SUPPORTING INFORMATION

The biological fate of magnetic protein-specific molecularly imprinted polymers: toxicity and degradation

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Maghemite nanoparticles and magnetic imprinted polymers preparation

Synthesis

Synthesis of functionalized magnetic nanoparticles

Maghemite nanoparticles were synthesized as previously described by Massart[1], using a co-precipitation method. Briefly, 180 g of ferrous chloride (VWR) and 1.59 mol of ferric chloride (VWR) were dissolved in 6% hydrochloric acid (Merck). 1 L of ammonia at 22.5% (Carlo Erba) was added to the medium, under vigorous magnetic stirring at room temperature. Reaction was able to perform for 30 minutes. Then, the as-obtained magnetite was oxidized using 323 g of ferric nitrate (VWR). The suspension was heated at 100°C under magnetic stirring for 30 minutes. Maghemite nanoparticles were then washed three times with acetone (VWR) and two times with diethyl ether (VWR), before being dispersed in water.

The surface of the nanoparticles was directly functionalized with an iniferter agent, using a protocol slightly modified from the one of Gonzato et al.[2]. In short, 60 mg of the iniferter agent, 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (Sigma), were dissolved in 4 mL of ethanol (96%, VWR), followed by the addition of 26 mL of distilled water and 500 mg of γ-Fe_2O_3 nanoparticles. The reaction was allowed to proceed at room temperature for 18 hours under continuous orbital stirring. Then, functionalized nanoparticles were dialyzed using a 5/5 water/ethanol mixture until no more molecules were detected by conductivity measurements. The particles were characterized by XRD (Figure S1D).

Synthesis of γ-Fe_2O_3@MIP

The synthesis of protein imprinted polymers (MIP) was carried out as previously described. Briefly, 10 µmol of GFP and 30 mmol of acrylamide (Sigma) were dissolved in 150 mL of HEPES buffer (200mM, pH = 8, Sigma). The mixture was allowed to react and form a pre-polymerization complex for 2 hours at room temperature under constant magnetic stirring. Then, 3 mmol of N,N-methylene-bis-acrylamide (Sigma), 300 mg of functionalized nanoparticles and 25 mg of APS (Sigma) were added and the mixture was nitrogen purged for 15 minutes under magnetic stirring. Lastly, 75 µL of N,N,N',N'-tertramethyl-ethylene-diamine (Sigma) were added to the mixture and the reaction was allowed to proceed for 18 hours at room temperature under magnetic stirring. The final product was washed and template proteins were extracted using dialysis until no more fluorescence remains in the solution and no more proteins or molecules were detected by conductivity measurements. Dialysis baths were alternatively a 5/4/1 water/methanol/acetic acid mixture (methanol: VWR, acetic acid: Carlo Erba) and distilled water. Finally, particles were transferred into HEPES buffer (200mM, pH = 8).

Non-imprinted polymers (NIP) were synthetized using the same way but without the GFP as template.

We also synthesized some non-magnetic imprinted polymers by adding pure Iniferter agent instead of functionalized maghemite nanoparticles.

