Supporting Information

PEGylated and functionalized aliphatic polycarbonate polyplex nanoparticles for intravenous administration of HDAC5 siRNA in cancer therapy

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1. siRNA complexation, size, and zeta potential

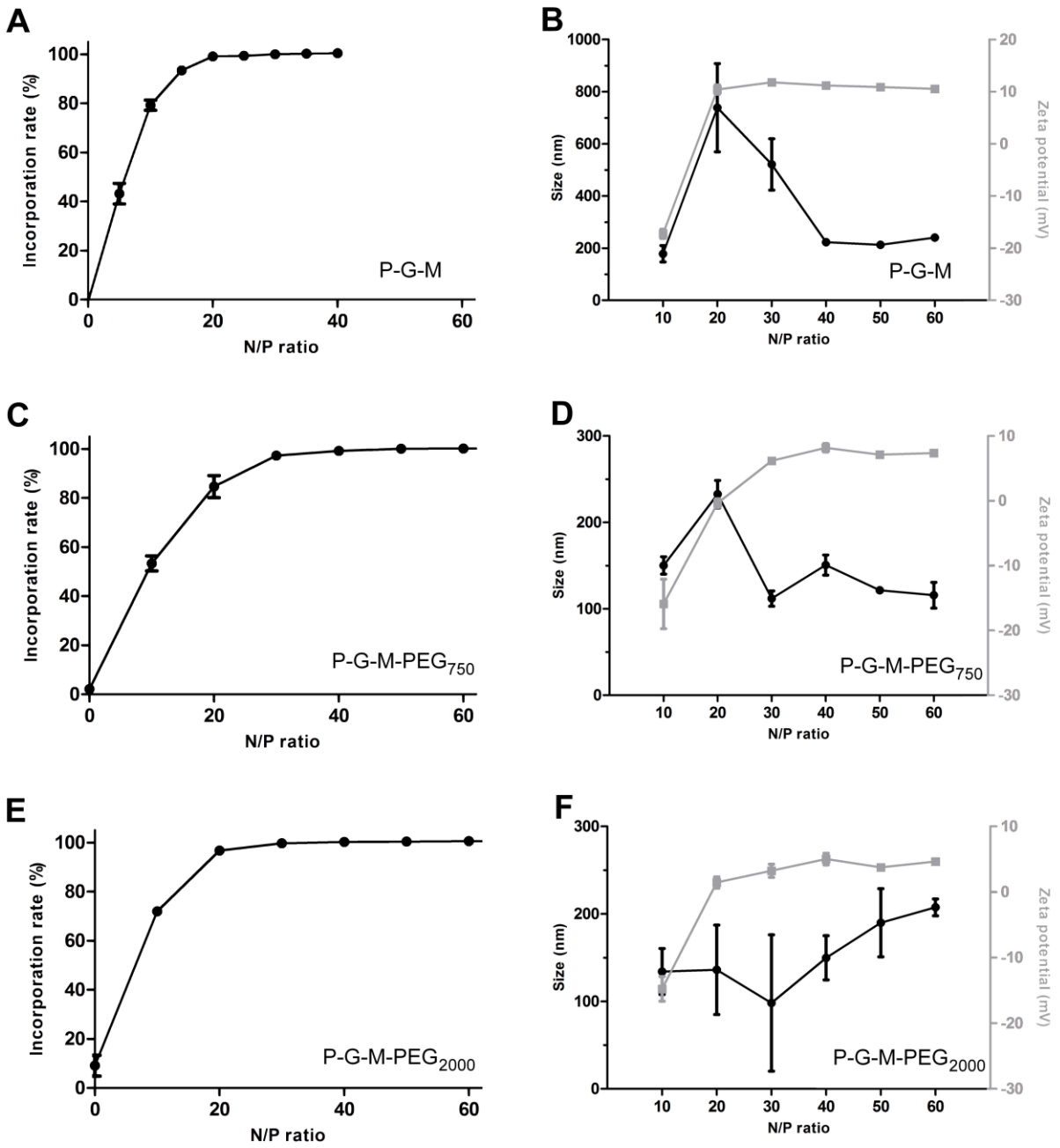

Fig. S1. siRNA complexation rate, size, and zeta potential of P-G-M **(A; B)**, P-G-M-PEG$_{750}$ **(C; D)**, and P-G-M-PEG$_{2000}$ **(E; F)** polyplexes as a function of the N/P ratio.

