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A Polyamidoamine Dendrimer-Capped Mesoporous Silica Nanosphere-Based Gene Transfection Reagent

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Recent reports in the literature have demonstrated that polyamidoamine (PAMAM) dendrimers can serve as nonviral gene transfection reagents.[1] However, only those PAMAMs of high generations (G > 5) have been shown to be efficient in gene transfection.[1a] The required procedures for the synthesis and purification of these high-G PAMAMs are usually tedious and low-yield. In contrast, the low-G PAMAMs (G < 3) are nontoxic and easy to synthesize. Despite the benefits, the smaller molecular sizes and the limited surface charges of the low-G PAMAMs prohibit efficient complexation with plasmid DNAs in solution due to the entropy penalty.[1a]

Herein, we report a novel gene transfection system, where second generation (G2) PAMAMs were covalently attached to the surface of a MCM-41-type mesoporous silica nanosphere (MSN)[2] material as depicted in Figure 1. The G2-PAMAM-capped MSN material (G2-MSN) was used to complex with a plasmid DNA (pEGFP-C1) that codes for an enhanced green fluorescence protein.[3] We have investigated the gene transfection efficacy, uptake mechanism, and biocompatibility of the G2-MSN with neural glia (astrocytes), human cervical cancer (HeLa), and Chinese hamster ovarian (CHO) cells.

In contrast to other recently reported silica nanoparticle-based gene transfer systems,[4] the mesoporous structure of the MSN allows membrane-impermeable molecules, such as pharmaceutical drugs and fluorescent dyes, to be encapsulated inside the MSN channels.[2,5] The system renders the possibility to serve as a universal transmembrane carrier for intracellular drug delivery and imaging applications. To the best of our knowledge, no uptake study of MCM-41-type mesoporous silicas into eukaryotic cells have been reported before.

To construct the G2-MSN gene transfer system, a MSN material with an average particle size of 250 nm (avg pore diameter = 2.7 nm) was synthesized via our previously reported method.[2] 3-Isocyanatopropyltriethoxysilane (0.25 mL, 1 mmol) was grafted onto the pore surface of the MSN (1 g) in 80 mL of toluene for 20 h to yield the isocyanatopropyl-functionalized MSN (ICP-MSN) material. To visualize the interaction of MSNs and cells, we prepared a G2-MSN material loaded with a fluorescent dye (Texas Red). ICP-MSN (0.15 g) was added to an anhydrous ethanol solution of Texas Red (5 mM), and the mixture was stirred for 20 h. The amine-terminated G2-PAMAMs were used as caps to encapsulate Texas Red molecules inside the porous channels of ICP-MSN. An ethanol solution of G2-PAMAM (0.11 mmol) was added to the MSN/Texas Red solution for 20 h to form urea linkage between amines of PAMAM and ICP groups of MSN (Figure 1 inset). The structure of G2-PAMAM-capped, Texas Red-encapsulated MSN was scrutinized by X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), N_2 sorption isotherms, and ^{13}C CP-MAS NMR spectroscopy.[6]

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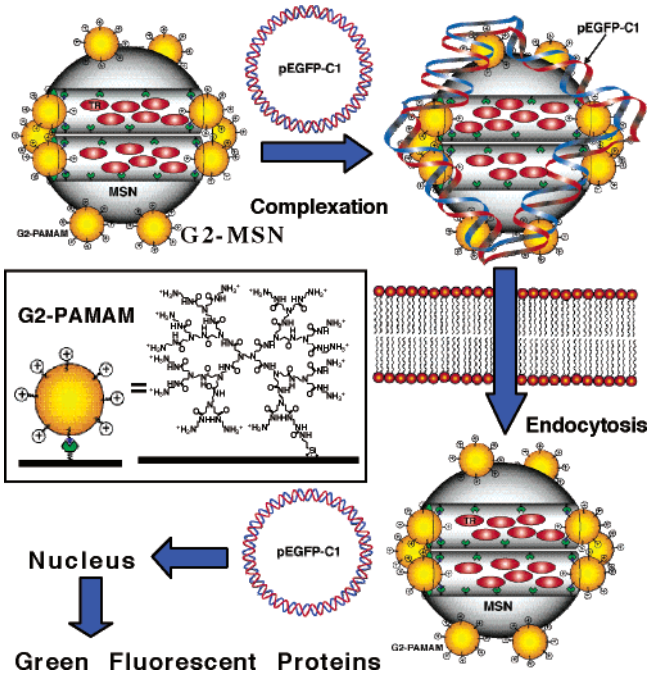

Figure 1. Schematic representation of a nonviral gene transfection system based on a Texas Red (TR)-loaded, G2-PAMAM dendrimer-capped MSN material complexed with an enhanced green fluorescence protein (*Aequorea victoria*) plasmid DNA (pEGFP-C1).

