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Nonviral gene delivery

Thinking of silica

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We have known for a while that silica is a promising candidate gene delivery vector. New *in vivo* data from Dhruba Bharali and co-workers raise the hopes a notch for such approaches by showing that organically modified silica nanoparticles (that the authors call ORMOSIL) can be used to deliver genes to the brain.[1]

Silica, a major and natural component of sand and glass, has been employed in material sciences and engineering for many years. It is a versatile material due to the variety of available chemical and physical modifications that are available. Silica is also a relatively benign material due to its biocompatibility – many people eat it as a dietary supplement. Recently, silica has been used for gene delivery, as researchers around the world have pursued more efficient DNA delivery vectors for both basic research and clinical trials.

For nonviral gene delivery, DNA must be complexed with transfection reagents before it can be efficiently delivered into cells. Most DNA transfection reagents utilize the cationic nature of synthetic polymers, dendrimers, and lipids to condense plasmid DNA. Pure silica nanoparticles without surface modifications do not seem to be able to condense and deliver DNA. However, in cultured cells they are able to aid other cationic transfection reagents for enhanced gene delivery, primarily due to a 'concentration' mechanism where dense silica nanoparticles concentrate DNA-transfection reagent complexes at the surface of cell monolayers due to gravity.[2] These silica-mediated DNA delivery systems turned out to be very versatile, and were further developed into modular transfection systems, in which different components (including fluorescent dyes) can be incorporated to aid in intracellular tracking.[3–5]

Additional surface modifications greatly extended the utility of silica nanoparticles in DNA delivery. For example, silica nanoparticles modified with aminosilanes (either *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane or *N*-(6-aminohexyl)-3-aminopropyltrimethoxysilane) were able to condense and deliver DNA, very much like cationic polymers,[6,7] without the addition of other cationic transfection reagents. Other cationic silica nanoparticles with surfaces modified by amino-hexyl-aminopropyltri-methoxysilane (AHAPS) were also reported recently to be successful transfection reagents.[8] Moreover, a combination of polyamidoamine (PAMAM) dendrimer and silica nanoparticles was reported recently: mesoporous silica nanoparticles were covalently attached to low generation PAMAM dendrimers, leading to a transfection reagent that complexed and delivered DNA.[9]

Building upon their earlier success in fabricating ORMOSIL – which are essentially amino-functionalized ultra-fine silica nanoparticles[5] – Bharali *et al*.[1] now report its use for *in vivo* gene delivery in the brain. ORMOSIL's transfection efficiency was equal to or even better than Herpes Simplex Virus-1 (HSV-1). Moreover, ORMOSIL-mediated delivery does not cause the tissue damage or immunological side effects that have been commonly observed with viral-mediated gene delivery.[10,11]

According to this new report, the procedures for ORMOSIL-mediated gene delivery are relatively simple. Ultra-fine silica nanoparticles are first surface modified with cationic amine groups. DNA is then complexed with these particles through a 30-min incubation in phosphate-buffered saline (PBS). The DNA-complexed ORMOSIL is then directly injected into the substantia nigra pars compacta (SNc): an area of the brain that is richly populated with neuronal cells.

In the studies reported in *PNAS*, a reporter gene, enhanced green fluorescent protein gene (EGFP), was first used to assess the overall efficiency of *in vivo* transfection. After the initial validation, a functional gene, the nucleus-targeting fibroblast growth factor receptor type 1 (FGFR-1), was used to further demonstrate the efficient gene delivery capability of ORMOSIL. In particular, an area of the brain that generates neural stem cells was targeted: the subventricular zone (SVZ) of the lateral ventricle (LV). Unlike surface FGFR-1, nucleus-targeting FGFR-1 causes cells to withdraw from the cell-cycle, which results in neuronal differentiation (as opposed to proliferation).

