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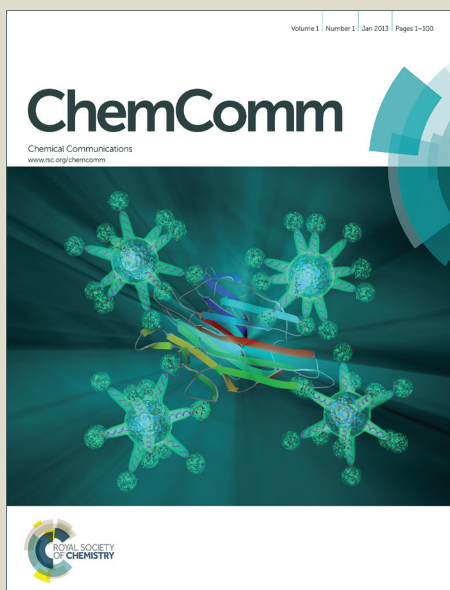

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ARTICLE TYPE

Dendrimersomes: a new vesicular nano-platform for MR-Molecular Imaging applications

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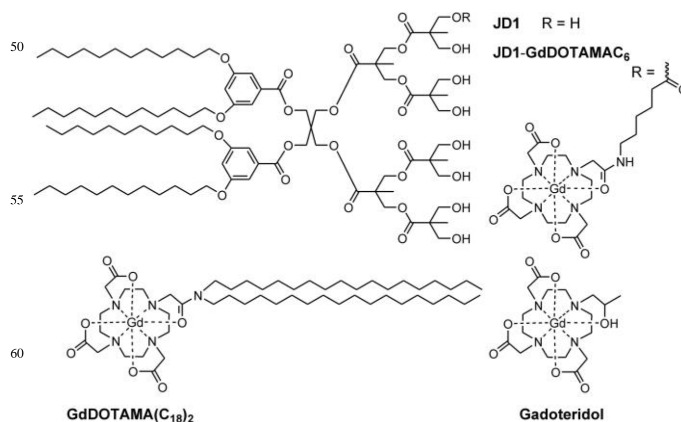
A new class of nanovesicles formed by self-assembly of amphiphilic Janus dendrimers, dendrimersomes, loaded with hydrophilic or amphiphilic Magnetic Resonance Imaging chelates shows promising properties as a novel, efficient, and versatile nanoplatform for biomedical imaging.

Dendrimers are synthetic molecules with highly branched architectures characterized by a tree-like appearance.[1] Although most dendrimers exhibit a roughly spherical symmetry, a new structural class of dendrimers has been recently obtained by joining together two chemically distinct dendritic building blocks.[2] The resulting molecule is a double-faced compound with bifunctional character, named Janus dendrimer, constituted by diverse peripheral groups on opposite sides. In particular, amphiphilic Janus dendrimers possessing one hydrophobic and one hydrophilic moiety are reminiscent to the typical structure of phospholipids, thus favouring their self- assembling in water into supramolecular aggregates of different morphologies, including nanosized bilayered vesicles named dendrimersomes.[3-5] Until now, different vesicular systems such as polymersomes and liposomes have been extensively investigated for biomedical applications, mainly as nanocarriers for drug delivery or as diagnostic or theranostic agents.[6-9] The main advantage associated with the use of polymersomes is their high stability and mechanical resistance,[10] while liposomes show excellent biocompatibility and marked ability to interact with natural membranes, thus providing an interesting opportunity to investigate many biological mechanisms at a cellular level.[11] Although dendrimersomes should combine all these properties,[3-5] their potential in biological scenarios is still completely unexplored. In particular, their use may hold promises for Molecular Imaging applications.[12] Inspired by the versatility of liposomes that can encapsulate hydrophilic probes into the aqueous core or embed amphiphilic chelates into the phospholipid bilayer, this work aims at assessing the ability of dendrimersomes to load hydrophilic and/or amphiphilic Gd-complexes as MRI probes. The clinically approved agent Gadoteridol (GdHPDO3A) was selected as hydrophilic probe, whilst two approaches were pursued for the incorporation of amphiphilic agents in the membrane vesicles: i) the synthesis of a novel Janus dendrimer covalently conjugated to a Gd-chelate (JD1-GdDOTAMAC$_6$), and ii) the incorporation in the bilayer of the lipophilic Gd-DOTAMA(C$_{18}$)$_2$ complex (Scheme 1).



