Phosphonate PEG Copolymers to control the USPIO stealthiness

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Abstract – For nanomedicine applications, there is a need to design advanced and cost-effective coatings, resistant to protein adsorption leading to increased biodistribution in vivo. In this study, phosphonate poly(ethylene glycol) copolymers were synthesized and used to coat 6nm and 13nm iron oxide particle. In vitro, PEGylated particles exhibit exceptional stability and low cellular uptake, of the order of 100 femtograms of iron per cell. In vivo, we developed an in vivo Dynamic Susceptibility Weighted MRI (7T) protocol to monitor the hepatic capture and clearance of the particles injected to mice as a function of the time. PEGylated coat of molecular weight 2000 gmol^{-1} show a 2hrs delay to liver uptake as compared to the commercially available USPIO Cliavist, indicating a direct correlation between the surface properties of the contrast agents and their stealthiness.

Index terms– Magnetic Resonance Imaging, Nanomedicine, Contrast agents USPIO, PEG, polymer coating.

I. INTRODUCTION

Cancer is the second leading cause of premature deaths in Europe, after cardiovascular diseases [1]. Magnetic Resonance Imaging (MRI) enables to have precise information about the size, the location and the spread of the tumor in order to diagnose it as fast as possible. In most applications intrinsic contrast is sufficient to obtain a well contrasted image with MRI, but in almost 40% of clinical studies, extrinsic contrast agents able to enhance the signal of the lesions are required.
Iron oxide nanoparticles offer many properties that make them attractive as MRI contrast agents [3].
When disperse in biological fluids (plasma, lung fluid), engineered nanoparticles are selectively coated with proteins, resulting in the formation of a protein corona, recognition by the RES and a rapid cellular capture. For imaging applications, there is a need to design advanced and cost-effective coatings that are resistant to protein adsorption and that increase the biodistribution *in vivo*. In this study, engineered copolymers made with poly(ethylene glycol) and phosphonate anchors were synthesized and used to coat 6 nm iron oxide particles [1]. MRI dynamic methods have been developed to study their biodistribution in the liver, especially their liver uptake and release, giving indications on the nanoparticle stealthiness.

II. MATERIALS AND METHODS

II.1. **Nanoparticles**: the iron oxide nanoparticles were received from Paris 6. They were synthesized according to the Massart method (3). The nanoparticles were then size-sorted by subsequent phase separation. For this study, two batches of γ-Fe_2O_3 nanoparticles with diameters of 6.8 nm and 13.2 nm were synthesized.

Polymers and coating: The phosphonate PEG copolymers were synthesized by SPECIFIC POLYMERS (http://www.specificpolymers.fr/) to supply mixed dispersions of 6.8 and 13.2 nm iron oxide nanoparticles. The copolymer composition was optimized to provide simple and scalable protocols as well as long-term stability in culture media.

Nanoparticles characterization*: Dynamic Light Scattering (DLS)* was performed to measure the D_H of nanoparticles. *Ultra-Violet (UV) spectrometry* was performed on a Varian Cary 50 scan to measure the iron concentration.

II.3. **Cytotoxicity Assay**
The cytotoxicity of USPIOs are analyzed *in vitro* by a WST assay on hepatocytes.
II.4. **Stability**: All the samples were diluted to obtain a concentration of 1 mM to be analyzed for D_H during 2 h and at 24 h, and 1 week. The stability of the nanoparticles

was assessed by measuring the D_H with DLS as a function of the time.

II.4. **In vitro studies**

Relaxivities r_1 and r_2 of USPIOs were measured in saline and cells culture medium at 7T.

II.5. **In vivo studies**

In vivo, we developed an in vivo Dynamic Susceptibility Weighted MRI (7T) protocol to monitor the hepatic capture and clearance of the particles injected to Balbc C mice as a function of the time**.**

III. RESULTS

In vitro, PEGylated particles exhibit long-term stability in biological media and low cellular uptake, of the order of 100 femtograms of iron per cell.

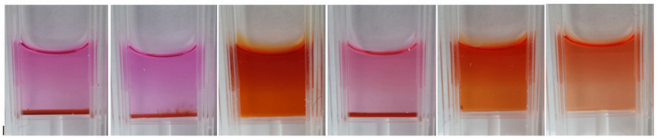

Figure 1 : *Photos of the nanoparticles in DMEM. Macro aggregates can be seen for the bare nanoparticles and the 13.2 nm citrate nanoparticle (red arrows).*

In vivo, Dynamic Susceptibility Weighted MRI (7T) protocol to monitor the hepatic uptake and clearance of the particles injected to Balbc C mice as a function of the time demonstrated that commercial contrast agents (such as Cliavist) were captured by the liver within 5 minutes after injection. With a PEGylated coat of molecular weight 2000 g mol-1, the capture was postponed by 2 hours and the uptake level lower, showing a direct correlation between the surface properties of the contrast agents and the biodistribution.

IV. DISCUSSION – CONCLUSION

The present survey demonstrates that the surface chemistry of engineered particles is a key parameter in the interactions with cells. This work demonstrates that the functionalization by PEG and phosphonate groups to be attached to the particles is an effective way to develop highly stable nanoparticles and to increase their circulation time in blood. The RES uptake is delayed, allowing the particles to stay longer in the blood pool. Thus, these new PEG polymer coated nanoparticles open up new avenues for detection of tumors with MRI.

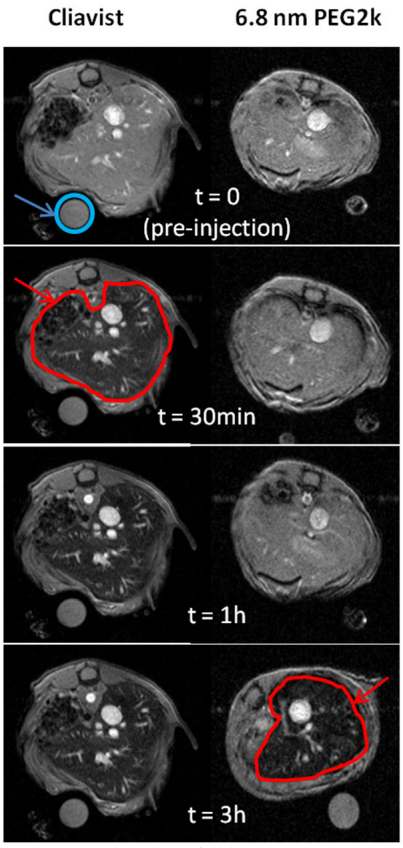

Figure 2: Liver sections of mice at different time points (pre-injection, 30min, 1h and 3h). On the left hand-side, Cliavist was injected, on the right hand-side, 6.8 nm PEGylated iron oxide nanoparticles was injected. An hyposignal is observed in both cases but at different time point.

Beyond applications to imaging and nanomedicine, the approach also opens up new avenues for the efficient functionalization of inorganic surfaces.

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