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CELL TRACKING

Functionalized superparamagnetic iron oxide nanoparticles provide highly efficient iron-labeling in macrophages for magnetic resonance–based detection *in vivo*

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Abstract

Background aims. Tracking cells during regenerative cytotherapy is crucial for monitoring their safety and efficacy. Macrophages are an emerging cell-based regenerative therapy for liver disease and can be readily labeled for medical imaging. A reliable, clinically applicable cell-tracking agent would be a powerful tool to study cell biodistribution. *Methods.* Using a recently described chemical design, we set out to functionalize, optimize and characterize a new set of superparamagnetic iron oxide nanoparticles (SPIONs) to efficiently label macrophages for magnetic resonance imaging–based cell tracking *in vivo*. *Results.* A series of cell health and iron uptake assays determined that positively charged SPIONs (+16.8 mV) could safely label macrophages more efficiently than the formerly approved ferumoxide (−6.7 mV; Endorem) and at least 10 times more efficiently than the clinically approved SPION ferumoxytol (−24.2 mV; Rienso). An optimal labeling time of 4 h at 25 μg/mL was demonstrated to label macrophages of mouse and human origin without any adverse effects on cell viability whilst providing substantial iron uptake (>5 pg Fe/cell) that was retained for 7 days *in vitro*. SPION labeling caused no significant reduction in phagocytic activity and a shift toward a reversible M1-like phenotype in bone marrow–derived macrophages (BMDMs). Finally, we show that SPION-labeled BMDMs delivered *via* the hepatic portal vein to mice are localized in the hepatic parenchyma resulting in a 50% drop in T2⋆ in the liver. Engraftment of exogenous cells was confirmed via immunohistochemistry up to 3 weeks posttransplantation. *Discussion.* A positively charged dextran-coated SPION is a promising tool to noninvasively track hepatic macrophage localization for therapeutic monitoring.

Key Words: *cell therapy, cell tracking, liver fibrosis, macrophage, MRI*

Introduction

Cell-based therapy offers an exciting new approach in the field of regenerative medicine to treat a range of diseases. Tracking cells after transplantation through medical imaging is considered an indispensable tool to ensure appropriate cell localization, migration and engraftment in the host tissue. There is a pressing need

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to develop new techniques to noninvasively detect and track transplanted cells *in vivo* using sensitive and safe tracking agents. Superparamagnetic iron oxide nanoparticles (SPIONs) are a versatile class of magnetic resonance imaging (MRI)-based contrast agents that have been used clinically to detect hepatocellular carcinomas in patients [1,2] and have potential as magnetic fluid hyperthermia treatment for cancers as well as magnetic targeting of drugs [3]. More recently, several reports have successfully employed SPIONs as a means to label cells *in vitro* (primarily macrophages, which readily ingest SPIONs) before transplantation and subsequently track cells via MRI [4,5]. Obtaining sufficient iron uptake in cells is a challenge and the limiting factor for MRI sensitivity. To improve uptake, several approaches have been used previously, including post-modification steps [6] or use of transfection agents [7], which can elicit toxicity [8]. In 2009, two clinically approved SPIONs were removed from the market for commercial reasons, ferumoxide (Endorem, Guerbet) and ferucarbotran (Resovist, Schering). Ferumoxytol (Rienso, Takeda/AMAG Pharmaceuticals) is still available clinically for the treatment of anemia in parts of Asia and the United States, but not in Europe. Therefore, there is currently a lack of suitable MRI-based contrast agents that can be used in both the laboratory and the clinic for cell-tracking purposes.

Our group recently described a technique to synthesize SPIONs with a modified dextran coating that contains diethylaminoethyl (DEAE) and fluorescein isothiocyanate (FITC) moieties that confer a positive charge and green fluorescent properties (GFPs) to the nanoparticles, respectively [9]. Chemically and structurally, the novel SPIONs are comparable to the formerly clinically approved SPIONs in having a dextran-coating and a similar sized iron core of approximately 60 nm. Here, we have used these novel SPIONs to label macrophages of murine and human origin. Macrophages represent a promising cell therapy for the treatment of liver fibrosis by reducing fibrotic scarring and improving liver regeneration and function through several mechanisms, including the expression of metalloproteinase enzymes, which degrade fibrotic scars, clearance of cellular debris and increased expression of cytokines implicated in the regenerative processes of the liver such as vascular epithelial growth factor [10,11]. Phase I/II clinical trials are currently in progress (Macrophage Therapy for Cirrhosis [MATCH] study). The availability of a suitable cell-tracking agent for macrophage therapy would potentially complement the design of prospective clinical trials and support clinical use by monitoring appropriate localization and engraftment of cells in the host.

Therefore, to assess whether the SPIONs are suitable MRI-based contrast agents for macrophage therapy, we have performed a series of investigations to examine the effect of SPION-labeling on these cells. Primary macrophages were differentiated *in vitro* from mouse bone marrow (bone marrow–derived macrophages [BMDMs]) or from human monocytes (monocyte-derived macrophages [hMDMs]) using defined protocols. Both sources provide highly enriched populations of mature and functionally active macrophages as previously reported [11,12]. Our group has recently described a method to control the synthetic approach to change the size and electrostatic charge of dextran-coated SPIONs [9]. Here we have investigated how modulation of these physicochemical properties at the particle level can influence the suitability of these molecules as macrophage contrast agents. Hence, macrophages were labeled with either novel functionalized SPIONs, previously clinically approved, or clinically approved SPIONs that served as comparators. Safety studies (cell viability/cytotoxicity) and iron uptake experiments were performed with respect to dose and time to identify the most appropriate labeling protocol in these cells. Due to the plasticity of macrophages, we further sought to test whether iron labeling caused any change in cell phenotype or function. Finally, we performed a longitudinal MRI study to monitor the biodistribution of SPION-labeled BMDMs *in vivo* after transplantation. In this way, we have been able to show that macrophages localize into the liver parenchyma after transplantation, which can be monitored for several weeks. We have thus developed a SPION that can efficiently label macrophages, is biocompatible and can serve as a suitable tracking agent to noninvasively monitor hepatic localization of macrophages *in vivo*.

Methods

BMDMs

Femurs and tibias of C57/BL6 male mice (8–10 weeks old) were collected in Hank's Balanced Salt Solution (Gibco) containing penicillin/streptomycin. In a sterile fume hood, muscle tissues were removed. Bone marrow was flushed out with Dulbecco's Modified Eagle's Medium (DMEM):F12 (1:1) cell culture media (Gibco) supplemented with 10% fetal bovine serum (v/v), 1 × glutamine and 1 × penicillin/streptomycin. Mouse bone marrow was centrifuged (400*g*, 5 min) and then resuspended in fresh supplemented DMEM:F12 media (20 mL) containing 20 ng/mL murine recombinant macrophage colony-stimulating factor (mCSF; Peprotech). Bone marrow suspensions were then transferred to sterile ultra-low attachment flasks (Corning) and incubated at 37°C, 5% CO_2. Every second day, 10% of the media was replaced with fresh media containing 400 ng mCSF. After 7 days, mature macrophages were fully differ-