A NEW ACTIVE AND SPECIFIC DIAMOND NANOPROBE DESIGNED FOR MEDICAL IMAGING APPLICATIONS

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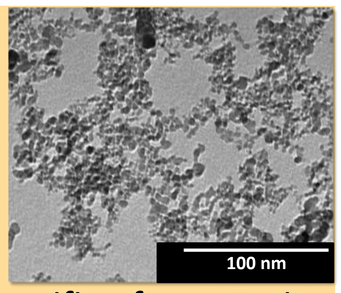
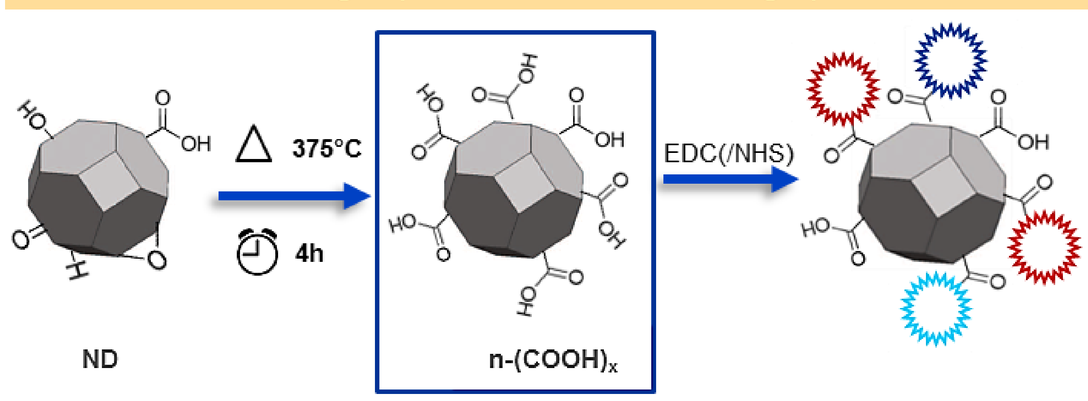
INTRODUCTION

The popularity of nanodiamonds has risen over the last few years because they have proved to be safe, biocompatible and significantly less toxic than other well-known carbon nanomaterials [1]. According to some studies, diamond nanoparticles seem to be a good candidate for biomedical purposes and more precisely as a tool for therapeutic and diagnosis applications in the medical imaging context [2, 3].

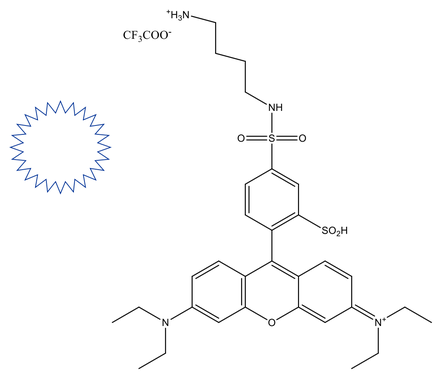


In this work, the surface of diamond nanoparticles is subjected to a thermal oxidation in order to introduce more oxygen containing functional groups like carboxylic acid functions. EDC(/NHS)-coupling processes make the platform active and specific of apoptosis thanks to different molecules of interest (fluorochrome, contrast agent & peptide). The benefit of this original bimodal nanoprobe is the combination of high spatial resolution and high penetration tissue of Magnetic Resonance Imaging and the high sensitivity of Optical Imaging.

