

Imaging the Distribution of High Colloidal Stability Mesoporous Silica Nanoparticles (MSNs) with PET and MRI

M. Laprise-Pelletier^{1,2,3,4}, J-L. Bridot^{3,4}, R. Guillet-Nicolas^{1,2,3,4}, J. Lagueux¹, F. Kleitz^{3,4}, M-A. Fortin^{1,2,4*}

¹Centre de Recherche du Centre Hospitalier Universitaire de Québec (CR-CHUQ), Canada

²Department of Mining, Metallurgy and Materials Engineering, Université Laval, Québec, Canada

³Department of Chemistry, Université Laval, Québec, Canada

⁴Centre de Recherche sur les Matériaux Avancés (CERMA), Université Laval, Québec, Canada

*marc-andre.fortin@gmn.ulaval.ca

ABSTRACT

Mesoporous silica nanoparticles (MSNs) are promising materials currently being developed for cell labeling and tracking, as well as for drug delivery applications. In this work, we report on the functionalization of MSNs with diethylenetriaminepentaacetic acid (DTPA), followed by co-labeling with Gd³⁺ and ⁶⁴Cu²⁺. A colloiddally stable PET/MRI nanoprobe was therehows successfully developed, with high relaxometric properties ($r_1=33.8 \text{ mM}^{-1}\text{s}^{-1}$, r_2/r_1 ratio of 1.86). Short labeling times and high radiolabeling yields allowed for co-labeling and size-exclusion chromatography purification within a short (2h) period. The relaxometric performance of Gd-DTPA-MSNs was studied, followed by *in vivo* MRI studies (*i.v.* injections). MSNs injected in mice provided MRI signal enhancement and strong contrast effects in blood. Finally, a mouse biodistribution study was performed with PET (dynamic and static acquisitions) using injections of ⁶⁴Cu/Gd-DTPA-MSNs. PET biodistributions allowed the quantification of amounts of MSN delivered to organs *in vivo*.

Keywords: mesoporous silica nanoparticles (MSNs), magnetic resonance imaging (MRI), positron emission tomography (PET), radioactive nanoparticles biodistribution

1 INTRODUCTION

The development of theranostic agents is an emerging field that contributes to the improvement of medical therapies and diagnosis procedures. Mesoporous silica nanoparticles (MSNs) figure among the most promising products developed as drug vectors. They are small (50 - 250 nm diam.), have high specific surface areas, show high biocompatibility, and allow the grafting of functional molecules at their surface. Therefore, MSNs are currently being developed as multifunctional biomedical formulations for a variety of drug delivery applications. The biocompatibility of MSNs was recently studied and described in the literature [1-3], as well as their great potential for theranostic applications[4-8]. In the perspective of precisely monitoring drug delivery processes in patients injected with such products, it will be necessary

to track MSNs vectors over time with high resolution and high sensitivity clinical medical imaging techniques. Among all the imaging modalities, PET/MRI provides an optimal and thus powerful combination of anatomical resolution (MRI: Magnetic Resonance Imaging) and sensitivity (PET: Positron Emission Tomography). These techniques can provide quantitative and high sensitivity information about organ accumulations of nanoparticles (PET biodistribution studies) as well as high-resolution images with blood signal enhancement effects (anatomical and vascular MRI). Tracking systemically injected MSNs with imaging would enable a better understanding of their biodistribution, pharmacokinetics, as well as the assessment of efficient drug delivery to target organs. This would provide an efficient way to quantify the local amount of MSN delivered *in vivo*, a very important biological data in the perspective of establishing quantitative diagnostic and/or therapy with drug eluting MSNs. Tracking drug delivery vectors could become one of the most promising applications of the recently commercialized PET/MRI clinical systems.