The results were encouraging: after intraventricular injection of ORMOSIL-FGFR-1 complexes, a significant inhibition was observed of the *in vivo* incorporation of bromodeoxyuridine into the DNA of the cells in the SVZ and the adjacent rostral migratory stream, which suggest that the proliferation of these cells was indeed greatly inhibited. These new experiments clearly demonstrate that ORMOSIL can be used not only for tracking the fate of delivered silica nanoparticles in the brain via delivery of reporter genes such as EGFP, but also for monitoring or even controlling the cell biology *in situ* via efficient *in vivo* delivery of clinically relevant genes such as nucleus-specific FGFR-1. That these new genetic manipulations occurred without the use of viral vectors, and were enabled by silica nanoparticles, is provocative. These studies will surely encourage others to investigate the role of nanoparticle chemistry and morphology on gene transfer in animals.

From work accumulated over the past 5 years, it is now clear that no single nonviral vector will serve as a universal gene delivery vehicle. Different clinical settings and different anatomic sites require different materials and different approaches to deliver genes. Bharali *et al*., have demonstrated for the first time that silica nanoparticles are promising candidates for efficient DNA delivery in the brain. This work and other similar studies also makes it clear that there is little correlation between *in vitro* and *in vivo* gene delivery using most transfection systems. Traditional ways of testing new DNA delivery agents, by using cultured model cell lines with reporter genes, may need to be re-evaluated and changed. Instead, specific cells

that best represent tissue and intended genes, should be assessed in the beginning. Better yet, direct animal experiments should be carried out at a very early stage. ■

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1 Bharali DJ, Klejbor I, Stachowiak EK, Dutta P, Roy I, Kaur N *et al.* Organically modified silica nanoparticles: a nonviral vector for *in vivo* gene delivery and expression in the brain. *Proc Natl Acad Sci USA* 2005; **102**: 11539–11544.
2 Luo D, Saltzman WM. Enhancement of transfection by physical concentration of DNA at the cell surface. *Nat Biotechnol* 2000; **18**: 893–895.
3 Luo D, Han E, Belcheva N, Saltzman WM. A self-assembled, modular DNA delivery system mediated by silica nanoparticles. *J Control Release* 2004; **95**: 333–341.
4 Gemeinhart RA, Luo D, Saltzman WM. Cellular fate of a modular DNA delivery system mediated by silica nanoparticles. *Biotechnol Prog* 2005; **21**: 532–537.
5 Roy I, Ohulchanskyy TY, Bharali DJ, Pudavar HE, Mistretta RA, Kaur N *et al.* Optical tracking of organically modified silica nanoparticles as DNA carriers: a nonviral, nanomedicine approach for gene delivery. *Proc Natl Acad Sci USA* 2005; **102**: 279–284.
6 Kneuer C, Sameti M, Bakowsky U, Schiestel T, Schirra H, Schmidt H *et al.* A nonviral DNA delivery system based on surface modified silica-nanoparticles can efficiently transfect cells *in vitro*. *Bioconjug Chem* 2000; **11**: 926–932.
7 Kneuer C, Sameti M, Haltner EG, Schiestel T, Schirra H, Schmidt H *et al.* Silica nanoparticles modified with aminosilanes as carriers for plasmid DNA. *Int J Pharm* 2000; **196**: 257–261.
8 Kumar MNVR, Sameti M, Mohapatra SS, Kong X, Lockey RF, Bakowsky U *et al.* Cationic silica nanoparticles as gene carriers: Synthesis, characterization and transfection efficiency *in vitro* and *in vivo*. *J Nanosci Nanotechnol* 2004; **4**: 876–881.
9 Radu DR, Lai CY, Jeftinija K, Rowe EW, Jeftinija S, Lin VS. A polyamidoamine dendrimer-capped mesoporous silica nanosphere-based gene transfection reagent. *J Am Chem Soc* 2004; **126**: 13216–13217.
10 Assessment of adenoviral vector safety and toxicity: report of the National Institutes of Health Recombinant DNA Advisory Committee. *Hum Gene Ther* 2002; **13**: 3–13.
11 Muruve DA. The innate immune response to adenovirus vectors. *Hum Gene Ther* 2004; **15**: 1157–1166.