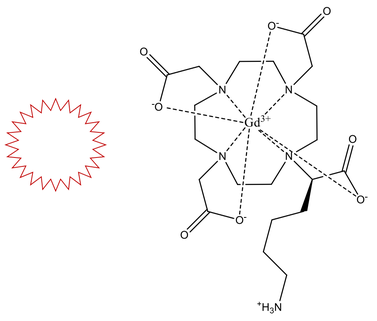


EXPERIMENTAL

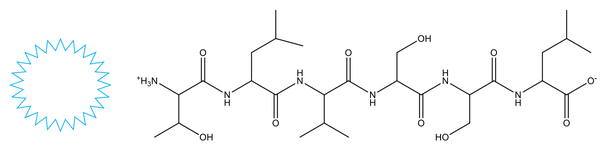
Specific Agents



Rhodamine derivative



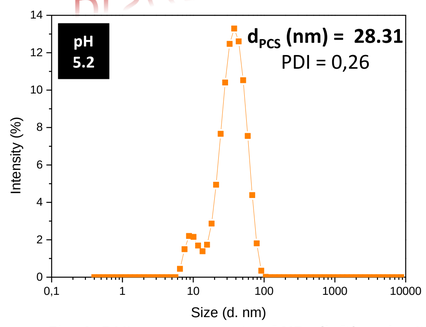
Gd-DOTA derivative



E3 peptide

RESULTS

Nanoparticle Characterization - DLS/Zeta Potential & XPS



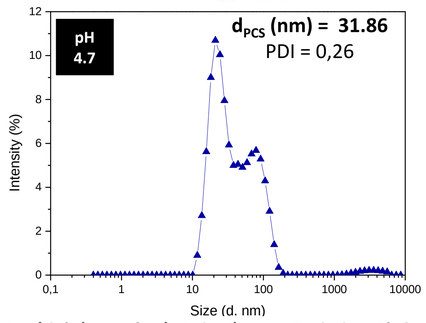


Fig. 1: DLS measurements of NDs (left) and n-COOH (right) samples (0.1% wt). US A.70%, time 1h & 10 000rpm – 90mins

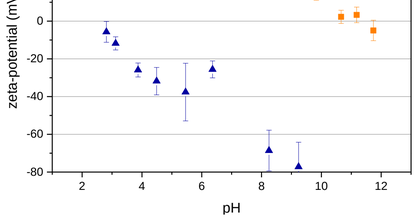


Fig. 2: Zeta potential vs. pH measurements of NDs and n-COOH samples (0.1% wt). US A.70%, time 1h & 10 000rpm - 90mins

Table 1 – Atomic percentages (%) of carbon and oxygen elements, ratio O/C and weight loss of carbon after annealing

Samples	C1s (%)	O1s (%)	Ratio O/C	Weight loss (%)
Untreated ND	94.67	3.10	0.03	-
n-COOH (375°C, 4h)	**87.7**	**10.24** +7.1%	**0.12**	**3.03**
n-COOH (400°C, 4h)	87.84	10.1	0.11	7.57
n-COOH (425°C, 4h)	87.43	11.23 +8.1%	0.13	17.46

Ligand Synthesis & Relaxometric Properties of Paramagnetic Gd-Complexes

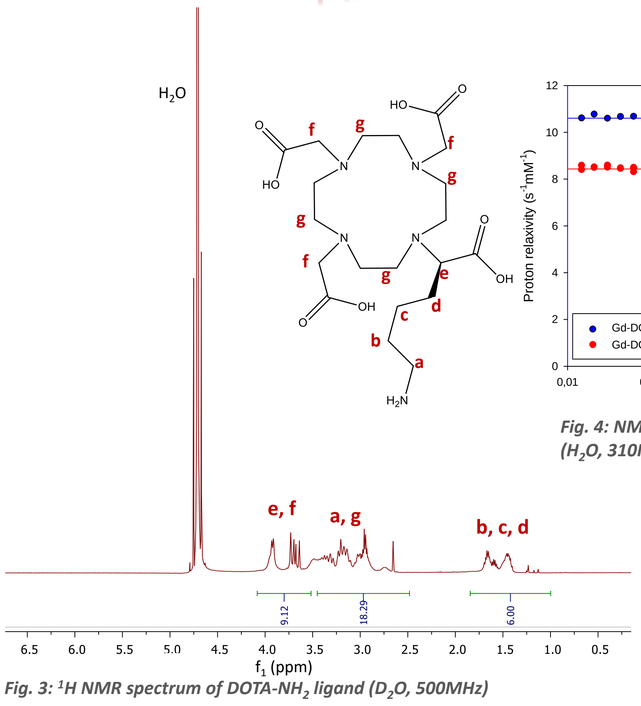


Fig. 3: ^{1}H NMR spectrum of DOTA-NH_2 ligand (D_2O, 500MHz)

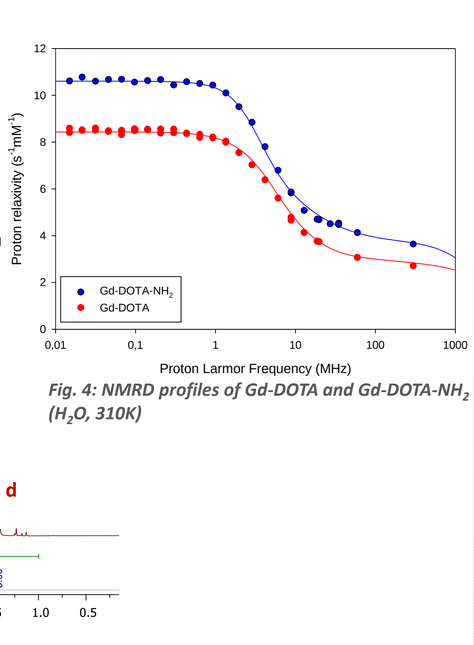


Fig. 4: NMRD profiles of Gd-DOTA and Gd-DOTA-NH_2 (H_2O, 310K)