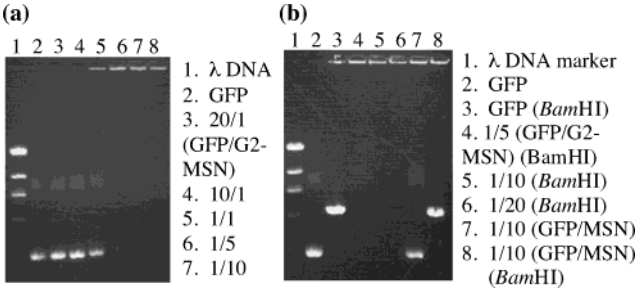

Figure 2. Complexation of G2-MSN with pEGFP-C1 DNA **(**GFP**)**: (a) electrophoretic gel shifts of GFP in the absence of (lane 2) and in the presence of increasing amounts of G2-MSN (lane 3−8); (b) electrophoretic gel shifts of GFP complexed with 1a and uncapped MSN (MSN). Lane 2 is undigested free GFP, whereas lane 3 is the GFP digested with *Bam*HI endonuclease. Lanes 4−6 are GFP complexed with different ratios of G2-MSN and treated with *Bam*HI. Lanes 7 and 8 are the GFP complexed with uncapped MSN untreated and treated with *Bam*HI, respectively.

To study the complexation between the pEGFP-C1 DNA and G2-MSN in different weight ratios at physiological pH, agarose gel electrophoresis (0.8%, 45 mM TBE buffer) of pEGFP-C1 in the presence of G2-MSNs was performed for 3.5 h at 155 V. Figure 2a shows the electrophoretic shifts for pEGFP-C1 in the absence (lane 2) of G2-MSN and presence of increasing amounts (lane 3−8)

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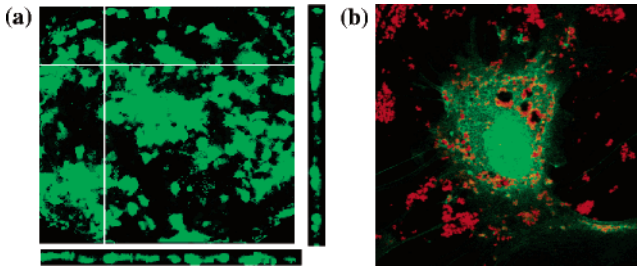

Figure 3. Fluorescence confocal micrographs of cells transfected by the pEGFP-C1-coated G2-MSN system. Panel a presents GFP-transfected HeLa cells showing cross-sections through a cell layer. Orthogonal images indicated the monolayer packing of cells. Panel b presents Texas Red-loaded G2-MSNs inside a GFP-transfected rat neural glia cell (astrocyte).

of G2-MSN. The results demonstrated that the G2-MSN could bind with plasmid DNA to form stable DNA−MSN complexes at weight ratios larger than 1:5, as illustrated by the retention of pEGFP-C1 around the sample wells (lane 6−8). Similar to the high-G PAMAMs with large numbers of positive surface charges, each G2-MSN particle was covered with numerous covalently anchored G2 PAMAMs. Thus, the resulting polycationic G2-MSN could complex with the polyanionic pEGFP-C1 efficiently without paying a large entropy penalty as in the case of free G2 PAMAMs complexing with plasmid DNAs.[1a]

To examine whether the G2-MSN are efficient in protecting pEGFP-C1 DNA against enzymatic cleavage, a restriction endonuclease (*Bam*HI, 2 units) was introduced to the DNA and the stable G2-MSN−DNA complexes containing 1 μg of DNA. The samples were incubated for 2 h at 37 °C, followed by deactivation of enzyme at 70 °C for 15 min. As shown in Figure 2b, free pEGFP-C1 (lane 2) was digested and cleaved by *Bam*HI, whereas the DNAs when complexed with MSNs (lane 4−6) were not cleaved by the enzyme under the same condition. We also extracted the pEGFP-C1 from the *Bam*HI-treated G2-MSN−DNA complex with 2 M NaCl(aq). The electrophoretic shift of the extracted DNA was the same as that of the free pEGFP-C1 suggesting that the G2-MSN−DNA complex could protect DNA from enzymatic degradation.[6] Interestingly, pEGFP-C1 DNA was digested by *Bam*HI in the presence of MSN without PAMAM caps (Figure 2b, lane 8).

