Research Highlights

Highlights from the latest articles in nanomedicine

Aptamer-conjugated gold nanoparticles for intracellular targeted molecular imaging

Evaluation of: Zheng D, Seferos DS, Giljohann DA, Patel PC, Mirkin CA: Aptamer nano-flares for molecular detection in living cells. *Nano Lett.* 9(9), 3258–3261 (2009).

Intracellular targeted molecular imaging for disease detection is an important topic in nanomedicine and has been challenging due to two aspects. One is the availability of the molecular probes for the target molecules, which are of high selectivity and affinity, and another is the cellular internalization of the molecular probes. Aptamers are nucleic acids or peptides that exhibit high selectivity and affinity for target molecules and, thus, can be an ideal molecular probe for targeted molecular imaging. It has been reported that an aptamer-based probe, which consists of a fluorophore-labeled DNA competitor and a quencher-labeled aptamer, can be used for small-molecule detection. Aptamers are also well suited for cellular applications, including diagnostics and therapeutics. For example, an aptamer–gold nanoparticle-based system, which makes use of a marker for PDGF in homogeneous solution, has been developed for sensitive detection of cellular growth and proliferation. However, cellular uptake of an aptamer-based system capable of entering a cell and remaining active in the intracellular environment has proved to be a problem and it is difficult to affect the cellular uptake of nucleic acid-based agents without additional agents for improving cellular internalization. Moreover, oligonucleotides can become unstable and degraded in cellular environments and, thus, their application *in vivo* has been challenging.

In this article, Mirkin *et al.* at Northwestern University International Institute of Nanotechnology (IL, USA)

developed a novel molecular probe of aptamer-conjugated gold nanoparticles, which they termed aptamer nano-flares, for detection and quantification of intracellular molecular analytes. These nanostructures consist of a gold nanoparticle that is functionalized with a dense monolayer (~ 8.4 pmol/cm²) of chemisorbed aptamer oligonucleotides hybridized to fluorophore-labeled flares. The dense shell of oligonucleotides is expected to provide enhanced stability and a mechanism for cellular entry, and the flare is expected to be capable of transducing a binding event into a fluorescence signal. In addition to synthesis and characterization, they determined the sensitivity and selectivity of the approach in the detection of ATP and evaluated the ability to quantify the intracellular concentration of this target molecule.

In this work, ATP was chosen as a target molecule for proof-of-concept experiments aimed at developing nanoconjugates that quantify concentrations of small-molecule analytes inside living cells. ATP is important for regulating cellular metabolism and biochemical pathways. The intracellular ATP level is used as an indicator of living organisms and their biological activity. Current methods of ATP detection require the lysis of cells and only measure the ATP concentration of a bulk sample. Such aptamer nano-flares were designed in a way that the flare oligonucleotide can bind to the aptamer attached on the surface of the gold nanoparticle (FIGURE 1). In this bound state, the fluorescence of the flare strand is quenched by the gold nanoparticle. In the presence of the ATP target molecules, ATP binds to the aptamer, causing a conformational change and resulting in a new folded secondary structure. This folded structure disrupts the Watson–Crick base pairing

Si-Shen Feng

National University of Singapore,
Department of Chemical & Biomolecular
Engineering and Division of Bioengineering,
Block E5, 02-11, 4 Engineering Drive,
117576 Singapore
Tel.: +65 6516 3835;
Fax: +65 6779 1936;
chefss@nus.edu.sg

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Figure 1. Synthesis of aptamer nano-flares, which are designed so that the flare oligonucleotide binds to the aptamer that is attached to the surface of the gold nanoparticle. (A) Aptamer nano-flares are gold nanoparticles functionalized with thiol-terminated aptamer sequences hybridized to a short complementary Cy5-labeled reporter strand. The reporter is capable of being displaced by a conformation change in the aptamer that is induced by the target molecule. **(B)** Oligonucleotide sequences used to prepare the aptamer nano-flares. Reproduced with permission from [1].

between the aptamer and the flare, which causes flares to be liberated with an increase in fluorescence owing to the greater distance of the flare from the gold surface.

The authors demonstrated that their aptamer nano-flares showed a significant fluorescence increase upon target addition. When 2 mM ATP was added, an immediate 3.8-fold increase in fluorescent signal was observed. They also quantified the intracellular signaling of the aptamer nano-flares by analytical flow cytometry of the cells treated with probes, which revealed that the HeLa cell population treated with

aptamer nano-flares was highly fluorescent and 2.6-times more fluorescent than the population treated with mismatch control particles. These flow cytometry experiments were in excellent agreement with confocal imaging and demonstrated the uniform cellular internalization and intracellular signaling of the aptamer nano-flares.

Reference

- 1 Zheng D, Seferos DS, Giljohann DA, Patel PC, Mirkin CA: Aptamer nano-flares for molecular detection in living cells. *Nano Lett.* 9(9), 3258–3261 (2009).

Mesoporous hybrid silica nanoparticles for targeted intracellular delivery of hydrophobic imaging and therapeutic agents

Evaluation of: Rosenholm JM, Peuhu E, Eriksson JE, Sahlgren C, Linden M: Targeted intracellular delivery of hydrophobic agents using mesoporous hybrid silica nanoparticles as carrier systems. *Nano Lett.* 9(9), 3308–3311 (2009).