Characterization

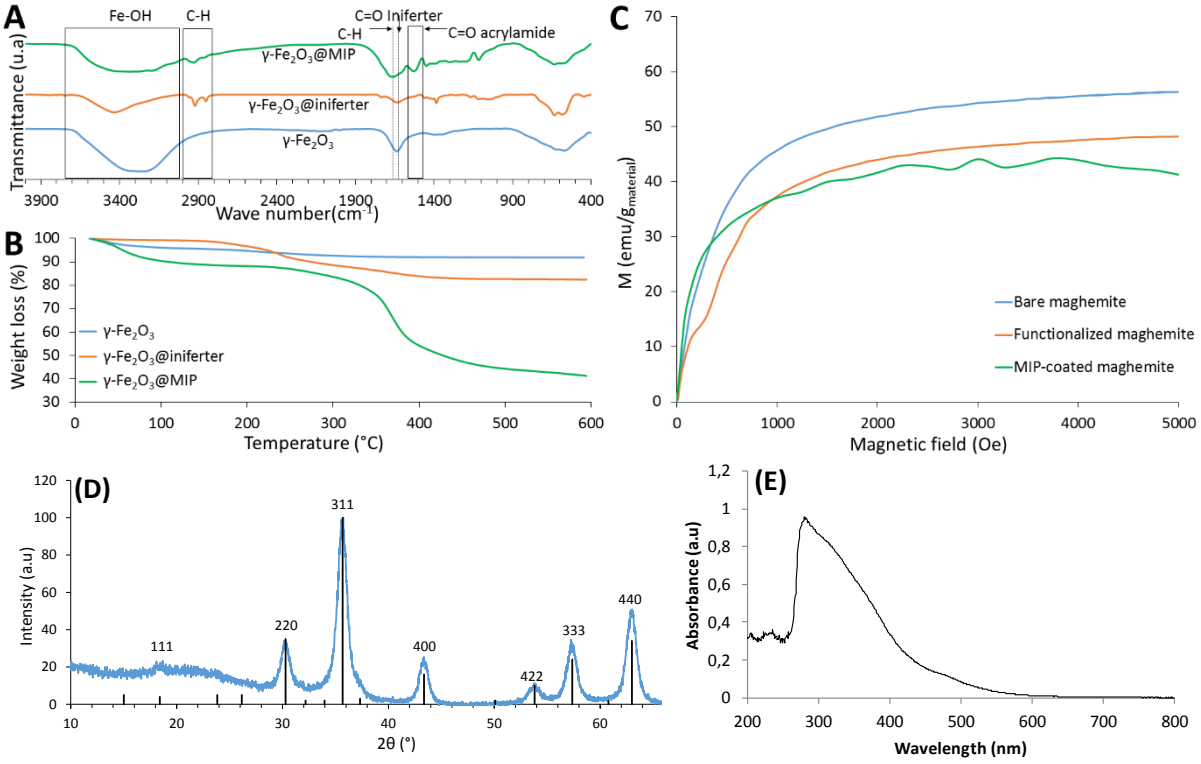


Figure S1: Characterizations of the different γ-Fe_2O_3 hybrid nano-objects. (A) FTIR spectra of bare, iniferter-functionalized and MIP functionalized maghemite nanoparticles. (B) TGA thermograms of bare, iniferter-functionalized and MIP functionalized maghemite nanoparticles. (C) Magnetization curves of bare, iniferter-functionalized and MIP-coated maghemite nanoparticles. (D) XRD data for bare magnetic nanoparticles. Black lines: theoretical data for maghemite nanoparticles (JCPDS file 39-1346). (E) UV-Visible absorption spectra for bare maghemite nanoparticles.

Effectiveness of the protein imprinting process

To assess the protein imprinting, adsorption capacities (Q) of both magnetic imprinted and non-imprinted polymers were determined using the following protocol. 5 mg of γ-Fe_2O_3@MIP or NIP were dispersed in 3 mL of protein solutions at 0.75 mg/mL to be sure to saturate the adsorption sites. The resulting mixtures were shaken at room temperature for two hours. Particles were then collected using an external magnetic field and supernatants were analysed using UV-Visible spectroscopy to determine the adsorption capacity according to equation $Q = \frac{(C_i - C_f)V}{m}$, where C_i (mg/mL) and C_f (mg/mL) are respectively the initial and final concentrations of the protein samples, determined using UV-Visible spectroscopy, V (mL) is the volume of the protein solution and m (mg) is the mass of hybrid nano-objects in suspension.

The calculated adsorption capacities show that protein imprinted polymer has a maximal adsorption capacity of 57.5 mg/g and adsorbed 2.28 times more GFP than the non-imprinted one, thus confirming the existence of imprints and their efficiency to recognize the template protein.

Nano-objects degradation within the lysosome-like buffer solution

Each degradation day, 1 mL of solution composed of various nanoparticles inside the lysosome-like buffer solution was taken for analysis using dynamic light scattering. Z-average values, providing a reliable value of the average size of the particles size distribution, evolved as displayed on the following figure during degradation.

