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Mannose-targeted Mesoporous Silica Nanoparticles for Photodynamic Therapy.

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The design of functionalized mesoporous silica nanoparticles (MSN) has been challenging over the last few years. Due to their interesting properties (tunable size, high specific surface area, and narrow size pores distribution) major developments in the field of biology have been recently described and reviewed[1-4]. In the course of our program dealing with one and two-photon light-activated MSN[5,6], we were interested in photodynamic therapy (PDT) [7] which is unprecedented with MSN. Several examples of silica-based nanoparticles for one[8-13] and two-photon[14] PDT applications have been described but the porosity of the silica was not controlled and the photosensitizer (PS) was physically entrapped inside the silica network which could lead to a premature release of the PS from the carrier, and thus to a reduced efficiency of treatment and to side effects. Covalent coupling of the PS inside the nanoparticles is expected to overcome this drawback. Only three recent examples involving the covalent attachment[15-17] of the PS through trialkoxysilane groups to the silica matrix have been reported. Nevertheless none of these nanoparticles were functionalized by a biomolecule able to target cancer cells. As specific bioreceptors are overexpressed at the surface of cancer cells in many tumors[18], functionalizing the nanoparticles in order to target these receptors would enhance the uptake of the nanoparticles by these cells. To date, only one example of silica-based nanoparticles functionalized with a monoclonal antibody in order to target breast cancer cells for PDT applications was described and the PS (merocyanine) was physically entrapped into the silica matrix[19]. Therefore, we present here the synthesis of novel MSN combining covalent anchoring of the photosensitizer to the mesoporous silica matrix and targeting of cancer cells with mannose attached on the surface of MSN. We show that those functionalized MSN were efficiently endocytosed through mannose receptors. PDT treatment ensured an efficient destruction of the cancer cells.

In order to prepare the MSN, the key point was the use of a water-soluble photosensitizer. The anionic porphyrin **1** was prepared according to literature procedures[20]. The trialkoxysilane function was then introduced by using isocyanatopropyltriethoxysilane. The **NP1** MSN synthesis was performed[5] with a proportion of 5 mg (5.44 μmol) of PS **1** for 3.25 g (15.7 mmol) of $Si(OEt)_4$. The surfactant (cetyltrimethylammonium bromide CTAB) was eliminated by treatment with HCl in EtOH at 60°C. UV-visible spectra allowed the determination of the loading of the PS inside the nanoparticles which was found to be 3.5 μmoles per gram of **NP1**. Transmission Electron Microscopy (TEM) showed monodispersed nanoparticles with a diameter of 100 nm for **NP1**. Dynamic light scattering (DLS) was in good agreement with TEM showing a hydrodynamic diameter of 150 nm. N_2 adsorption desorption confirmed the mesoporosity with a specific surface area of 860 m^2/g and mesopores of 2.2 nm diameter. The nanoparticles were able to generate singlet oxygen as shown by $^1O_2$ phosphorescence measurements in EtOH. Rose Bengal was used as the standard reference. The quantum yield of $^1O_2$ production was calculated to be 57%. The next step was the anchoring of the sugar moiety on the surface of the nanoparticles. Aminopropyltriethoxysilane (APTS) was first grafted on the surface of **NP1** as described earlier[6] to give **NP2**. Microanalysis and solid-state ^{29}Si DP MAS NMR showed a loading of about 1.5 mmol of amino groups per gram of **NP2**. The specific surface area dropped to 500 m^2/g and the pore diameter diminished to 2.1 nm, in agreement with a partial blocking of the pores by APTS treatment. DLS showed an increase of the hydrodynamic diameter to 170 nm which confirmed the grafting of APTS on the surface of **NP2**. Then diethyl squarate was used to link the supported amine groups with mannose. The ethyl squarate-functionalized mannose was synthesized[21], and reacted with the amines on the surface of **NP2**. Titration[22] of the supported carbohydrate by resorcinol in H_2SO_4 allowed determining a quantity of 0.180 mmoles of mannose per gram of **NP3**. ^{13}C CP MAS NMR confirmed the presence of the mannose moiety.

Scheme 1: Synthesis of **NP1** : 1) $O=C=N(CH_2)_3Si(OEt)_3$, EtOH. 2) $Si(OEt)_4$, NaOH, CTAB, H_2O. 3) HCl, EtOH

Scheme 2: Mannose functionalization of the surface of MSN

In order to study the efficiency of these MSN for PDT, human breast cancer cells (MDA-MB-231) were incubated at different times with or without 20 µg/ml of MSN in the presence or absence of 10 mM mannose and then submitted to monophotonic irradiation (630-680 nm; 6 mW / cm²) for 40 min. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[15] was performed two days after irradiation, to establish the cytotoxicity of MSN.