Scheme 1. Janus dendrimer JD1and its GdDOTAMAC$_6$ conjugate structures (upper part), Gd-DOTAMA(C$_{18}$)$_2$ and Gadoteridol (lower part).

A large number of Janus dendrimers based on different libraries of amphiphilic molecules have been reported to form self assembled architectures of variable size and morphologies.[3] Among them, JD1 (Scheme 1) was chosen as it forms dendrimersomes with size similar to liposomes (*ca.* 120 nm) and excellent polydispersity index (PDI = 0.06).[3] This is based on a pentaerythritol core with a hydrophobic moiety consisting of two 3,5 bis-dodecyl substituted benzoyl ethers and a hydrophilic segment made of a generation one polyester dendritic structure terminating with eight hydroxyl groups. While JD1 was synthesized as described elsewhere,[13] a novel synthetic procedure was followed to covalently conjugate to the dendrimer multiple GdDOTA-monoamide chelates (H$_4$DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid). In particular, one hydroxyl group of JD1 was esterified by reaction with one equivalent of a *t*-butyl protected bifunctional chelating agent bearing an activated carboxyl function (DOTAMA(*t*Bu)$_3$C$_6$-NHS).[14] The deprotection of the *t*-butyl esters, followed by complexation with GdCl$_3$, yielded the functionalized Janus dendrimer (JD1-GdDOTAMAC$_6$; see ESI for details).
Unlike most phospholipids, which easily self-organize into liposomes in physiological saline buffer, JD1 forms large,

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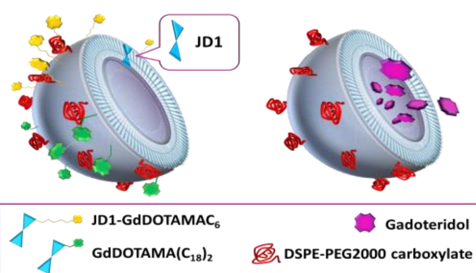

Figure 1. Dendrimersomes made of JD1 dendrimer incorporating JD1-$GdDOTAMAC_6$ or Gd-DOTAMA$(C_{18})_2$ into the membrane bilayer (left), and encapsulating Gadoteridol in the inner core (right).

micron-sized aggregates under the same isotonic conditions (see ESI). To overcome this problem, two main strategies can be followed: i) dendrimersomes are prepared in a 5% w/w glucose solution, then forming vesicles with a low PDI (0.095) and a mean hydrodynamic diameter of around 100-120 nm; ii) charged lipids (DMPG or DSPE-PEG-COOH) are incorporated into the membrane architecture to prevent the aggregation by electrostatic and/or steric repulsion.[15-17] The addition of DMPG effectively allowed to obtain a homogeneous suspension of 100-130 nm sized vesicles, but only using phospholipids in relatively large amount (20% in moles). On the other hand, the addition of a small percentage (5%) of DSPE-PEG-COOH resulted to be effective to improve sterically the stability of the nanovesicles then preventing aggregation in the isotonic buffer. Moreover, it has to be noted that PEG-2000 is normally used for coating the surface of vesicles in order to reduce their detection by the reticuloendothelial system, thus prolonging the circulatory lifetime and improving biodistribution in perfused tissues.[15-17] Furthermore, the carboxylic terminal functionality of the PEG chain can serve as an anchoring site for attachment of molecules capable of modulating the biodistribution of the nanoparticle (e.g. targeting vectors).

The dendrimersomes herein reported (Figure 1) were obtained by using the conventional film hydration method: a chloroform solution of the amphiphilic molecules was dried under vacuum to obtain homogeneous films that were hydrated at 50°C with an isotonic solution containing Gadoteridol (250 mM) at pH 7.4. After extrusion and exhaustive dialysis, vesicles with size ranging from 160 to 180 nm and PDI lower than 0.2 were obtained (see ESI). Dendrimersomes containing Gd-complexes embedded in the lipidic bilayer were obtained by adding 20% of JD1-$GdDOTAMAC_6$ or Gd-DOTAMA$(C_{18})_2$ to the membrane formulation.[18,19]

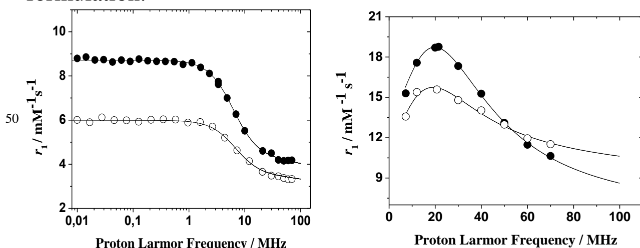

Figure 2. ^{1}H NMRD profiles measured at 298 K of Gadoteridol (●) and dendrimersomes encapsulating Gadoteridol (○) (left panel), and dendrimersomes incorporating GdDOTAMA$(C_{18})_2$ (●) and JD1-$GdDOTAMAC_6$ (○) (right panel).

