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Selective functionalization of mesoporous silica nanoparticles with ibuprofen and Gd(III) chelates: a new probe for potential theranostic applications.

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Organo-modified mesoporous silica nanoparticles, loaded with ibuprofen into the pores and functionalized on the external surface with a stable Gd(III)-DOTA-monoamide chelate, were prepared and explored as potential theranostic probes.

A theranostic device is a multifunctional nanoparticle (NP) that combines drug molecules and diagnostic agents in the same platform and it can be simultaneously exploited for diagnosis and therapy.[1] In the last decade, great efforts have been made in this field, as testified by more than 400 articles published in the literature.[2] Various nanosystems have been proposed for the construction of novel theranostic agents, including inorganic NPs (i.e. silica, gold), polymeric micelles and liposomes, often embedded with iron oxide or paramagnetic metal ions.[3]

Mesoporous silica nanoparticles (MSNs) functionalized or loaded with imaging probes or drugs (fluorescent dyes and paramagnetic species) have been actively investigated for biomedical applications in the last decade.[4] The great advantage of MSN as nanocarriers can be summarized by: i) improved delivery of poorly water-soluble drugs, ii) optimized drug biodistribution through targeted delivery of therapeutics, iii) low cytotoxicity, iv) sufficient chemical stability and v) high surface area and tuneable porosity, conferring to the system high chemical versatility.[2]

The first to publish their findings on these drug loaded MSNs was Vallet-Regí et al. in 2001.[5] More recently, MSNs carrying a number of different drugs (anti-inflammatory and anti-cancer molecules) and decorated with biocompatible agents (i.e. phospholipids layers)[6] and target molecules (folic acid, peptides,…)[7] have been reported.

In parallel, MSNs functionalized with Gd(III) chelates have been studied as potential T_1 MRI contrast agents. The efficacy of a MRI probe is measured in terms of the relaxivity parameter, r_1 [mM^{-1} s^{-1}], which indicates the variation in the relaxation rates of water protons normalized to 1 mM Gd^{3+} ion concentration. Initial studies on the use of Gd(III)-based chelates with MSN include different shapes and sizes of the NPs to improve the ^1H relaxivity of the system for cell tracking and angiographic MRI applications.[8] In this field we have optimized the relaxometric properties of Gd(III) chelates conjugated to mesoporous silica, obtaining high r_1 values per Gd^{3+} (79 mM^{-1} s^{-1}) and per particle (≈ 65800 mM^{-1} s^{-1}) at 20 MHz and 310 K. These remarkable values have been obtained by a careful design of the system aiming to reduce the interactions between the silica surface and the Gd(III) chelates and improve the water accessibility to the metal probe.[9-11]

Recently, the combination of silica particles with drug molecules (e.g. doxorubicin) and paramagnetic metal oxides (Fe_2O_3), typically working as negative contrast agents (T_2), have been greatly investigated in the literature.[12] On the contrary, theranostic systems functionalized with T_1-MRI Gd(III)-based contrast agents, generally used to improve the quality and diagnostic content of the MRI images in drug delivery and therapy applications, appears only marginally explored. In this contest, M.-A. Fortin et al. have very recently published a functional Gd-based theranostic agent grafting GdDTPA-like chelates and PEG on the external surface of MCM-48 NPs and loading daunorubicin into the pores.[13]

In this work, an organo-modified hybrid MSN consisting of NPs of 90±30 nm size and functionalized with -NH_2 groups, was selected as platform. A neutral GdDOTA-monoamide derivative (GdDOTAMA, Fig. 1) was selected as potential MRI probe. This macrocyclic complex, bearing a pendant arm with a terminal carboxylic functional group that can be used for conjugation to the silica surface, is characterized by high thermodynamic stability and kinetic inertness.[14,15] Ibuprofen was used as a typical drug model. The different silica domains were selectively exploited to link GdDOTAMA on the external surface of NPs and to accommodate ibuprofen into the channels.