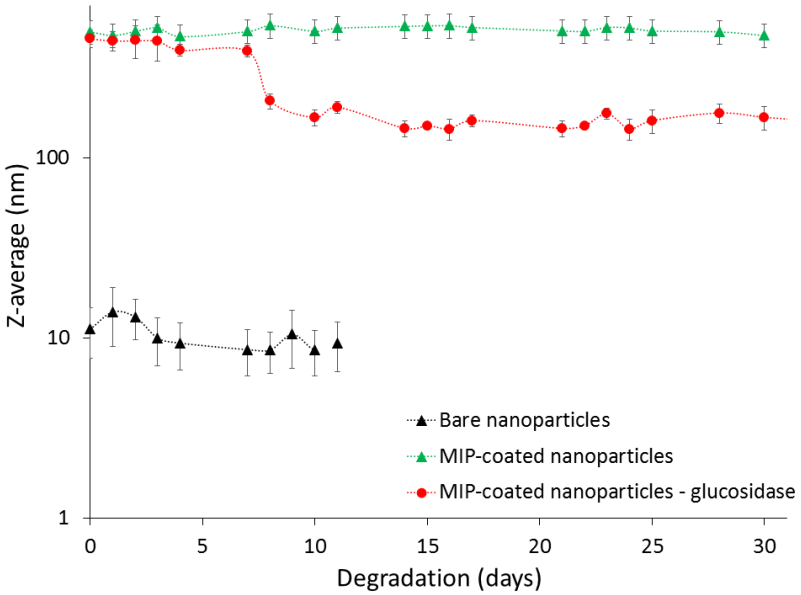


Figure S2: Degradation of hybrid nano-objects in lysosome-like buffer solution. Variation of the hydrodynamic diameter over time.

As one can see, the average size of the bare or MIP-coated nanoparticles (without enzyme) showed no significant evolution over time, while the addition of glucosidase induced a diminution of the z-average value. These results are coherent with an all-or-nothing degradation mechanism for iron oxide nanoparticles[3] but also suggest the possible action of the glucosidase enzyme to cleave the MIP-coating.

Follow-up of magnetic properties of nano-objects during maturation of spheroids

Spheroids (one at a time) were placed in nuclear magnetic resonance tubes containing 2 mL of PBS buffer. Transverse relaxation time T_2 were recorded as described in the experimental section.

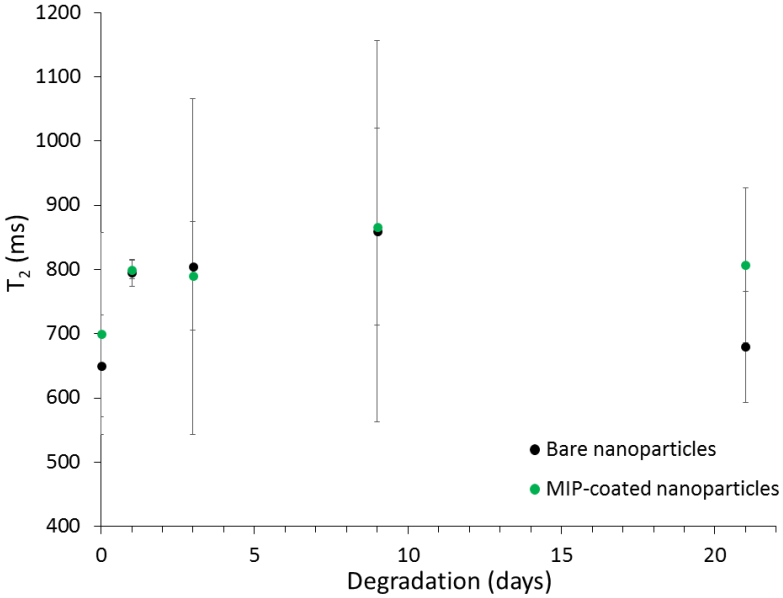
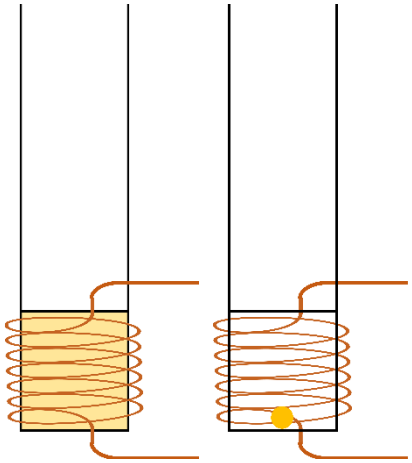


Figure S3: Evolution of the transverse relaxation times of spheroid after internalisation of bare or MIP-coated maghemite nanoparticles at different days during the spheroid maturation. Error bars correspond to standard deviation of measurements on three different spheroids.