First, as showed in Figure 1, the irradiation of cancer cells alone did not induce any toxicity. We then compared the cytotoxic efficiency of **NP1** and **NP3** on MDA-MB-231 cancer cells incubated for 24 h with MSN. **NP1** incubated with cancer cells for 24 h and not submitted to irradiation, induced 7% cytotoxicity whereas their irradiation induced 45% cell death. In the same conditions, mannose-functionalized **NP3** induced 19% cell death without irradiation and 99% cell death when irradiated. The higher efficiency of mannose-functionalized MSN must be due to an active endocytosis via unidentified mannose receptors[23].
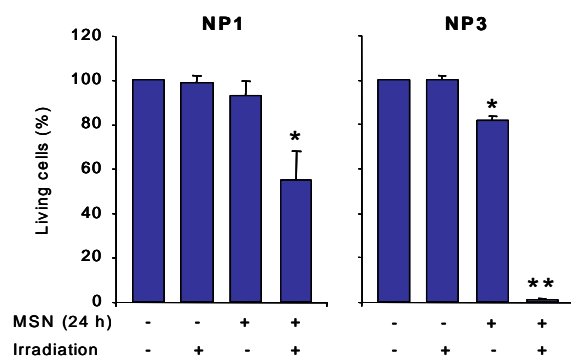

Figure 1 : PDT-induced cytotoxicities of **NP1** and **NP3**.
Means ± SD of 3 experiments. * p< 0.05; ** p< 0.01 Student's *t* test.

To prove this, cancer cells were incubated for 1 h in a serum free medium with MSN, in presence or in absence of 10 mM mannose. Table 1 shows that cell irradiation without MSN pre-incubation did not induce any significant toxicity and neither did cell incubation with **NP1** or **NP3** without irradiation. By contrast, cell treatment with **NP3** and submission to irradiation as already described, generated a significant cell death of 30%. This effect was totally reversed (no significant cell death occurred) by incubating **NP3** in the presence of mannose. This indicates that mannose acts as a competitor of **NP3**. Therefore, mannose receptors are involved in the active endocytosis of **NP3**, which thus have a higher therapeutic efficiency than **NP1**.

MSN (1 h)	-	NP3	NP3	NP3	NP3	NP1	NP1
Mannose	-	+	-	-	+	-	-
Irradiation	-	-	-	+	+	-	+
% cell viability	100	99 ± 1	101 ± 2	70 * ± 6	97 ± 5	99 ± 2	98 ± 2

Table 1 : MSN cytotoxicity mediated through mannose receptors. Means ± SD of 3 experiments. * p< 0.05 Student's *t* test.

In conclusion, a new class of MSN was elaborated by covalent incorporation of a water-soluble PS and by covering the external surface with mannose residues. We have proved that these MSN presented a much higher *in vitro* photoefficiency in MDA-MB-231 cancer cells through mannose-dependent endocytosis than non functionalized nanoparticles. Studies are in progress to adapt these nanotools to *in vivo* PDT applications.

Notes and references :
PM and CF are members of GDR 3049 "Medicaments Photoactivables-Photochimiothérapie (PHOTOMED)".