MSNs were prepared through a sol–gel procedure reported in the literature.[11] The amino functional groups were introduced during the gel synthesis by using aminopropyltriethoxysilane. NPs with spherical morphology and size of 90±30 nm (as indicated by TEM micrographs, Fig. 1A, C) were obtained. Micrographs collected at high magnifications (Fig. 1A) indicate the presence of a pore array,

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supported also by N_2 physisorption analysis (Fig. 1D). The sample shows a bimodal pore-size distribution, determined by the Barrett–Joyner–Halenda (BJH) method, presenting maximum values at 22 and 37 Å and pore volumes of 1.52 cm^3/g (Fig. 1D). These values are in full agreement with data reported for similar materials.[16]

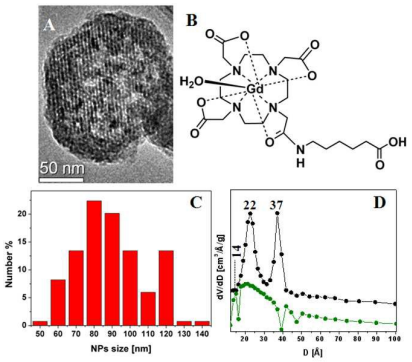
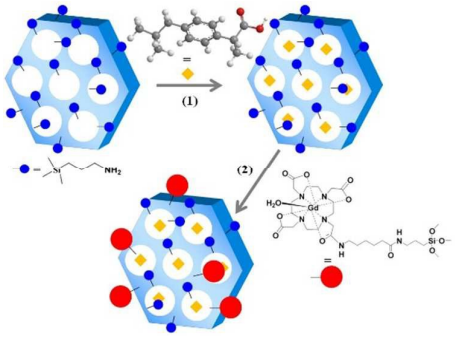


Figure 1. HR-TEM micrograph at high magnifications of MSN nanoparticles is reported in A; B) chemical structure of GdDOTAMA chelate: C) particles size distribution; and D) Pore-size distribution of MSN before (black) and after ibuprofen impregnation (green).

The theranostic probe was then obtained following two consecutive synthetic steps (Scheme 1). The first one consists of ibuprofen impregnation into the silica channels. This reaction was carried out in hexane at 298 K for 24 h. The second step is based on the chemical linkage of GdDOTAMA to the -NH_2 functional groups exposed on the external surface of the NPs. This reaction was carried out using a standard *N*'-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC)/*N*-hydroxy-2,5-dioxopyrrolidine-3-sulfonic acid sodium salt (SulfoNHS) coupling procedure in water for 2 h, thus avoiding ibuprofen release (Scheme 1; details are reported in ESI).



Scheme 1. Schematic illustration of the synthesis of theranostic probe starting from the organo-modified mesoporous silica.

The ibuprofen uptake was quantified by measuring the amount of free drug in the washing medium hexane with UV-Visible spectroscopy at 273 nm (see Fig. S1). The ibuprofen concentration absorbed by MSN is 0.60 (±0.02) mmol/g (12.4 wt%). A physico-chemical analysis of the hybrid material was carried out before Gd-chelate functionalization to determine the effective drug impregnation. Thus, the mesopores filling with ibuprofen molecules was evaluated by N_2 adsorption. The ibuprofen inclusion produces a significant change of the pore size distribution of MSN. The two pores populations at 22 and 37 Å of free MSNs decrease in intensity after drug impregnation. A new peak at ca. 14 Å (Fig. 1D) and a decrease of the pores volume from 1.52 cm^3/g to 0.87 cm^3/g were observed. These results confirm that the ibuprofen molecules are located inside the pores.

Further confirmations come from infrared spectroscopy analysis. IR spectrum of the functionalized material shows the peaks typical of the organo-modified silica (*i.e.* bands at 3350 and 3290 cm^{-1} and at 1595 cm^{-1} (Fig. S2), ascribed to the asymmetric and symmetric stretching and bending modes of the -NH_2 species, respectively) along with a sharp band at 1710 cm^{-1}, attributed to the stretching mode of the COOH group of ibuprofen. Two peaks at 1510 and 1390 cm^{-1}, typical of C=C stretching of the aromatic ring and the bending mode of CH_3 groups of drug, respectively, are also detected (Fig. 2, right and Fig. S3).

The percentage of ibuprofen released from MSNs over time was calculated by using UV-Visible spectroscopy (Figure 2). Prior to the analysis, the sample was pelleted and immersed into SBF solution (simulated body fluid),[17] at neutral pH and 298 K. These experimental conditions promote the deprotonation of the COOH group of ibuprofen and the conversion of the NH_2 functionalities of MSN to NH_3^+ (see IR data reported in Fig. S4).