The main evidences of PET/MRI probes developed thus far were based on the functionalization of iron oxide nanoparticles with chelates (DOTA or NOTA), followed by labeling with the positron emitter ⁶⁴Cu²⁺ [9-14]. However appropriate for targeted molecular and cellular imaging, iron oxide nanoparticles cannot be used to deliver drugs, and they are associated with certain limitations such as magnetic susceptibility-induced image artifacts. Labeling MSNs with the paramagnetic element gadolinium allows “positive” contrast enhancement in blood vessels containing a certain concentration of the labeled nanoparticles. Therefore, Gd labeling could be used to monitor MSNs blood retention and liver uptake as indicators of vector delivery. Few studies have reported thus far on the use of MSNs in PET and/or MRI [15-21]; none have used 3-D high porosity materials, and none have performed dynamic PET biodistribution studies. Huang et al. reported the synthesis of tri-modal (optical/MRI/PET) mesoporous silica nanoparticles (Dye@MSN@Gd@⁶⁴Cu) to study sentinel lymph nodes in the mice model [16]. They also performed an optical biodistribution, which demonstrated the uptake of MSN-probes by the liver and the spleen within 30 min post-injection.

In this study, a recently developed [22] high colloidal stability MSN system showing an intricate 3-D porous network, narrow particle size distribution (150 nm mean diam.) and very high porosity (up to 60-70% vol.), was grafted with diethylenetriaminepentaacetic acid (DTPA), enabling efficient chelation of $^{64}\text{Cu}^{2+}$ and Gd^{3+} . DTPA-MSNs were co-labeled with $^{64}\text{Cu}^{2+}$ and Gd^{3+} . We demonstrated the possibility to co-label and purify DTPA-MSNs in not more than 2 hours (at RT). The labeled MSN compound formed a stable and robust colloid, ready-to-use in PET/MRI studies. An efficient size-exclusion chromatography procedure was developed to allow rapid purification (30 minutes) of unchelated metal ions, compatible with the use of short-lived radioactive metal ions used in PET/MRI procedures. In this report, labeled MSNs suspensions were injected *i.v.* in mice, and scanned in dynamic MRI (1.5 hour) and in dynamic PET (1 hour), followed by static scans at time points. An organ biodistribution study was performed with PET acquisitions, to quantify the amounts of MSNs internalized in the liver, spleen, lungs, as well as the very limited presence of ^{64}Cu /Gd-DTPA in the urine. Dynamic PET acquisitions performed in this study are a first step toward the integration of MSNs with pharmacokinetic models.

2 MATERIALS AND METHODS

Nanoparticles synthesis: Nanospheres with a 3-D cubic network of pores were synthesized according to a recently reported methodology [22]. **DTPA grafting on MSNs:** MSN nanoparticles (100 mg) were dispersed in dry DMSO, followed by addition of diethylenetriaminepentaacetic acid (DTPA) bis-anhydride (>99%, Sigma-Aldrich). This solution was stirred overnight. The particles (DTPA-MSNs) were isolated by centrifugation (7500 G, 10 min) and washed thrice with DMSO. 1.5 mL of DTPA-MSNs in DMSO was sampled and washed thrice in nanopure water (1:4.5 v/v each time, 4300G, 15 min) prior to labeling with Gd^{3+} (and $^{64}\text{Cu}^{2+}$). **Gd³⁺ labeling:** One (1) mL of 100 mM solution of $\text{Gd}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$ (0.334 g / 10 mL nanopure water) was added dropwise to the DTPA-MSN suspension. The solution was left 30 minutes for chelation at 22°C (RT), under moderate agitation. **Size-exclusion chromatography purification:** The nanoparticles suspension (1mL) was