From the results presented on Figure S3, there was no clearly discernible trend. Dispersity of results and very high values of error bars may be due to the extremely small size of the spheroid containing the iron compared to the size of the volume being monitored and analysed for T_2 recording.
Scheme S1 represents an imaged view of the magnetic coil responsible of T_2 measurements, placed inside the relaxometer around the tube containing the sample.



Scheme S1: Representation of tubes containing different samples once inside the relaxometer, and more precisely inside the magnetic coil responsible for the T_2 measurements. Left: Magnetic nanoparticles degradation in the lysosome-like buffer medium. Solution containing iron occupies the whole volume to be measured. Right: Magnetic nanoparticles degradation in the model tissue. Spheroid containing iron occupies only a small portion of the volume to be analyzed.

Same problem was not observed and error bars were much smaller while monitoring the particles degradation inside the lysosome-like buffer solution, as magnetic iron oxides (bare or MIP-coated) occupied the entire volume to be measured.

As described in the experimental section of the main paper, magnetization curves were analysed to obtain the particles mean magnetic diameter and the polydispersity index σ. Evolution of the polydispersity index during degradation for both pristine and MIP-coated maghemite nanoparticles is displayed on Figure S4.

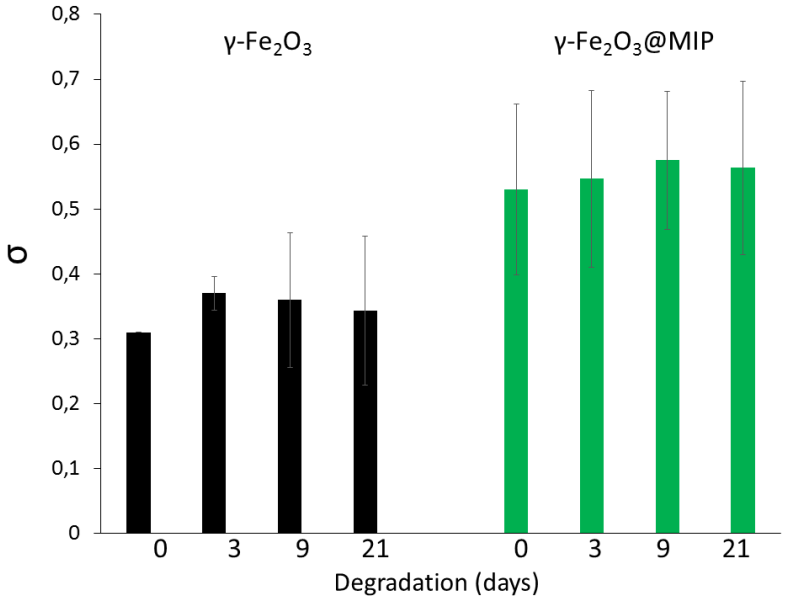


Figure S4: Variation of the polydispersity index σ of bare or MIP-coated maghemite nanoparticles during maturation within the model tissue.

Magnetic nanoparticles inside the hybrid nano-objects have a higher polydispersity index than bare maghemite nanoparticles, maybe due to their possible aggregation inside the protein imprinted polymer coating. But no clear evolution of σ is visible for either kind of objects during degradation.

Long-term imaging of bare and MIP-coated nanoparticles within the model tissue

TEM images supplementary to those displayed in the main article, and obtained as described in the experimental section are visible on Figure S5.