To investigate the transfection efficacy of our system, G2-MSNs without Texas Red (10 μg) were mixed with 1 μg of pEGFP-C1.[6] The MSN/DNA suspension (60 μL/well) was further added to the cells on 24-well plates (6×10^4 cells per well in 0.6 mL growth medium) and incubated for 4 h at 37 °C. The cells were then washed with PBS buffer and cultured with DMEM with 10% calf serum (CS) medium and antibiotics for 2 days. The transfected cells were trypsinized and resuspended in 0.5 mL of PBS for direct flow cytometric analysis to evaluate the expression of GFP.[3] Control experiments utilizing cultures of untransfected cells and "mock"-transfected cells (cells incubated with MSNs without pEGFP-C1) were measured with an EPICS-ALTRA flow cytometer. As shown in Figure 3, significant GFP expression was observed. To compare the transfection efficiency of G2-MSN with other commercial transfection reagents, we conducted pEGFP-C1 transfection experiments on HeLa cells with PolyFect, SuperFect, and Metafectene under the same experimental conditions.[6] Flow cytometry analyses on the 48 h post-transfection cells showed efficiencies of 35% ± 5% (G2-MSN), 15% ± 2% (PolyFect), 10% ± 2% (SuperFect), and 16% ± 2% (Metafectene).[6] The transfection enhancement of the G2-MSN could be attributed to the *particle sedimentation effect* as previously reported by Luo et al.[4a,d]

To examine the mammalian cell membrane permeability, Texas Red-loaded G2-MSNs were introduced to GFP-transfected rat

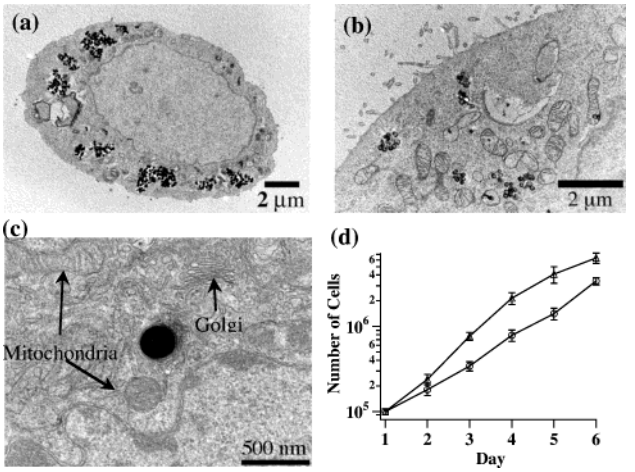

Figure 4. TEM micrographs of G2-MSN−DNA complexes (black dots) endocytosed by CHO (a), HeLa (b), and astrocyte (c) cells (see Supporting Information for enlarged micrographs). Subcellular organelles, for example, mitochondria and Golgi, were observed with MSNs nearby in panels b and c. Panel d shows a plot of logarithm of HeLa cell growth with (○) and without (△) G2-MSNs.

astrocytes. The confocal fluorescence micrograph (Figure 3b) clearly illustrated that the G2-MSNs (red fluorescent dots) entered into the cytoplasm of a green fluorescent neural glia cell. Transmission electron micrographs of the post-transfection cells also provided direct evidence that a large number of G2-MSN−DNA complexes were endocytosed by all three types of cells (Figure 4). It is noteworthy that many subcellular organelles, such as mitochondria and Golgi, were observed with MSNs nearby as shown in Figure 4b,c. Given the fact that these organelles disappear rapidly upon cell death, the result strongly suggested that the MSNs were not cytotoxic in vitro. To further investigate the biocompatibility of G2-MSN, we compared the cell growth profiles of HeLa cultures without and with G2-MSNs (0.1 mg/mL). The numbers of cells from cultures with and without G2-MSNs were counted daily for 6 days.[6] As shown in Figure 4d, the slopes of the cell growth curves from those cultures with and without G2-MSNs were very similar indicating that the increase of numbers of cells was not hindered by the presence of G2-MSNs. We envision that the G2-MSN material could serve as a new transmembrane delivery system for many biotechnological applications.

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Supporting Information Available: Syntheses and spectroscopic characterizations of ICP-MSN and G2-MSN, as well as cell cultures and gene transfection experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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