Delivery of siRNA to Ewing sarcoma tumor xenografted on mice, using hydrogenated detonation nanodiamonds: treatment efficacy and tissue distribution.

Sandra Claveau, Émilie Nehlig, Sébastien Garcia-Argote, Sophie Feuillastre, Grégory Pieters, Hugues A. Girard, Jean-Charles Arnault, François Treussart and Jean-Rémi Bertrand

Supplementary Materials

Sample ref.	hydrodynamic diameter in intensity (nm)	hydrodynamic diameter in number (nm)	PDI	Zeta Potential (mV)
H-DND-22	75	31	0.17	+40
H-DND-23	72	31	0.21	+46
H-DND-24	63	56	0.18	+55
T-DND	93	48	0.23	*n.d.*

Table S1: **Size measurement of hydrogenated and tritiated DND.** Hydrodynamic diameters are inferred from scattered light intensity time autocorrelation. The raw data are in the first column (from left); the size "in number" (second column) is obtained from raw data after correction from Rayleigh and Mie scatterings, which reinforces the contribution of the smallest nanoparticles having a lower scattering efficiency. PDI (third column): polydispersity index. Zeta potential is displayed in the fourth column. *n.d.*: not determined. H-DND-24 and T-DND are the two samples used for *in vitro* and *in vivo* siRNA delivery.

	DND suspension before centrifugation (counts per minutes, cpm)	Supernatant of centrifugation (cpm)	% of the initial dose present in the supernatant
T-DND	1382796	63341	5
purified T -DND	1219936	24223	2
Urine T-DND	113	131	117
Urine T-DND:siAS	89	106	119
Urine T-DND:siCt	115	104	90

Table S2: **Determination of free tritium in nanodiamond suspension and in mice urine after injection**. To evaluate the fraction of labile tritium the solutions were centrifugated during 3 h at acceleration 10600 *g* (50Ti rotor in XL90 Beckman ultracentrifuge). Aliquots of 100 µL were sampled before and after centrifugation and then diluted in 8 mL final volume of deionized water before radioactivity measurement by liquid scintillation. In the case of T-DND solution, we detected 5% of the initial deionized water dose present in the supernatant. We consider that this radioactivity is due to tritiated water, since it could be fully recovered on a paper filter after evaporation and re-condensation in a closed tube. The resulted centrifugated T-DND was the "purified T-DND" injected into the mice. Note that an additional centrifugation of this purified T-DND solution still reveals 2% of activity in the supernatant, which indicates that adsorbed tritium is still present at DND surface. Then, to determine if T-DND were present in urines, a similar centrifugation protocol was applied to 2 mL of urines collected 24 h after purified T-DND injection, and diluted in 8 mL final volume of deionized water. We did not detect a significant change of radioactivity in the aqueous phase after centrifugation. Furthermore, there was no solid T-DND pellet for all the 3 conditions tested (free T-DND and for the two T-DND coated with a siRNA).