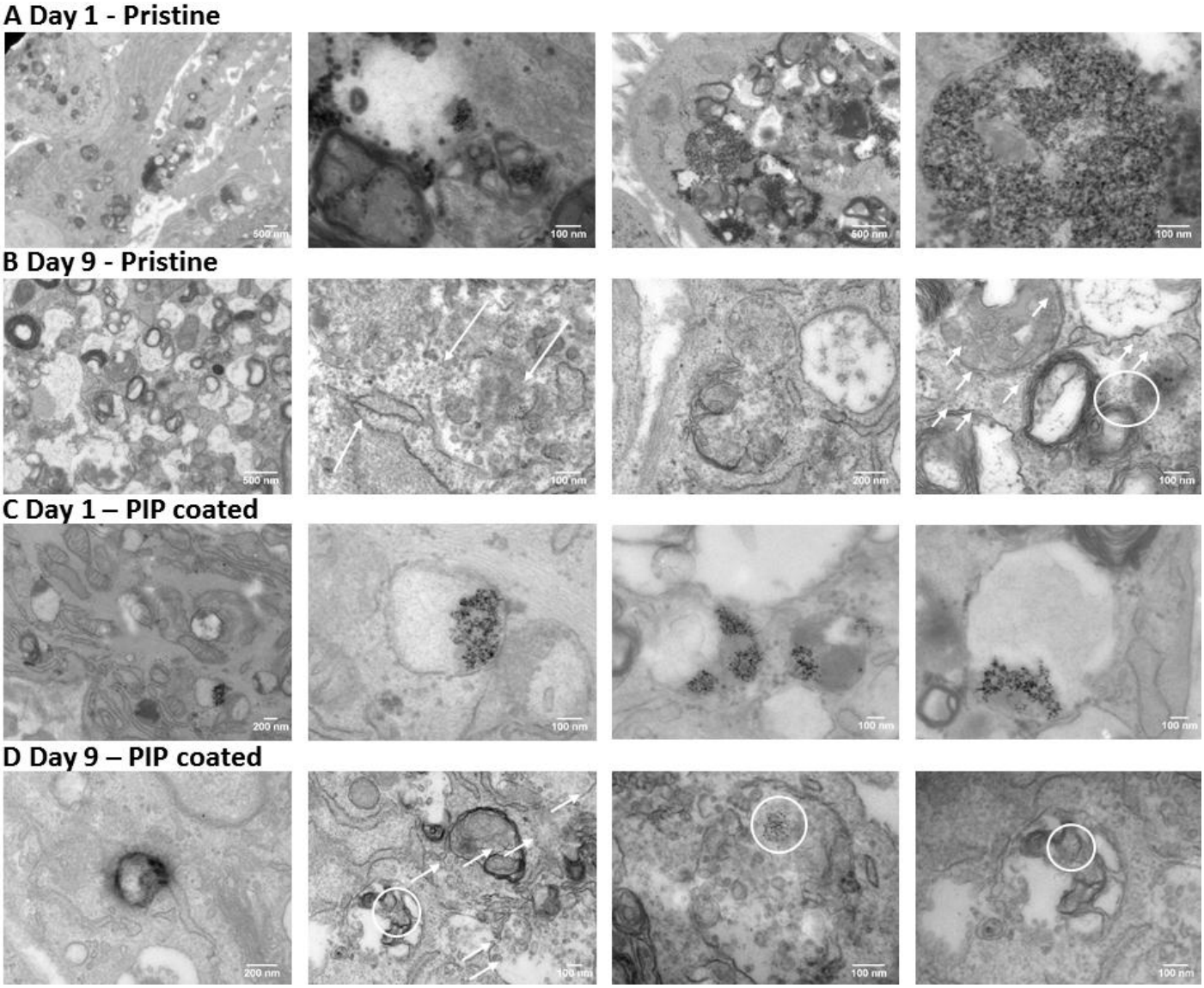


Figure S5: Bare and MIP-coated nanoparticles imaging within the model tissue. (A, B) Transmission electron microscopy of spheroid tissues containing bare maghemite nanoparticles at day 1 (A) and after 9 days of maturation (B). (C, D) Transmission electron microscopy of spheroid tissues containing γ-Fe_2O_3@MIP hybrid nano-objects at day 1 (C) and after 9 days of maturation (D). White arrows indicate ferritins.

References

(1) Massart, R. Preparation of Aqueous Magnetic Liquids in Alkaline and Acidic Media. *IEEE Trans. Magn.* **1981**, *17* (2), 1247–1248. https://doi.org/10.1109/TMAG.1981.1061188.

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(3) Mazuel, F.; Espinosa, A.; Luciani, N.; Reffay, M.; Le Borgne, R.; Motte, L.; Desboeufs, K.; Michel, A.; Pellegrino, T.; Lalatonne, Y.; Wilhelm C. Massive Intracellular Biodegradation of Iron Oxide Nanoparticles Evidenced Magnetically at Single-Endosome and Tissue Levels. *ACS Nano* **2016**, *10* (8), 7627–7638. https://doi.org/10.1021/acsnano.6b02876.