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# MRI-sensitive contrast agent with anticoagulant activity for surface camouflage of transplanted pancreatic islets

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ABSTRACT

Pancreatic islet implantation in the liver is a promising approach for diabetes therapy. However, 70% of the islet mass fails to be engrafted in the liver due to the instant blood-mediated inflammatory reactions (IBMIR) resulting from direct contact between islet cells and the bloodstream. To overcome this issue, direct monitoring is very important for establishing prognosis after islet cell therapy. Here we established a new type of MR contrast agent with anticoagulant activity via heparin-immobilized superparamagnetic iron oxide (HSPIO). The HSPIO was chemically conjugated onto islet surface *ex vivo* without damage of their viability and functionality. The conjugated HSPIO nanoparticles onto islet surface could attenuate IBMIR *in vitro* and *in vivo*. The HSPIO-conjugated islets could cure the blood glucose levels of diabetes animals after implantation. In addition, the HSPIO nanoparticles were well maintained on the transplanted islets for a long time during modulation of inflammation. Also, they allowed for stable visualization of the implanted islet cells for more than 150 days without reduction of the MRI signal. Furthermore, when HSPIO itself was intraportally injected, it was rapidly eliminated without accumulation in the liver, suggesting that HSPIO nanoparticles could only track the immobilized islet. Collectively, this HSPIO nanoparticle having MRI sensitivity and anticoagulant activity could be utilized for successful islet implantation.



## 1. Introduction

Insulin-dependent diabetes mellitus (IDDM) is generally thought to be an autoimmune disease that induces insulin hormone depletion in diabetic patients. To cure IDDM, insulin-secreting pancreatic islets isolated from a cadaveric donor pancreas are clinically infused into the liver of diabetic patients via the portal vein in order to rapidly control blood glucose levels of diabetic patients, because the liver is the major action point of insulin hormone [1,2]. However, there are many obstacles to successfully engraft the infused islets, such as the cellular damage during islet isolation procedure, relatively lower parenchymal oxygen tension of liver, toxicity of the administered immunosuppressive drugs, and immune reaction-mediated liver failure. Critically, a main hurdle for islet engraftment failure is the instant blood-mediated inflammatory reaction (IBMIR), which results in significant reduction (50–75%) of the total islet mass (Fig. S1A, Supporting Information) [3,4]. Thus, IBMIR attenuation is generally believed to be a 'Rosetta Stone' for successful outcome of clinical islet implantation. Heparin, a highly sulfated and negatively-charged polysaccharide, is clinically and widely used as an injectable anticoagulant. In particular, heparin immobilization on the surfaces of artificial materials and devices is useful for inhibition of the coagulation system, including platelet adhesion and complement activation, after implantation of devices into the body [5–7]. Therefore, when heparin is administered after intraportal islet transplantation, it could attenuate the IBMIR around the infused islets, thereby improving their viability.

Although there have been many efforts to study alternative sites for islet implantation in order to avoid IBMIR, intraportal islet implantation is currently inevitable in clinical practice. Thus many studies have been performed in hopes of attenuating IBMIR. In these circumstances, direct monitoring of the infused islets is useful

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for assessment of implanted islet cells. Also direct monitoring could assess therapeutic and/or adverse effects of immunosuppressive medications on the infused islets. Among various kinds of imaging modalities, magnetic resonance imaging (MRI) is beneficial and informative to track the fate of implanted cells in the body due to its high spatial and contrast resolution, unlimited penetration through tissues, and tomographic capability [8–11]. Unfortunately, the MRI sensitivity of islet cells themselves is rare, resulting in their wrong assessment *in vivo*. Therefore, to enhance the visualization of implanted islet cells, MRI-sensitive contrast agents such as Feridex® and Resovist® are used to enhance the MRI sensitivity. Specially, in the case of *ex vivo* cell implantation, these contrast agents are physically labeled into them via cellular endocytosis after one to three days of incubation [12–15]. However, this strategy is not systematic and limits quantitative tracking of islet cells *in vivo* due to the random cellular uptake of the contrast agents. There have been several trials to overcome this challenge by using transfection agents such as lipofectamine and polyethyleneimine [16,17], protamine sulfate [18], and dendron guanidine [13]. These could be well-defined remedies for intracellular islet labeling, but there are still limitations related to the availability and toxicity of such agents to cells. Therefore, there is a need to develop new types of MRI-sensitive contrast agent that can overcome these disadvantages.