2. Freeze-drying and long-term stability

The colloidal dispersions show a low stability over time **(Fig. S2A)**. Size increases from the early hours, especially for P-G-M polyplexes. A better stability is observed for both PEGylated

nanoparticles, particularly for P-G-M-PEG_{2000}, confirming the observations obtained by TEM. The stability of a colloidal suspension is related to the surface charge of nanoparticles. Under -30 mV and above +30 mV, the dispersion is considered stable due to high electrostatic repulsion between nanoparticles. The more the charge is close to neutrality, the less the suspension is stable. In this case, the surface charge of polyplex nanoparticles is too low to bring sufficient stability by electrostatic repulsions. With PEGylated polymers, stability is increased by steric repulsions between nanoparticles[1]. Nevertheless, it is not sufficient to guarantee stability for several months or years required for a pharmaceutical product. To increase shelf life, we freeze-dried the samples[2]. The use of low binding vials, Daikyo Crystal Zenith®, was essential. Prepared in classical glass vials, characteristics of polyplexes were modified, probably due to a slight polymer adhesion on the vial walls (data not shown). Secondly, to ensure the integrity of polyplexes, a cryoprotectant must be added to the suspension. Mannitol, as many sugars like trehalose and sucrose, possesses this characteristic, forming a glassy matrix around the nanoparticles during lyophilization process[2-3].

Polyplexes were prepared in Daikyo Crystal Zenith® vials (Daikyo Seiko, Tokyo, Japan), and freeze-dried (Heto-Holten DW 8030 freeze-dryer, Vacuubrand RZ8 pump) using the following lyophilization cycle process: freezing at -35°C and 1 bar for 3.5 hours; primary drying at -15°C and 0.8 bar for 3 hours and then at -10°C and 0.1 bar for 12 hours; and secondary drying at +10°C and 0.1 bar for 5 hours. Vials were then closed and sealed under vacuum and stored at -18°C.

In these conditions, PEGylated polyplexes show better stability in terms of size, zeta potential and siRNA complexation until at least three months (**Fig. S2B-C-D**). The integrity of siRNA was also similar after three months of storage (data not shown).

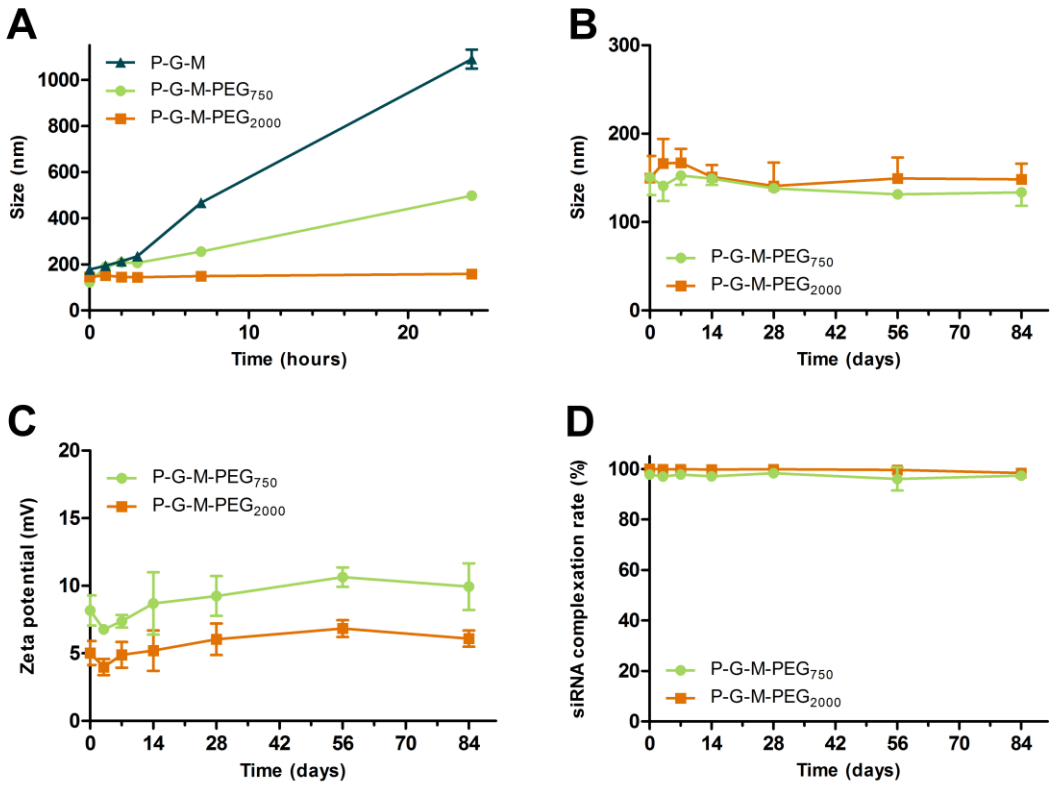

Fig. S2. (A) Size stability over time of polyplex dispersions in TE buffer isotonized with 5% mannitol. Size **(B)**, zeta potential **(C)**, and siRNA complexation rate **(D)** after freeze-drying and extemporaneous rehydration in water.

