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# Glycosaminoglycan-targeted iron oxide nanoparticles for magnetic resonance imaging of liver carcinoma

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ABSTRACT

To develop an efficient probe for targeted magnetic resonance (MR) imaging of liver carcinoma, the surface modification of superparamagnetic iron oxide nanoparticles (SPIONs) was carried out by conjugating a naturally-occurring glycosaminoglycan with specific biological recognition to human hepatocellular liver carcinoma (HepG2) cells. These modified SPIOs have good water dispersibility, superparamagnetic property, cytocompatibility and high magnetic relaxivity for MR imaging. When incubated with HepG2 cells, they demonstrated significant cellular uptake and specific accumulation, as confirmed by Prussian blue staining and confocal microscopy. The in vitro MR imaging of HepG2 cells and in vivo MR imaging of HepG2 tumors confirmed their effectiveness for targeted MR imaging of liver carcinoma.



## 1. Introduction

In recent years, superparamagnetic iron oxide nanoparticles (SPIONs) have attracted considerable attention in biomedical imaging [1–3]. For magnetic resonance (MR) imaging applications, SPIONs should be easily dispersible in aqueous system, colloidally stable, and non-cytotoxic in the given concentration range [4,5]. For such requirements, much effort has dealt with the surface modification of SPIONs by some hydrophilic polymers such as polyethylene glycol (PEG) [6,7], chitosan [8,9], dextran [10,11], polyethyleneimine (PEI) [12] and dendrimers [13]. However, the modified SPIONs by these polymers suffer usually from their non-specificity or non-recognition to treated cells, and are not applicable for tumor-specific MR imaging because they can be easily taken up by phagocytic cells and accumulated in the reticulo-endothelial system (RES) [14]. Therefore, there has been a growing demand to develop various tumor-targeted SPION systems for the MR imaging of tumor sites.

As a bioactive glycosaminoglycan, hyaluronic acid (HA) consists of repeating disaccharide units of *D*-glucuronic acid and *N*-acetyl-*D*-glucosamine [15], and plays an important role in some biological processes such as cell adhesion, cell migration, wound repair, cell proliferation and innate immunity [16]. The more interesting bioactivity of HA is an attractive targeting ligand that can bind CD44 receptors, which are over-expressed in many kinds of tumor cells [17,18]. Recently, Li et al. [19] reported a polyethyleneimine (PEI)-mediated approach to synthesizing HA-targeted magnetic iron oxide nanoparticles ($Fe_3O_4$ NPs), and confirmed the effectiveness of these modified $Fe_3O_4$ NPs for MR imaging of a human cervical carcinoma cell line (Hela cells). However, the used PEI has a high cytotoxicity [20], which will be unfavorable for in vivo MR imaging.

In this work, we carried out the surface modification of SPIONs via HA conjugation assisted by a biocompatible silane agent with amino group, and then used for the first time to develop an efficient probe for targeted MR imaging of liver carcinoma (HepG2 cells), one of the most lethal cancers globally [21]. It was found that such a modification provided SPIONs with good water dispersibility and cytocompatibility. In particular, the conjugated HA was confirmed to endow SPIONs with the specific targeting for the in vitro and in vivo MR imaging of HepG2 tumors.