Here we prepared heparin-immobilized superparamagnetic iron oxide (HSPIO) nanoparticle with anticoagulant activity and then it was chemically conjugated for surface camouflage of the islet *ex vivo* (Fig. 1). We evaluated the stable conjugation of HSPIOs onto the islet surface and the resultant inhibition of IBMIR. The beneficial effects of HSPIO nanoparticles were demonstrated through implantation of HSPIO-conjugated islets. This system could have several advantages in islet implantation (Fig. S1B). First, the biocompatible linker polymer itself could repel adhesion of platelets onto the islet surface via its natural hydrodynamic volume and flexibility. Second, the PEG polymer could extrude infiltrating immune cells and cytotoxic molecules in accordance with allogeneic or xenogeneic immune reactions. Third, the immobilized heparin could attenuate blood coagulation, thereby reducing blood-mediated inflammation. Finally, the MRI-sensitive HSPIO nanoparticle could be consistently attached to the islet surface and quantitatively visualized as a means of monitoring the implanted islet cells in the body.

## 2. Materials & methods

### 2.1. Modification of unfractionated heparin (UFH)

Unfractionated heparin (UFH; Nanjing King-Friend Biochemical Pharmaceutical CO., Nanjing, China) was modified to contain sulfhydryl groups with Traut's reagent (2-iminothiolane•HCl; Pierce Biotechnology, Rockford, IL, USA). To introduce free sulfhydryl groups, Traut's reagent (a cyclic thioimidate compound for thiolation) was reacted with primary amines. For this, 250 mg of UFH was dissolved in 10 ml of sodium borax buffer (20 mM, pH 10.0) and added to 3 mM EDTA to chelate divalent metals in the solution. A 20-fold molar excess of Traut's reagent was further added and incubated at room temperature (RT) for 1 h. Finally, the thiolated UFH (UFH-SH) from excess Traut's reagent was separated using a dialysis membrane (3500MWCO; Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) in pH 7.5 PBS buffer containing 3 mM EDTA.

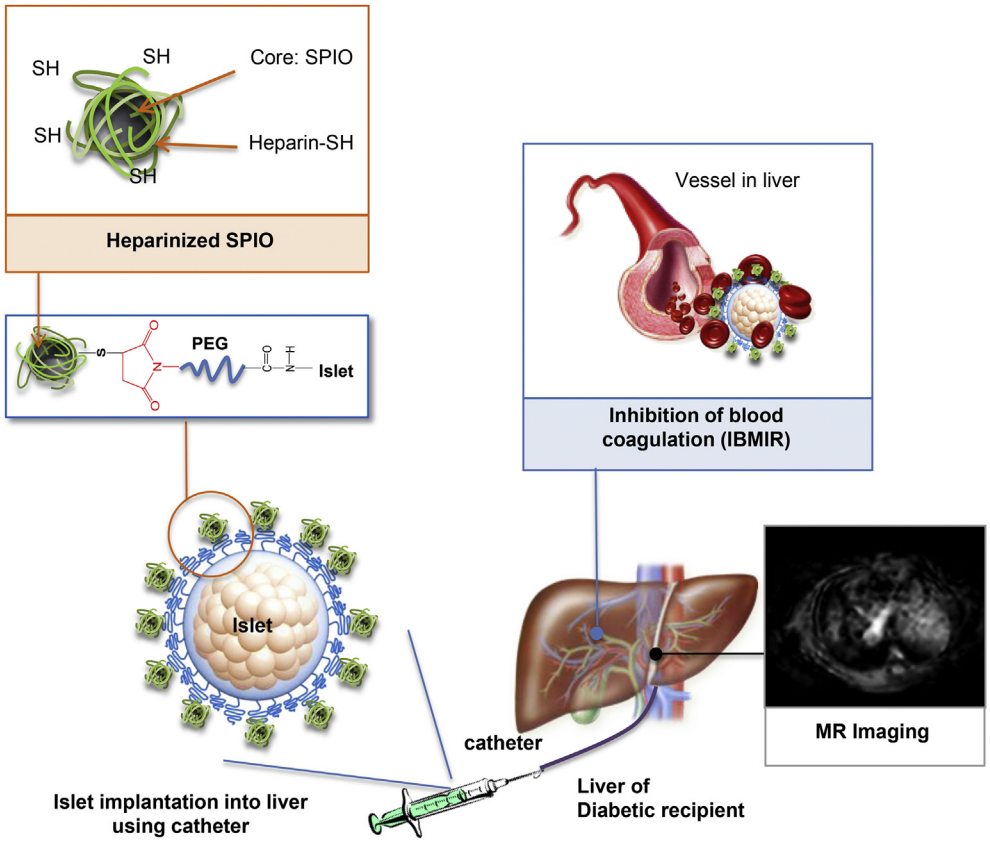


**Fig. 1.** Scheme of the inhibition of the instant blood-mediated inflammatory reaction (IBMIR) and MR imaging by immobilization of heparin-coated superparamagnetic iron oxide (HSPIO) particles after pancreatic islet transplantation in the liver.