purified on a Sephadex G-25 (2.6 x 28 cm, GE Healthcare Life Sciences, USA) filled column (model XK 26/40, Pharmacia, Uppsala, Sweden). Saline (0.15M NaCl; 0.22 μm filter, 431097, Corning, USA) was used to equilibrate the column and for nanoparticle elution. The column was coupled to a peristaltic pump, to allow elution at a constant flow rate (5mL/min). The first 48 mL corresponded to the void volume. The nanoparticles were collected in the next 22.5 mL fraction. Tangential filtration (10 kD, 11 cm², PS, MicroKros, Rancho Dominguez, Ca) was used to concentrate the nanoparticles suspension by a factor of 6 to 16. Altogether, the purification steps (chromatography and concentration) were performed in not more than 60 min. This nanoparticle suspension was referred to as Gd-DTPA-MSNs. ***In vivo MR signal enhancement studies:*** All animal experiments were conducted under the guidelines of Université Laval and CHUQ's animal ethical committee. Six week-old balb/c female mice (3, Charles River, Montreal, Canada) were injected with 100 μL of nanoparticle suspension (chromatographed Gd-DTPA-MSN in 154m mM NaCl, $T_1 = 19.4$ ms). The animals were scanned twice using a T_1 -weighted 2D spin echo sequence, with the following parameters: FOV: 90 mm, 24 slices, 0.7 mm, 0.1 slice gap, dwell time 25 μs , 400x320, $\text{f}\alpha$ 90°, TE/TR: 18/800ms, duration: 4min16s. At time $t = 0$, and the same MR sequence was repeated for at least 90 minutes. ***Dynamic in vivo PET biodistribution study:*** The radioisotope $^{64}\text{Cu}^{2+}$ was purchased from the Centre hospitalier universitaire de Sherbrooke (Quebec, Canada), in the form of copper acetate ($^{64}\text{Cu}(\text{CH}_3\text{CO}_2)_2$). To favor $^{64}\text{Cu}^{2+}$ chelation to DTPA, 500 μL (8.36 mCi) of $^{64}\text{Cu}^{2+}$ were first incubated (90 min, 22°C) with DTPA-MSNs. Then, 500 μL of $\text{Gd}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$ (100 mM) were added (30 min, 22°C) to complete de co-labeling procedure. The purification steps (SEC and tangential concentration) were performed in 60 minutes. The nanoparticle suspension was concentrated to 1.5mL. Before the first injection, 10 % v/v of human albumin (50mg HSA/5mL NaCl solution at 154mM) was added to the nanoparticle suspension. PET imaging were performed using a Micro-PET scanner (LabPET8, CHUS). All animal experiments were conducted under the guidelines of Université de Sherbrooke and CHUS's animal ethical committee. Four six week-old balb/c female mice were injected with 150 μL of ^{64}Cu -Gd-

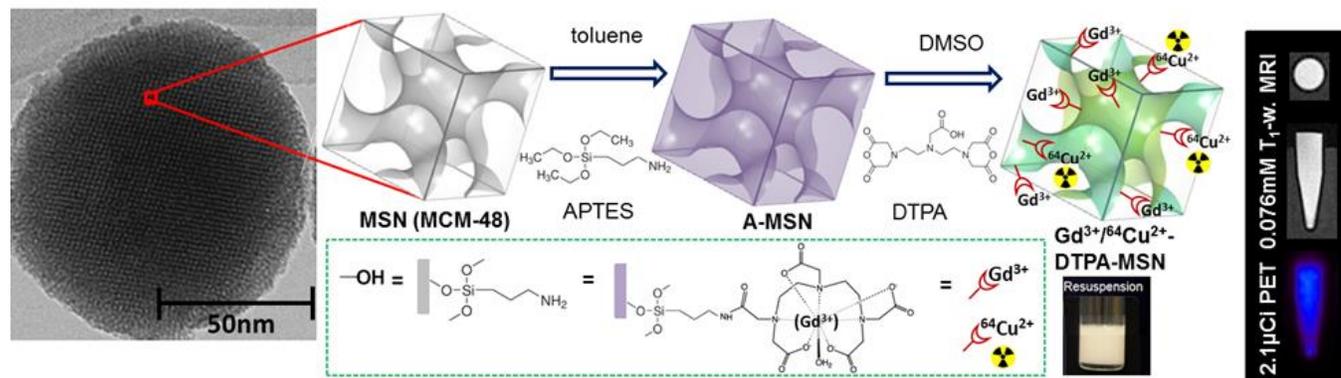


Figure 1: Schematic representation of MCM-48 MSN particles reacted with APTES, then grafted with DTPA, followed by labeling with Gd^{3+} and $^{64}\text{Cu}^{2+}$.

DTPA-MSNs. A dynamic study of 60 min was done for each animal. Static scans of injected mice were performed at time points of 6h, 12h, 24h and 48h with acquisition times of 30 minutes for the scans at 6h and 1h for the scans at 12h, 24h and 48h. Images were reconstructed by MLEM 3D with 15 iterations and analysed with the software LabTEP (Université de Sherbrooke). ROI analysis of blood, spleen and liver were performed for a better understanding of nanoparticles distribution *in vivo*. **Organs counts and final biodistribution study:** 48h after the injection of $^{64}\text{Cu}/\text{Gd}$ -DTPA-MSNs, mice were dissected. Organs of interest were weighed and their activity was measured by gamma counter (Cobra) to obtain %ID/g.