The ibuprofen released during the first 8 h followed a linear behaviour with zero order kinetics (Fig. S5), typical of systems in which the drug strongly interacts (through ionic bond) with the silica surface.[18] After the first day, the release further decreases with time and reaches a maximum value of 57% before levelling off.

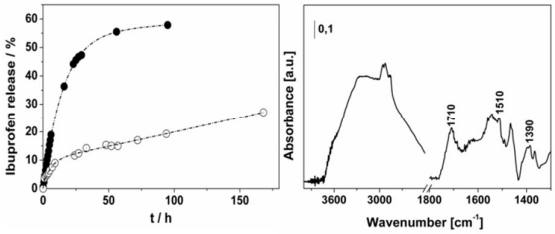


Figure 2. Left: Ibuprofen released from MSN (●) and GdDOTAMA-based functionalized material (○). Right: IR spectrum of MSN loaded with ibuprofen.

After conjugation of the GdDOTAMA chelates to the surface, the final concentration of Gd^{3+} present in the material was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). 0.01 mmol of Gd per gram of material was found, corresponding to approximately 1290 (±50) complexes per particle. Only a small fraction of NH_2 groups, mainly located on the external surface, were involved in the coupling procedure (ca. 0.3% of the total amount, estimated to be 3 mmol g^{-1}, by thermogravimetric analysis (Fig. S6)). The IR spectrum of the final material shows a main absorption at 1640 cm^{-1} attributed to the bending mode of the amide group (Fig. S7). The presence of this signal is a clear evidence that the Gd(III) chelates are bound to the organo-silica surface. In addition, it is important to note that the intensity of ibuprofen IR absorptions appear ca. 90% preserved after the coupling reaction (Fig. S7).

The kinetic release of ibuprofen was also evaluated after GdDOTAMA attachment following the same procedure described above. In this case, only 27% of ibuprofen was released after 175 h. This value is ca. two times lower than that obtained for the amino-functionalized MSNs (Fig. 2, left), suggesting that the GdDOTAMA complexes, located in close proximity to the pores entrance, partially limit the drug release. This result is in line with what observed in similar systems.[13]

Finally, the ^{1}H NMR relaxometric properties of the final hybrid material were investigated aiming to understand its efficacy as MRI nanoprobe. The average hydrodynamic diameter of the functionalized MSNs in aqueous suspension was ca. 180 nm (data obtained by DLS analysis, Fig. S8). In order to maintain the suspension stability for several days, a low amount (0.1 wt%) of xanthan gum was added. At 20 MHz and 310 K, the r_1 value (per Gd) measured for the xantham gum stabilized aqueous suspension of the hybrid material at neutral pH is 15.0 mM^{-1} s^{-1}. This value is more than three times higher than that measured for the discrete GdDOTAMA complexes.[14] On a per particle basis, the relaxivity assumes the value of ca. 19300 mM^{-1} s^{-1}, considering an average of 1300 Gd(III) chelate anchored to the surface of a NP. Based on what is known about macromolecular paramagnetic complexes, a strong increase in relaxivity is quite predictable for metal chelates anchored to slowly tumbling nanosystems. Nonetheless, the nuclear magnetic relaxation efficacy of the present system appears lower than that found for related GdDOTAMA functionalized MSNs, which exhibited r_1 values of 26.6 mM^{-1} s^{-1}.[9] We previously demonstrated for Gd-chelates attached onto the MSN surface that the transformation of the protonated free amino groups onto amides is responsible of a pronounced relaxivity increase due to a change in surface/Gd-complex interaction.[10] Thus, also in the present case, it could be hypothesized that an interaction of the Gd-chelate with functional groups on the surface or with the drug encapsulated into the pores might be responsible of the observed relaxivity decrease. To better clarify this point, the sample was left in water for five days, at neutral pH, in order to promote a partial release of the drug, and again characterized by ^{1}H NMR relaxometry. In these conditions, about 20% of drug was released as determined by UV measurement. Noteworthy, the pH remains substantially unchanged during the drug release. The r_1 value of the suspension (20 MHz, 310 K, pH = 7) increases to 23.7 mM^{-1} s^{-1} (r_1 = 30454 mM^{-1} s^{-1} per particle), corresponding to a ca. 60% increase over the initial value of the intact sample (before drug release) and similar to that recently reported for related GdDOTAMA-based MSNs (Table 1).[9-10]

