Comparative study of the influences of surface functionality and corona protein adsorption on the hemocompatibility of magnetite nanoparticles

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Statement of Purpose: The hemocompatibility is determined by the corona forming around the nanoparticles, which, in turn, is determined by the nanoparticle surface functionality. Previous studies showed that surface-charged polymeric nanoparticles influence platelet activation (Mayer et al., 2009; Thasneem et al., 2011). Another study revealed that positively charged magnetite nanoparticles show no significant hemolysis of human blood (Shieh et al., 2006). We focused on the effect of the functionality of magnetite nanoparticles, and the composition of their corona proteins, on their hemocompatibility. To our knowledge, this is the first comparative study in which this was done.

Methods:

Magnetite nanoparticles synthesis

We synthesized bare, positively and negatively charged magnetite nanoparticles, as previously described (Laurent et al., 2008).

Platelet aggregation testing

Freshly collected citrated human blood was centrifuged at 1000 rpm for 15 min and the platelet rich plasma (PRP) upper layer, was collected. The platelet concentration was then adjusted to $250x10^6$ per mL with platelet poor plasma prior to use. Platelet aggregation in PRP was then monitored on a four-channel optical aggregometer under shear (1000 rpm) at 37°C, where 1 mg/mL of bare or charged nanoparticles were used to induce platelet aggregation.

Hemolysis testing

For this study, freshly collected citrated human blood was centrifuged at 700 rpm for 10 min and the red blood cell (RBC) bottom layer, was collected and diluted (1:9) in normal saline. 100 µL of diluted RBC was incubated with 1mg/mL of magnetite nanoparticles. Normal saline was used as the negative control (0% lysis), and distilled water as the positive control (100% lysis). The samples were incubated for 30 min at 37 ºC and were then centrifuged at 700 rpm for 5 min. The supernatants were collected and their absorbance was measured at 541 nm by UV-Visible spectrophotometry.

MALDI-TOF mass spectroscopy

We used a Bruker Reflex IV MALDI-TOF mass spectrometer to determine the corona serum proteins adsorbed on the magnetite nanoparticles.

Results: The hemolysis assessment indicated that negatively charged magnetite nanoparticles were less hemocompatible than the positively charged and the bare ones, as they caused RBC lysis. The platelet aggregation testing revealed that negatively charged nanoparticles caused aggregation, whereas the positively charged ones inhibited it. The bare nanoparticles had no influence on platelet aggregation. Moreover, the study of the corona protein adsorption on the surface of these nanoparticles revealed that the corona composition depended on their surface functionality.

Conclusions: Nanoparticle functionalization and corona protein adsorption impact the hemocompatibility of magnetite nanoparticles. The hemocompatibility assessment permits the determination of which functional group on the surface of magnetite nanoparticles does not change their hemocompatibility. Comparing bare, positively and negatively charged magnetite naoparticles, we conclude that the negatively charged nanoparticles are less appropriate for use in *in vivo* studies, where these nanoparticles enter into contact with circulating blood. The positively charged or bare magnetite nanoparticles are good candidates for this purpose.

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