The relaxometric characteristics of these nanosized systems were assessed by evaluating the magnetic field dependence of their relaxivities (r_1, *i.e.* the longitudinal relaxation enhancement of the water protons induced by a 1 mM solution of Gd^{III}) at 298 K over a wide range of field strengths (from 0.00024 to 1.65 T, corresponding to 0.01–70 MHz proton Larmor frequencies) to obtain the so-called nuclear magnetic relaxation dispersion (NMRD) profiles. The NMRD profiles of Gadoteridol-loaded dendrimersomes at 298 K (Figure 2) exhibit the same shape of the free chelate, but with lower r_1 values over the entire range of frequency investigated. This represents a clear indication of the occurrence of a "limiting" effect on the relaxivity. It is well known that a reduced water permeability (P_W) through the vesicles bilayer results in a decrease of r_1 for the complex.[20] As the observed r_1 value is only slightly lower than for free Gadoteridol, we may conclude that the bilayer of dendrimersomes represents just a very weak "quenching" effect. NMRD data were analysed using the conventional Solomon-Bloembergen-Morgan (SBM) theory,[21] suitably modified to take into account the encapsulation of the chelate in the nanovesicles (see ESI). The values of parameters obtained from the data analysis for the free complex were fixed during the fit of the profile of the nanovesicles using only P_W as adjustable parameter. The value of 115 μm/s obtained for the water permeability (Table 1) is sensibly higher than those reported for conventional liposomes (0.3-70 μm/s).[22] Interestingly, the gain of r_1 due to the increase with temperature of membrane permeability results to be compensated by the decrease of r_1 observed for free Gadoteridol (due to faster rotation), resulting in almost unchanged r_1 values for Gadoteridol-loaded dendrimersomes at 310 K (Figure 3 and S5).

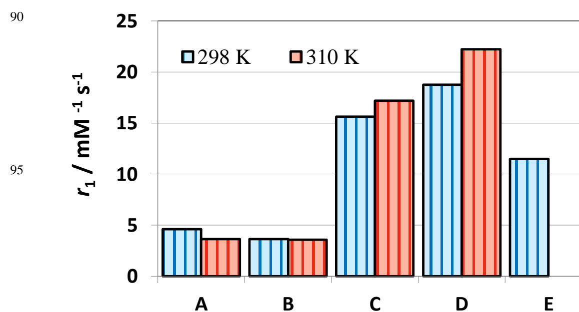

Figure 3. Relaxivity measured at 21.5 MHz and 298 K and 310 K of Gadoteridol in a free form (A) or encapsulated into dendrimersomes (B); dendrimersomes incorporating in the the membrane bilayer JD1-$GdDOTAMAC_6$ (C) or GdDOTAMA$(C_{18})_2$ (D) compared to liposomes incorporating GdDOTAMA$(C_{18})_2$ (E) into the lipidic bilayer.

NMRD profiles at 298 K of dendrimersomes incorporating 20% of JD1-$GdDOTAMAC_6$ or self-assembled with 20% of Gd-DOTAMA$(C_{18})_2$ showed the typical shape of slowly tumbling Gd-based systems, with a peak centred around 20 MHz corresponding to r_1 values of 15.6 (JD1-$GdDOTAMAC_6$) and 18.7 mM^{-1} s^{-1} (Gd-DOTAMA$(C_{18})_2$) (Figure 2, right). Data were fitted only in the high-field region (> 3 MHz) because of the known limitations of the SBM theory for slowly tumbling systems at low magnetic field strengths.[21] We used our recently published relaxation model for the analysis of r_1 in nanovesicles