3. Size stability measured by Dynamic Light Scattering (DLS) in presence of serum (FBS)

In addition to fSPT, size in presence of FBS was also determined using the Zetasizer Nano ZS®, exploiting the DLS technique. Samples were prepared with classic siRNA at a concentration of 100 nM. After the polyplexes formation, FBS was added to reach a concentration of 10%. Samples were analyzed immediately and after 1, 2, and 3 hours incubation at 37°C (**Fig. S3**). The size was evaluated taking into account the peak intensity corresponding to the nanoparticles, without the peaks related to the FBS.

However, 50% FBS was too concentrated in proteins to observe the peak corresponding to nanoparticles. As shown on this figure, it appears that the size increases directly after 10% FBS addition, but stabilizing after 1h and up to 3 h. The increase was greater for P-G-M compared to

PEGylated polyplexes, with a diameter around 600-700 nm. The size of P-G-M-PEG_{750} and P-G-M-PEG_{2000} was located around 300 nm directly after FBS addition, increasing slightly and stabilizing close to 400 nm, from 1 hour to 3 hours.

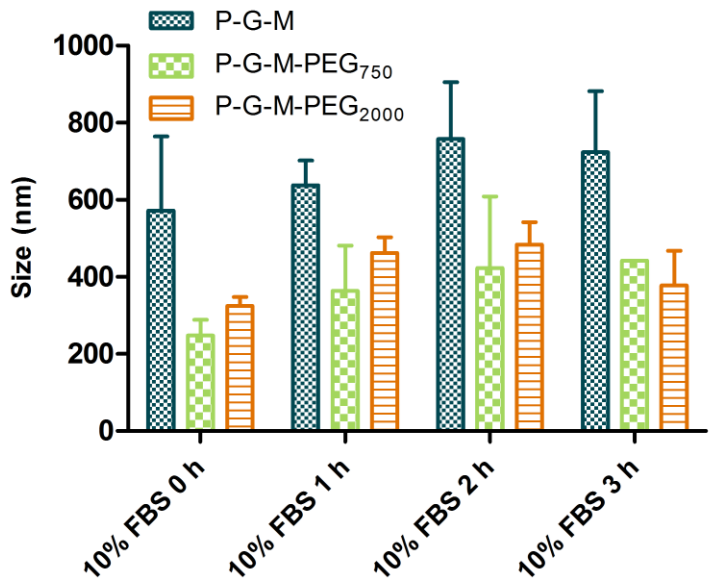

Fig. S3. Evolution of the size of polyplex nanoparticles determined by DLS, directly after addition of 10% FBS and after incubation with 10% FBS at 37°C for 1 h, 2 h, or 3 h.

However, DLS is not the optimal method to determine the size in complex medium. As shown in **Fig. S4**, proteins are also detected and their size peaks appear in the size distribution. Also, the presence of a few aggregates can greatly modify the size distribution through bigger size, because the intensity of the scattered light is proportional to the sixth power of the diameter.

