound to the nanoparticles were linked to increased cellular uptake and/or altered intracellular localization. The peptide–oligonucleotide-modified nanoparticles showed a 75% decreased expression of glyceraldehyde 3-phosphate dehydrogenase, whereas oligonucleotide-modified nanoparticles showed only a 50% decrease in expression *(4)*. Interestingly, the increased knockdown was not associated with greater numbers of nanoparticles per cell, but the nanoparticles were reproducibly associated more closely to the cell's nucleus.

Synopsis

A convincing body of work increasingly suggests that gold nanoparticles could see wide application for intracellular reporting and gene expression control. The reports surveyed here suggest the evolution of nanoparticle-based therapeutic approaches, offering a more efficient knockdown of gene expression but without the need for the transfection agents that often negatively augment cellular function. As with other emerging technologies, and particularly with nanotechnology-based tools, short- and long-term toxicity must be studied, as well as the effect on cellular processes in vivo and on the immune system. As recently noted by Austin and Lim, the perils of nanotechnology in medicine must be carefully weighed *(5)*. Although gold is chemically inert and generally regarded as safe in biological organisms, its properties in nanoparticle form are less understood and must be evaluated. Even with the inertness of gold, its pharmacokinetics and pharmacodynamics in any nanoparticle-based approach will have to be evaluated and understood. However, that should not taint our view of the potential advances in oligonucleotide delivery described in these reports.

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