To strengthen our understanding on the relaxometric properties of the material before and after the release of ibuprofen, r_1 was measured at 310 K as a function of the magnetic field over the frequency range 1-70 MHz, obtaining the so-called Nuclear Magnetic Relaxation Dispersion (NMRD) profiles (Fig. 3).[19] The nature of the magnetic field dependence of relaxivity for both materials is very similar and quite typical of slowly tumbling systems, characterized by a broad peak with a maximum around 20 MHz. However, the amplitudes of the two NMRD profiles are clearly different. We can observe a distinct increase in relaxivity over the entire frequency range investigated following the partial release of the drug. The contributions of the different physicochemical influences on r_1 can be evaluated quantitatively by analyzing the NMRD profiles according to well-established theoretical models. For molecular gadolinium agents the shape and amplitude of the frequency dependence of r_1 are well described by the equations of Solomon-Bloembergen-Morgan.[19] The key parameters that determine the relaxivity observed are: the number q of metal-bound water molecules, the correlation time for tumbling of the agent (τ_R), the mean lifetime of the water molecule in the coordination sphere of the paramagnetic ion (τ_M), the parameters characterizing the electron spin relaxation, i.e. the electronic correlation time for the modulation of the zero-field-splitting interaction (τ_V) and the mean square zero-field-splitting energy (Δ^2). In the case of metal chelates conjugated to scaffolds of nanometric size, the rotational dynamics is more appropriately evaluated in terms of the Lipari–Szabo model-free approach that accounts for the occurrence of a rapid local rotation of the complex (τ_{RL}) superimposed to the global motion of the nanoparticle (τ_{RG}).[20] This description is particularly appropriate for our GdDOTAMA functionalized MSNs due to the presence of a flexible linker connecting the Gd complexes to the MSN surface. The coupling of local and global motions is described by the order parameter S^2 whose value lies between 1 (global and local motions coincide) and 0 (local and global motion are completely independent).

Using known values for q (q = 1) and for the distance r_{GdHw} between the metal ion and the protons of the coordinated water molecule (r_{GdHw} = 3.0 Å) and fixing τ_{RG} to 0.1 ms, in analogy with previous studies,[10,11] we have analyzed the experimental data in terms of five adjustable parameters: Δ^2, τ_V, τ_{RL}, τ_M, and S^2 as (Table 1). The parameters for electronic relaxation were used as empirical fitting parameters and have no real physical meaning for macromolecular systems. Hence, low field data, those most affected by electronic relaxation, were not included in data analysis, in accordance with a well-established approach. Δ^2 has a value of (7.7±0.6)×10^{18} s^{-2} and τ_V of (16±1) ps, quite similar to those found for strictly related systems.[11]

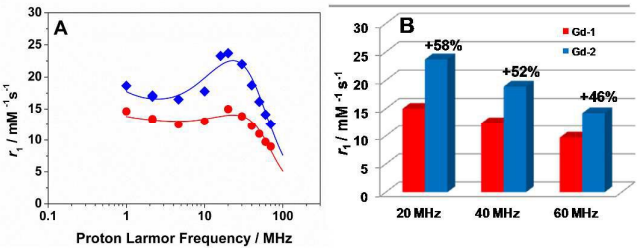


Figure 3. A) $1/T_1$ NMRD profiles at 310 K for the hybrid material impregnated with ibuprofen and functionalized with GdDOTAMA chelates before (red circles) and after partial release of the drug (blue diamonds). The best-fit curves were calculated using the parameters of Table 1. B) r_1 values for GdDOTAMA functionalized MSNs before (Gd-1) and after partial drug release (Gd-2) at 20, 40 and 60 MHz.