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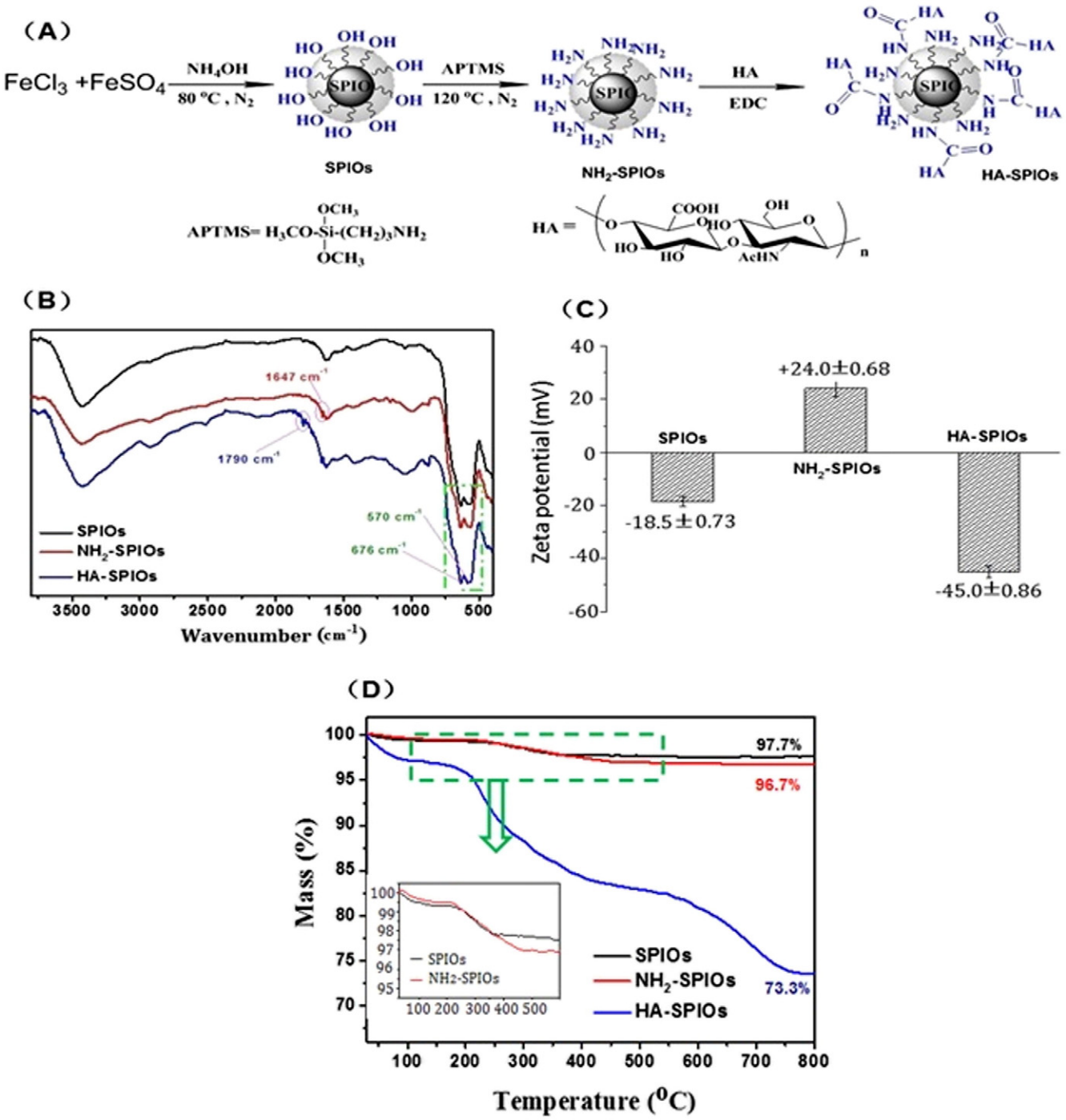


**Fig. 1.** (A) The preparation route to HA-modified SPIOs (HA-SPIOs); (B) IR spectra of SPIOs, $NH_2$-SPIOs and HA-SPIOs; (C) zeta potentials of SPIOs, $NH_2$-SPIOs and HA-SPIOs in aqueous dispersions; (D) thermogravimetric curves of SPIOs, $NH_2$-SPIOs and HA-SPIOs (the inset figure shows the decomposition of introduced aminosiloxane part).

## 2. Experimental section

### 2.1. Materials

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O > 99\%$), ferrous sulfate ($FeSO_4$), ammonium hydroxide, 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide (EDC), N-hydroxysuccinimide (NHS), and fluorescein isothiocyanate (FITC) were purchased from Aladdin Reagent Database Inc. (Shanghai, China). Hyaluronic acid (HA, MW 320 kDa) was purchased from Freda Biochem Co., Ltd. (Shandong, China). 3-Aminopropyltrimethoxysilane (APTMS) was purchased from Sigma-Aldrich (St. Louis, MO). Phosphate-buffered saline (PBS; 10 mM, pH 7.4), fetal bovine serum (FBS, 10%), Dulbecco's modified Eagle's medium (DMEM) and Roswell Park Memorial Institute medium (RPMI) were purchased from Hyclone/Thermo Scientific. All other chemicals and reagents were of analytical grade.

### 2.2. Preparation of SPIONs

SPIONs were prepared by a co-precipitation in alkaline media starting from a mixed $FeSO_4/FeCl_3$ solution with a molar ratio of $Fe(II)/Fe(III) = 0.5$ (typically 0.5 mmol $Fe^{2+}$ and 1 mmol $Fe^{3+}$ in 100 ml water) [22]. The mixed solution was stirred under nitrogen for 1 h to eliminate oxygen from the reaction flask at 30 °C, and 48 ml of