Linden's group successfully developed a strategy by using mesoporous hybrid silica nanoparticles as a carrier for targeted intracellular delivery of hydrophobic imaging and therapeutic agents. Many of the currently investigated nanotechnology approaches for the delivery of hydrophobic drugs rely on surfactant or polymer-related

formulations. However, cell-specific targeting often remains a challenge. Since the beginning of the millennium, much research has focused on applying mesoscopically ordered nanoporous silica materials as drug-delivery systems, as these materials can carry a large amount of payload as a consequence of their high

specific surface area and pore volume. Thus, current efforts lie in introducing cell-specificity to these materials. A successful intracellular delivery system should be able to be internalized by the right population of cells and then release its cargo into the cytoplasm once taken up by the cell. Simultaneously, premature leaking of cargo before cellular internalization is not desirable. In this work, the authors demonstrated that poly(ethylene imine)–mesoporous silica hybrid particles could specifically target cancer cells under co-culture conditions with normal cells using folic acid as the targeting ligand. The targeted intracellular drug-delivery ability of this system was verified using two hydrophobic fluorophores, DiI and DiO, as model drugs, which made it possible to follow the intracellular release by confocal fluorescence microscopy. Furthermore, the use of fluorophores as model cargo allows quantification of the intracellular delivery using flow cytometry.

Partially aminofunctionalized mesoporous silica nanoparticles with a mean diameter of approximately 300 nm were synthesized by using a mixture of aminotrimethoxysilane and tetramethoxysilane as the silica precursors and cetyltrimethylammonium chloride as the structure-directing agent. An ethanolic fluorescein isothiocyanate (FITC) solution was added in the synthesis step to create inherently fluorescent nanoparticles. Poly(ethylene imine) (20 wt%) was grown onto the mesoporous silica particle by hyperbranching surface polymerization according to procedures described in their earlier publications [1,2]. Folic acid was conjugated to the particles. The mesoscopic ordering of the pore system was verified by x-ray diffraction analysis, and the particle morphology was determined by scanning-electron microscopy (FIGURE 2A). Confocal microscopy analysis of the FITC fluorescence demonstrated a dotted pattern of fluorescent particles indicating that the particles were located in intracellular vesicles (FIGURE 2B). As they showed before, the preferential uptake mechanism of the folic acid-tagged particles by HeLa cells was by receptor-mediated endocytosis. To further verify the active endocytosis mechanism, the uptake of nanoparticles was analyzed under conditions where cellular endocytosis was

perturbed, by incubating the cells at low temperatures or by inhibiting the dynamics of the actin cytoskeleton. Both methods of blocking endocytosis efficiently inhibited cellular uptake of particles, as demonstrated by flow cytometric analysis of intracellular FITC fluorescence (FIGURE 2C). The potential of the system to deliver poorly water-soluble drugs to cells and the subsequent endosomal escape of the cargo was analyzed using particles loaded with lipophilic dyes. DiI or DiO dissolved in

Figure 2. Folic acid/poly(ethylene imine)/fluorescein isothiocyanate nanoparticles are actively endocytosed by cells. (A) X-ray diffraction analysis and scanning-electron microscopy imaging of mesoporous silica nanoparticles. The scale bar corresponds to 1 μm . **(B)** HeLa cervical carcinoma cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin in 37°C, 5% CO_2 . Nanoparticles were suspended in medium at a concentration of 10 $\mu\text{g}/\text{ml}$ and incubated with the cells for 24 h. The cells were fixed with 3% paraformaldehyde for 10 min and mounted with DAPI Vectashield® (Vector Laboratories) for nuclear labeling. Cells were viewed by Zeiss LSM 510 META laser-scanning confocal microscope (40 $\times$ oil objective, 405/488 nm excitation, 3D projection). **(C)** HeLa cells were incubated for 3 h with 10 $\mu\text{g}/\text{ml}$ nanoparticles. To inhibit actin polymerization, the cells were pretreated with 10 μM CytD for 30 min and incubated in 5 μM CytD during nanoparticle uptake. Alternatively, the cells were kept at +4°C at all times. Intracellular fluorescein isothiocyanate fluorescence (FL-1 channel) was analyzed by flow cytometry (FacsCalibur, BD Pharmingen; mean $\pm$ standard deviation; n = 3–5). CytD: Cytochalasin D. Reproduced with permission from [3].

cyclohexane were loaded (1 wt%) into the mesoporous particles. No residual fluorophore remained in the supernatant after loading, indicative of quantitative loading. After vacuum drying overnight to remove any excess solvent, the particles were washed in a 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution (25 mM, pH 7.2) and resuspended in HEPES buffer at a concentration of 1 mg/ml. The particles were easily dispersible in HEPES, indicating that the amount of hydrophobic dye present on the outer surface of the particles was small. Initially, the nanoparticles, along with their cargo, were compartmentalized in cellular vesicles, as demonstrated by highly overlapping green (FITC) and red (DiI) fluorescence. However, after 24 h of incubation, a strong red coloration of the cellular body demonstrated successful release of DiI into the cytoplasm (FIGURE 2B), whereas the FITC-labeled particles remained in the vesicular

compartment. Importantly, fixation of cells led to an immediate endosomal escape of the dye, emphasizing the importance of performing the analysis of nanoparticulate intracellular delivery in live cells.

References

- 1 Rosenholm JM, Penninkangas A, Lindén M: Amino-functionalization of large-pore mesoscopically ordered silica by a one-step hyperbranching polymerization of a surface-grown polyethyleneimine. *Chem. Commun.* 37, 3909–3911 (2006).
- 2 Rosenholm JM, Lindén M: Wet-chemical analysis of surface concentration of accessible groups on different amino-functionalized mesoporous SBA-15 silicas. *Chem. Mater.* 19, 5023–5034 (2007).
- 3 Rosenholm JM, Peuhu E, Eriksson JE, Sahlgren C, Linden M: Targeted intracellular delivery of hydrophobic agents using mesoporous hybrid silica nanoparticles as carrier systems. *Nano Lett.* 9(9), 3308–3311 (2009).