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incorporating paramagnetic Gd-complexes implemented with the Lipari–Szabo (LS) approach.[19] The LS model was proven effective to treat systems in which a local molecular rotation of a chelate (characterized by a correlation time τ_{RL}) is motionally coupled to the global tumbling motion of the nanoparticle (τ_{RG}).[23] The motional coupling is defined by the value of the order parameter S^2 (0 = no coupling, 1 = maximum coupling). Data were fitted by adjusting the parameters Δ^2, τ_V, τ_{RG}, τ_{RL}, τ_M, and S^2, whereas P_W was left to vary only in the range of 100-250 µm/s. On the basis of the results reported in Table 1, the lower relaxivity observed for the vesicles based on JD1-$GdDOTAMAC_6$ complex with respect to those ones assembled with $GdDOTAMA(C_{18})_2$ is due to the faster rotational tumbling (τ_{RL}) correlated with a poor motional coupling with the global rotation of the nanovesicle. Likely, such a difference stems from the flexible C6 linker that connects the coordination cage to the Janus dendrimer in JD1-$GdDOTAMAC_6$. The other relaxation parameters assume the values typical of slowly tumbling GdDOTA-monoamide complexes.[19,24] The P_W values obtained are slightly larger than the permeability determined for Gadoteridol-loaded dendrimersomes. However, the low statistical weight of P_W in the analysis of the profiles does not allow drawing reliable conclusions about the role of the membrane composition on the water permeability.

Table 1. Selected parameters obtained from the analysis of the $1/T_1$ NMRD profiles at 298 K for dendrimersomes encapsulating Gadoteridol or incorporating $GdDOTAMA(C_{18})_2$ or JD1-$GdDOTAMAC_6$.[a]

Parameter	Gadoteridol	GdDOTAMA $(C_{18})_2$	JD1-$GdDOTAMAC_6$
${}^{20MHz}r_1$ [$mM^{-1}s^{-1}$]	3.65	18.7	15.6
Δ^2 [10^{19} s^{-2}]	2.7±0.3	0.64±0.05	0.68±0.06
τ_v [ps]	16.0±1.1	23.0±1.3	45.0±1.8
τ_{RL} [ps]	65.0±7.0	550±15	405±12
τ_{RG} [ns]	--	80±11	71±9
S^2	--	0.65±0.1	0.24±0.08
τ_M [ns]	700±30	925±40	924±35
P_w [µm/s]	115±15	175±65	166±60

[a] The following parameters were fixed to common values during the fitting procedure: r_{Gd-H} = 3.0 Å, $D = 2.25\times10^{-5}$ cm^2 s^{-1}, q = 1, a = 3.8 Å.

At 310 K, the relaxivity increases for both dendrimersomes incorporating Gd-amphiphilic agents, likely as consequence of both τ_M shortening and increased water permeability (Figure S6). Interestingly, $GdDOTAMA(C_{18})_2$ complex exhibits a much higher relaxivity (*ca.* 50%) when incorporated in dendrimersomes as compared to the case where it is embedded in conventional liposomal bilayers (based on DPPC, cholesterol and DSPE-PEG2000).[19] This enhancement can be mostly attributed to the higher water permeability of the bilayer made of Janus dendrimers that limits the quenching effect on the relaxivity of the paramagnetic units exposed towards the inner core of the vesicles.

Finally, dendrimersomes encapsulating carboxyfluorescein at high concentration (20 mM) were also prepared (see ESI for details). They elicit a self-quenching effect of the fluorescence due to the contact and to the consequent non radiative transfer of energy among adjacent dye molecules.[25,26] The quenching effect eventually vanished when vesicles breakage with a surfactant (Triton-X100) led to probe release confirming the compartmentalization of the fluorescent dye in the aqueous core.

In virtue of their similarity to liposomes, whose versatile properties are being successfully exploited in various fields, dendrimersomes also have considerable potential for effective practical use in biomedicine. Our preliminary data indicate that the vesicles assembled from Janus dendrimers can be considered as new potential vectors of MRI agents, drugs and multimodal imaging probes. However, further studies are needed to develop and fully realize the great potential of this innovative nanosized platform.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Synthesis of Gd-$DOTAMAC_6$-Janus-dendrimer (JD1-$GdDOTAMAC_6$), technical protocol followed for dendrimersomes assembly, DLS, stability tests in buffer, experimental information about relaxivity measurements and ^{1}H NMRD acquisition, results about quenching of the relaxivity and internalization of fluorescent probes. See DOI: 10.1039/b000000x/

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