(1) Slowing, I. I.; Vivero-Escoto, J. L.; Wu, C.-W.; Lin, V. S. Y. *Adv. Drug. Deliv. Rev.* **2008**, *60*, 1278-1288.
(2) Trewyn, B. G.; Giri, S.; Slowing, I. I.; Lin, V. S. Y. *Chem. Com.* **2007**, 3236-3245.
(3) Slowing, I. I.; Trewyn, B. G.; Giri, S.; Lin, V. S. Y. *Adv. Func. Mater.* **2007**, *17*, 1225-1236.
(4) Johansson, E.; Choi, E.; Angelos, S.; Liong, M.; Zink, J. I. *J. Sol-Gel Sci. Technol.* **2008**, *46*, 313-322.
(5) Lebret, V.; Raehm, L.; Durand, J. O.; Smaihi, M.; Gerardin, C.; Nerambourg, N.; Werts, M. H. V.; Blanchard-Desce, M. *Chem. Mater.* **2008**, *20*, 2174-2183.
(6) Lebret, V.; Raehm, L.; Durand, J. O.; Smaihi, M.; Werts, M. H. V.; Blanchard-Desce, M.; Methy-Gonnod, D.; Dubernet, C. *J. Sol-Gel Sci. Technol.* **2008**, *48*, 32-39.
(7) Bechet, D.; Couleaud, P.; Frochot, C.; Viriot, M.-L.; Guillemin, F.; Barberi-Heyob, M. *Trends in Biotechnology*, In Press.
(8) Liu, F.; Zhou, X.; Chen, Z.; Huang, P.; Wang, X.; Zhou, Y. *Mater. Lett.* **2008**, *62*, 2844-2847.
(9) Tada, D. B.; Vono, L. L. R.; Duarte, E. L.; Itri, R.; Kiyohara, P. K.; Baptista, M. S.; Rossi, L. M. *Langmuir* **2007**, *23*, 8194-8199.
(10) Tang, W.; Xu, H.; Kopelman, R.; Philbert, M. A. *Photochem. Photobiol.* **2005**, *81*, 242-249.
(11) Yan, F.; Kopelman, R. *Photochem. Photobiol.* **2003**, *78*, 587-591.
(12) Zhou, J.; Zhou, L.; Dong, C.; Feng, Y.; Wei, S.; Shen, J.; Wang, X. *Mater. Lett.* **2008**, *62*, 2910-2913.
(13) Roy, I.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Bergey, E. J.; Oseroff, A. R.; Morgan, J.; Dougherty, T. J.; Prasad, P. N. *J. Am. Chem. Soc.* **2003**, *125*, 7860-7865.
(14) Kim, S.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Pandey, R. K.; Prasad, P. N. *J. Am. Chem. Soc.* **2007**, *129*, 2669-2675.
(15) Ohulchanskyy, T. Y.; Roy, I.; Goswami, L. N.; Chen, Y.; Bergey, E. J.; Pandey, R. K.; Oseroff, A. R.; Prasad, P. N. *Nano Lett.* **2007**, *7*, 2835-2842.
(16) Lai, C.-W.; Wang, Y.-H.; Lai, C.-H.; Yang, M.-J.; Chen, C.-Y.; Chou, P.-T.; Chan, C.-S.; Chi, Y.; Chen, Y.-C.; Hsiao, J.-K. *Small* **2008**, *4*, 218-224.
(17) Rossi, L. M.; Silva, P. R.; Vono, L. L. R.; Fernandes, A. U.; Tada, D. B.; Baptista, M. S. *Langmuir* **2008**, *24*, 12534-12538.
(18) Low, P. S.; Henne, W. A.; Doorneweerd, D. D. *Acc. Chem. Res.* **2008**, *41*, 120-129.
(19) Zhang, P.; Steelant, W.; Kumar, M.; Scholfield, M. *J. Am. Chem. Soc.* **2007**, *129*, 4526-4527.
(20) Chen, Y.; Parr, T.; Holmes, A. E.; Nakanishi, K. *Bioconjug. Chem.* **2008**, *19*, 5-9.
(21) Sperling, O.; Fuchs, A.; Lindhorst, T. K. *Org. Biomol. Chem.* **2006**, *4*, 3913-3922.
(22) Park, I. Y.; Kim, I. Y.; Yoo, M. K.; Choi, Y. J.; Cho, M.-H.; Cho, C. S. *Int. J. Pharma.* **2008**, *359*, 280-287.
(23) East, L.; Isacke, C. M. *Biochem. Biophys. Acta* **2002**, *1572*, 364-386.

Graphical abstract
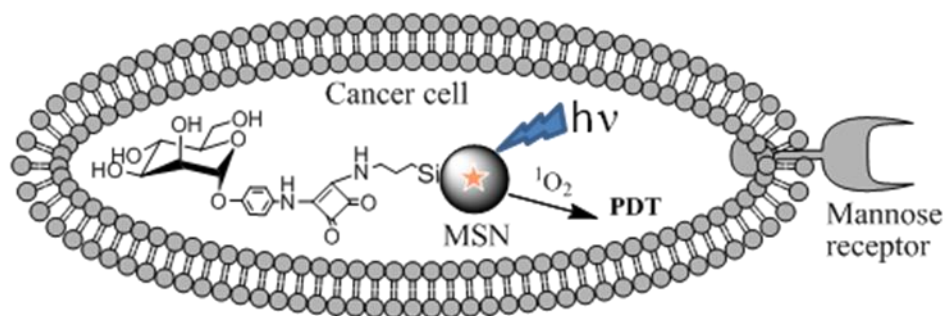
An efficient method for the synthesis of mesoporous silica nanoparticles with a covalent attachment of a photosensitizer inside the silica matrix for PDT applications is described. The surface of the nanoparticle was functionalized with mannose and the mannose-derivatized particles were much more efficient than non functionalized ones for the treatment of cancer cells.