3 RESULTS AND DISCUSSION

TEM measurements of the 3-D porosity network MCM-48 silica nanoparticles showed a mean diameter size of 150 nm. The particles were non-aggregated, well-defined and spherical (Figure 1a). The hydrodynamic diameter of labeled MSNs (Gd-DTPA-MSNs) was measured in nanopure water and in saline by dynamic light scattering (DLS). Gd-loaded materials revealed a mean hydrodynamic diameter value of 266 ± 76 nm, all with high quality reports. Taking into account the hydration corona around the particles, which can represent up to 30% of the hydrodynamic diameter, the particle size measured by DLS correlates well with the TEM particle size distributions. **Relaxometric properties:** The efficacy of a MRI contrast agent is provided by measurements of its longitudinal and transversal relaxation rates. The shorter the relaxation time, the higher the contrast. Relaxivities r_1 and r_2 were determined at 1.41 T and 37 °C by measuring the relaxation time of aqueous solutions containing $^{64}\text{Cu}/\text{Gd}$ -DTPA-MSNs at various concentrations. The Gd concentrations

(mM) of imaged suspensions were measured by ICP-MS. The corresponding values for longitudinal (r_1) and transverse (r_2) relaxivities were 33.8 and 62.8 $\text{mM}^{-1} \text{s}^{-1}$ respectively with a relaxivity ratio (r_2/r_1) of 1.86. This ratio is close to unity so the nanoparticle suspension presents efficient properties as positive MR contrast agent. **MRI/PET imaging of labeled-MSNs:** MRI signal enhancement was observed by imaging tubes containing various concentrations of $^{64}\text{Cu}/\text{Gd}$ -DTPA-MSNs suspensions (Figure 2a). As shown in Figure 2a, positive contrast enhancement effect was observed for concentrations ranging from 0.025 mM to 0.076 mM of Gd. Under this concentration threshold, the signal is too low to be exploited. These results confirm that these particles could be used as a “positive” contrast agent. No evidence of sedimentation nor aggregation were found either by DLS or in MRI images. The labeling of MSN by $^{64}\text{Cu}^{2+}$ ($^{64}\text{Cu}/\text{Gd}$ -DTPA-MSNs) was demonstrated by a static acquisition (Figure 2a). These results confirmed the suitability of $^{64}\text{Cu}/\text{Gd}$ -DTPA-MSNs for *in vivo* PET/MRI bimodal imaging procedures. ***In vivo* MRI signal enhancement study:** Figure 2b shows the contrast enhancement obtained in mice, after the injection of Gd-DTPA-MSNs. Coronal images show the inner-plane longitudinal section of the abdominal aorta and bladder. Signal enhancement is clearly visible in the aorta 6 min post-injection, however attenuated within 30 min. We observed a low signal enhancement of the bladder 68 min post-injection. Vivero-Escoto and al. [15] have already showed rapid renal clearance of cleaved Gd chelates from MSNs. They observed a strong signal enhancement in the bladder 15 min post-injection. In this work, the amount of Gd leaching seems to be relatively low and suggests only a very limited accumulation of small amounts of detached Gd-DTPA in the bladder over time. ***In vivo* PET biodistribution study:** *In vivo* PET imaging is a much more sensitive modality than MRI to perform organ