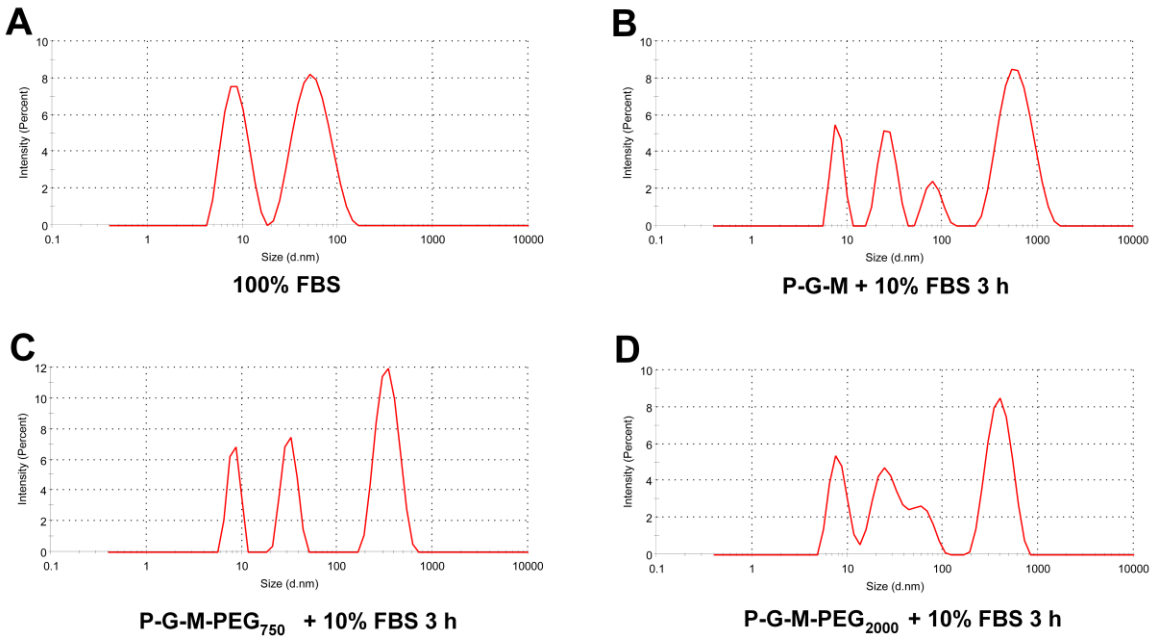

Fig. S4. Distribution of size by intensity observed by DLS for FBS alone **(A)** and for polyplex nanoparticles after 3 hours incubation with FBS at 37°C for P-G-M **(B)**, P-G-M-PEG_{750} **(C)**, and P-G-M-PEG_{2000} polyplexes **(D)**.

4. Hemocompatibility

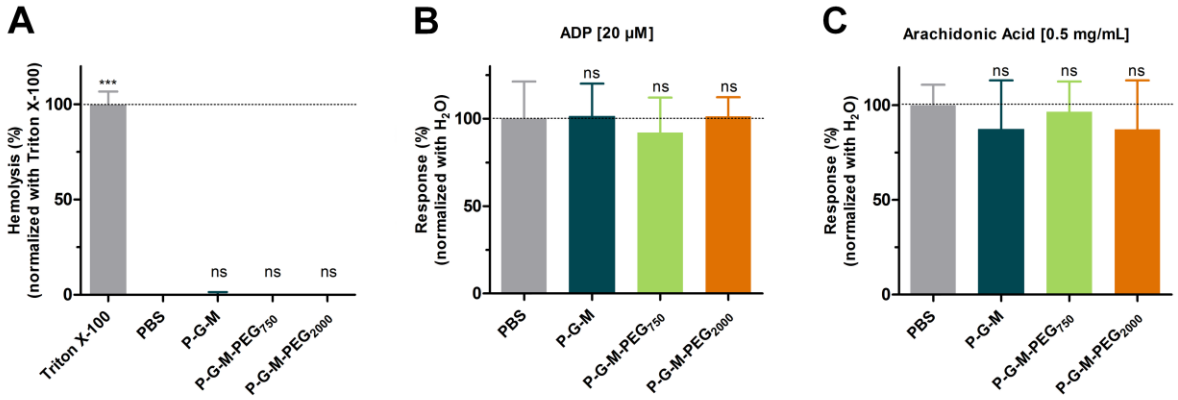

Fig. S5. Supplementary hemocompatibility assays of polyplex nanoparticles, also performed at a final concentration of siRNA of 100 nM. **(A)** Human RBC lysis (% of hemolysis) in washed RBC after 1.5 hour incubation. Triton X-100 (1%) and PBS were respectively used as positive and negative controls. Platelet aggregation induced by ADP **(B)** or AA **(C)** in presence of the different formulations. PBS is used as negative control. Results are expressed as % of response, normalized with H_2O. Statistical

comparisons with negative controls were performed by using one-way ANOVA, followed by the Dunnett's test.

5. References

(1) Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. Poly(Ethylene Glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives. *Angew. Chem., Int. Ed. Engl.* **2010,** *49*, 6288-6308.

(2) Fonte, P.; Reis, S.; Sarmento, B. Facts and Evidences on the Lyophilization of Polymeric Nanoparticles for Drug Delivery. *J. Controlled Release* **2016,** *225*, 75-86.

(3) Jain, A.; Thakur, K.; Sharma, G.; Kush, P.; Jain, U. K. Fabrication, Characterization and Cytotoxicity Studies of Ionically Cross-Linked Docetaxel Loaded Chitosan Nanoparticles. *Carbohydr. Polym.* **2016,** *137*, 65-74.