Table 2 – Relaxometric properties of gadolinium complexes

Material	20 MHz (37°C)	
	r_1 (s^{-1} mM^{-1})	r_2 (s^{-1} mM^{-1})
Gd-DOTA	3,6	4,4
Gd-DOTA-NH_2	4,7	5,6
	60 MHz (37°C)	
	r_1 (s^{-1} mM^{-1})	r_2 (s^{-1} mM^{-1})
Gd-DOTA	3,3	4,4
Gd-DOTA-NH_2	4,1	5,1

In vitro studies - Coupling with a peptide linker

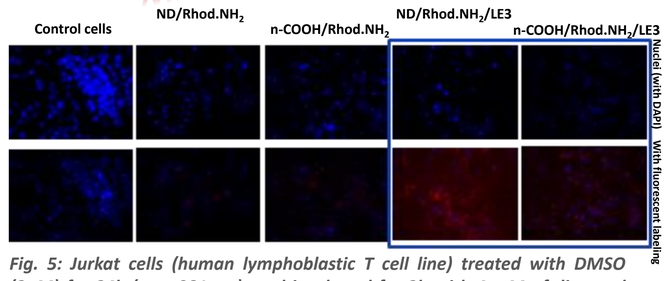


Fig. 5: Jurkat cells (human lymphoblastic T cell line) treated with DMSO (2µM) for 24h (exp. 381 ms); and incubated for 2h with 4 mM of diamond nanoparticles

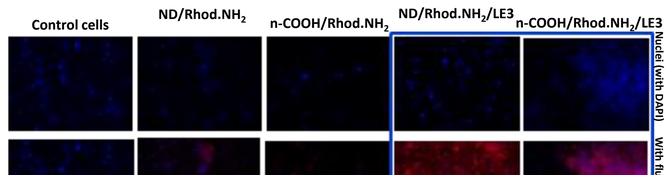


Fig. 6: Jurkat cells stimulated with camptothecin (2µM) for 24h (exp. 381 ms); and incubated for 2h with 4 mM of diamond nanoparticles

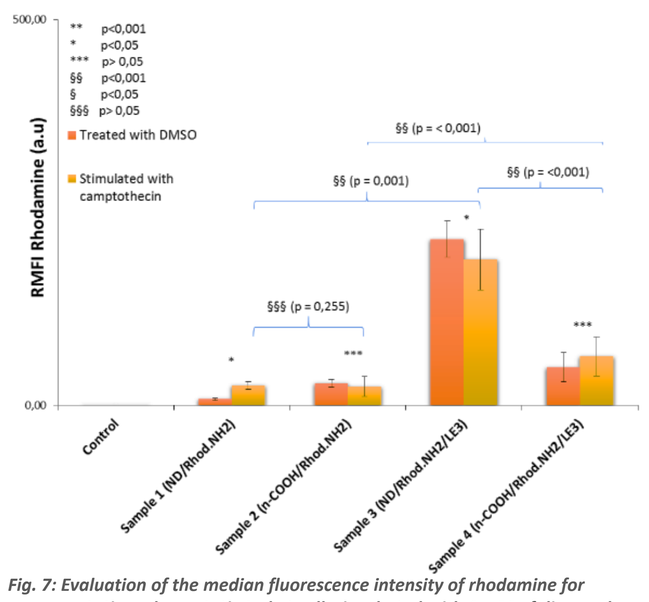


Fig. 7: Evaluation of the median fluorescence intensity of rhodamine for non-apoptotic and apoptotic Jurkat cells, incubated with 4 mM of diamond nanoparticles

DISCUSSION/CONCLUSIONS

- Oxygen rate (O-C=O, C-O) ↗ with temperature. 375°C = ideal temperature for thermal oxidation without drastic weight loss of carbon (3%).
- The behaviour of the zeta potential vs. pH confirms the increased presence of oxygenated groups (i.e. carboxylic acid functions). The typical size for a suspension of untreated and oxidized nanodiamonds is around 30 nm.
- The proton relaxivity of Gd-DOTA-NH_2 complex is greater than that of the parent, and throughout the range of the magnetic field studied. These results are explained by the fact that the rotational mobility of the new complex is slowed.
- The specificity for apoptosis of grafted-ND and n-COOH has been checked by fluorescence microscopy on Jurkat cells (stimulated with DMSO or camptothecin). Results confirm the well-done coupling of peptide with the diamond platform, compared to control cells and to nanoparticles without peptide.

References: [1] Zhang X. et al., Toxicology Research, **2012**, 1(1), 62-68.
[2] Manus L.M. et al., Nano Letters, **2009**, 10(2), 484-489.
[3] Chow E.K. et al., Science translational medicine, **2011**, 3(73), 73ra21, 1-10.

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