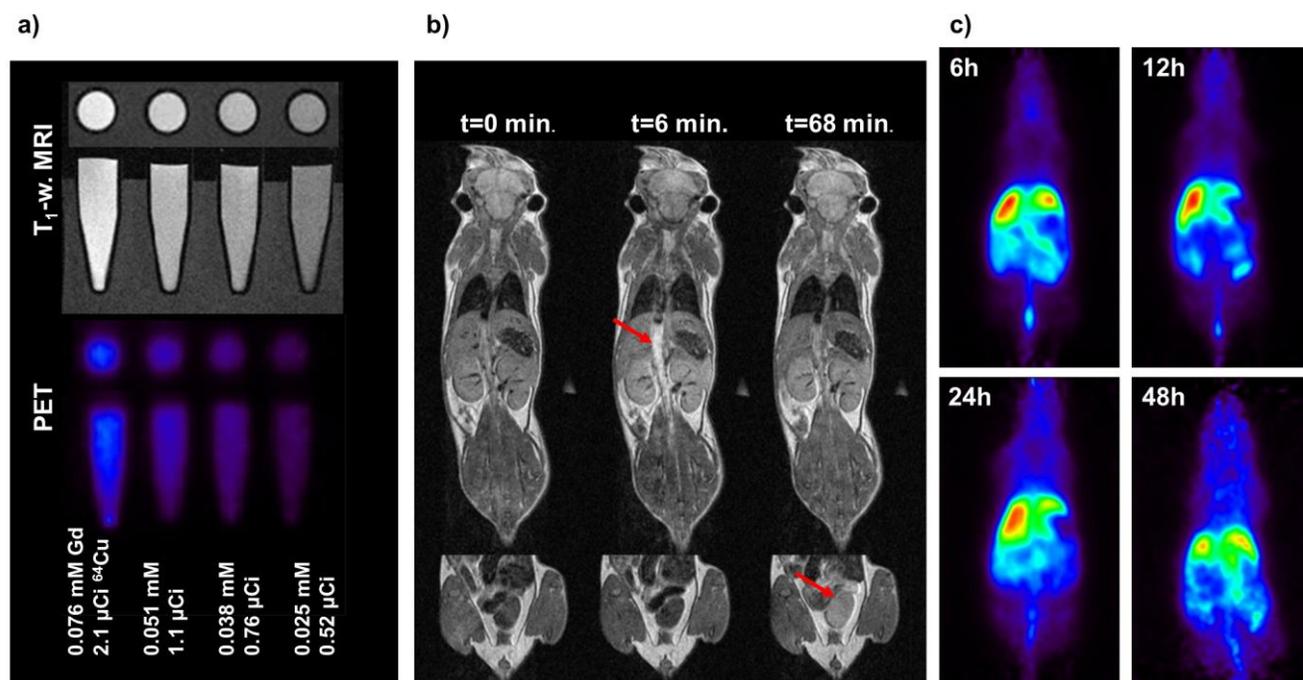


Figure 2: (a) $^{64}\text{Cu}/\text{Gd}$ -DTPA-MSNs nanoparticle suspensions imaging with PET and T_1 -weighted MRI (b) MRI signal enhancement of Gd-DTPA-MSNs injected in mice over time (c) *In vivo* PET biodistribution study of $^{64}\text{Cu}/\text{Gd}$ -DTPA-MSNs over time.

biodistribution studies. Figure 2c shows the strong accumulation of MSNs in the liver, the spleen and the gastro-intestinal system, consistent with other MSNs biodistribution studies, not based on imaging [23-25]. No evidences were found of free $^{64}\text{Cu}^{2+}$ or ^{64}Cu -DTPA because no accumulation of activity was found in the bladder (^{64}Cu -DTPA) nor in the kidneys (the presence of free $^{64}\text{Cu}^{2+}$ would have been detected in the kidneys, seconds after i.v. injection). In fact, Schipper and al. [26] demonstrated the rapid clearance of ^{64}Cu -DOTA and free $^{64}\text{Cu}^{2+}$ in the bladder and kidneys 80s post-injection. In this work, results from the PET dynamic study did not evidence $^{64}\text{Cu}^{2+}$ in the kidneys 5 min post-injection. Therefore, we can assume that DTPA is strongly grafted at the nanoparticle surface, and the biodistribution study does strictly reflect the biodistribution of ^{64}Cu /Gd-DTPA-MSNs.

4 CONCLUSIONS

Bimodal MSNs nanoparticles were synthesized, labeled and efficiently purified to allow their visualisation in MRI and PET imaging. MRI *in vivo* studies demonstrated a strong vascular signal enhancement and limited excretion in the bladder. The PET biodistribution study confirmed a blood half-life of 4.47 min, and showed that MSNs were mainly uptaken by the liver and spleen a few minutes after injection. Strong evidences of excretion via the gastro-intestinal system were also found, as well as a prolonged retention of radioactive MSNs in the blood. No evidence was found, of free $^{64}\text{Cu}^{2+}$ leaching from the particles, which confirmed that $^{64}\text{Cu}^{2+}$ /Gd $^{3+}$ salts are strongly chelated and DTPA molecules are adequately grafted to the particles. These results confirm the great potential of labeled MSNs as drug delivery vectors.

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