The increase of r_1 for the sample after partial drug release is associated with the variation of two dynamic parameters that characterize the system: the local motion of the complex around the linker and the exchange rate of the coordinated water molecule. This change in r_1 does not result from a variation of the q number during the drug release because GdDOTA-monoamide chelates are typically very stable and the coordinated water

molecule is not affected by the presence of ibuprofen in close proximity. Indeed, the relaxation rate value (measured at 20 MHz and 298 K) of pure GdDOTAMA solution, treated with increase amount of ibuprofen sodium salt, remained constant (Fig. S9). Specifically, the release of the drug from the silica is accompanied by an acceleration of the water exchange (τ_M decreases by ca. 45%) and by a faster local rotation of the complex (τ_{RL} decreases by ca. 25%). For the sample with ibuprofen impregnated into the pores, τ_M assumes the value of 1.3 μs (310 K), which is sensibly longer than those found for related GdDOTA-monoamide complexes.[14,19] Then, the combination of a long τ_M with a restricted local motion of the complex (τ_{RL} = 23 ns) is indicative of the occurrence of an interaction of the Gd(III) complex with the surface of MSN.

Clearly, the value of τ_M plays a dominant role and its decrease largely offsets the negative effect associated with the less restricted local rotation of the complex. Furthermore, the r_1 enhancement and the variation of the dynamic parameters of the Gd-chelates strongly suggest the presence of interactions between the complexes and the drug molecules. In particular, these interactions must involve complexes located on the edge or in close proximity of the entrance of the pores. It is fairly obvious to assume that interactions of electrostatic nature, also involving a number of H-bonded water molecules, make the exchange of the coordinated water with the bulk more difficult and limit the rotational degree of freedom of the complex around the linker. The partial release of the drug, especially the molecules nearest the entrance of the pores, removes this type of interactions allowing easier access of water to the metal site and increasing the rotational flexibility of the chelates. Previous studies have shown that the local ionic environment of the complex influences the process of coordinated water exchange (both prototropic and of the whole molecule) and then r_1.[21] Such an effect is particularly relevant in the case of systems in slow exchange (τ_M > ca. 5 μs) but it is likely that also in this case a similar mechanism might contribute to the variations in r_1 observed. In particular, the release of the drug changes the ionic environment and thus the nature of the second sphere of hydration of the Gd(III) centers through the replacement of the carboxylate groups with other anions, to a large extent chlorides. In turn, this influences the value of τ_M and hence relaxivity.

Table 1. Selected best-fit parameters[a] obtained from the analysis of the $1/T_1$ NMRD profiles (310 K) of the sample before (Gd-1) and after partial release of the drug (Gd-2).

	$^{20}r_1$ [$mM^{-1}s^{-1}$]	$^{20}r_1$ per NP [$mM^{-1}s^{-1}$]	τ_{RL} [ns]	τ_M [μs]	S^2
Gd-1	15.0±0.7	19275±900	23±7	1.3±0.2	0.48±0.03
Gd-2	23.7±1.1	30454±1410	12±3	0.71±0.04	0.33±0.02

[a] Parameters were determined by fitting data above 2 MHz, consistently with previous practice.[11] The outer sphere contribution to relaxivity was estimated by using standard values for the distance of closest approach, a (4 Å) and the relative diffusion coefficient of solute and solvent, D ($3.1x10^{-5}$ cm^2 s^{-1}).

Conclusions

Multifunctional mesoporous silica nanoparticles, selectively functionalized on the external surface with thermodynamically stable and kinetically inert Gd(III) chelates and loaded inside the pores with ibuprofen molecules, were successfully obtained and characterized. The experimental results suggest that mutual interactions between ibuprofen, Gd-chelates and the silica surface determine the effectiveness of the final theranostic system. Both the relaxivity of the Gd-based MSNs and the kinetics of the drug release are markedly influenced by the presence of one or the other component of the final material. Therefore, the release of ibuprofen is slowed down by anchoring the Gd-chelates on the surface of the NPs and the relaxivity increases significantly following the partial release of the drug. The ability to modulate the functional properties of the system, through controllable chemical procedures, is of great interest for the development of intelligent theranostic agents. For example, it might be possible to follow the process of drug release by monitoring the resulting changes in the relaxation rate. For a development in this direction, an optimization of all the components of the theranostic probe is required. New formulations based on Gd(III) chelates with improved efficacy, the modulation of the interaction MRI probes-drug molecules through a suitable choice of the length and chemical nature of the linker and of the electrical charge of the metal complex, the modification of the surface properties of NPs through the chemical transformation of the exposed groups are being studied and will be the subject of future work.

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