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Hyaluronic acid conjugated superparamagnetic iron oxide nanoparticle for cancer diagnosis and hyperthermia therapy

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ABSTRACT

Recently, superparamagnetic iron oxide nanoparticles (SPIONs) have been prepared for magnetic resonance (MR) imaging and hyperthermia therapy. Here, we have developed hyaluronic acid (HA) coated SPIONs primarily for use in a hyperthermia application with an MR diagnostic feature with hydrodynamic size measurement of 176 nm for HA-PEG10-SPIONs and 149 nm for HA-SPIONs. HA-coated SPIONs (HA-SPIONs) were prepared to target CD44-expressed cancer where the carrier was conjugated to PEG for analyzing longer circulation in blood as well as for biocompatibility (HA-PEG10 SPIONs). Characterization was conducted with TEM (shape), DLS (size), ELS (surface charge), TGA (content of polymer) and MRI (T2-relaxation time). The heating ability of both the HA-SPIONs and HA-PEG10-SPIONs was studied by AMF and SAR calculation. Cellular level tests were conducted using SCC7 and NIH3T3 cell lines to confirm cell viability and cell specific uptake. HA-SPIONs and HA-PEG10-SPIONs were injected to xenograft mice bearing the SCC7 cell line for MRI cancer diagnosis. We found that HA-SPION-injected mice tumors showed nearly 40% MR T2 contrast compared to the 20% MR T2 contrast of the HA-PEG10-SPION group over a 3 h time period. Finally, in vitro hyperthermia studies were conducted in the SCC7 cell line that showed less than 40% cell viability for both HA-SPIONs and HA-PEG10-SPIONs in AMF treated cells.

In conclusion, HA-SPIONs were targeted specifically to the CD44, and the hyperthermia effect of HA-SPIONs and HA-PEG10-SPIONs was found to be significant for future studies.

1. Introduction

Head and neck cancer is considered to be the sixth most common type of cancer. About 90% of the cancers affecting the head and neck are diagnosed as squamous cell carcinomas (SCC7) (Siegel, Naishadham, & Jemal, 2012). Hyaluronic acid (HA) is a biocompatible, readily available, non-toxic glycosaminoglycan polymer that is naturally found in our body which has got many functional groups required for chemical modification. Another important aspect of HA is its lower immunogenicity, and therefore, it is very well tolerated by the human body (Boeriu, Springer, Kooy, van den Broek, & Eggink, 2013; Choi et al., 2009; Liao, Jones, Forbes, Martin, & Brown, 2005; R. Thomas, Park, & Jeong, 2013; Thomas, Moon, Lee, & Jeong, 2015). CD44 receptor is recognized to be one of the major cell

Abbreviations: ATCC, American Type Culture Collection; CO_2, carbon dioxide; DLS, dynamic light scattering; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; EPR, enhanced permeability and retention effect; FBS, fetal bovine serum; MCF-7, human mammary carcinoma cells; NHS, N-hydroxy succinimide; PBS, phosphate buffered saline; pH, Potenz hydrogen; RPMI, Roswell park memorial institute medium.

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surface markers in epithelial tumors such as head and neck cancer (Kokko et al., 2011).

Superparamagnetic iron oxide nanoparticles (SPIONs) for magnetic resonance imaging (MRI) are very promising for biomedical application because of their excellent biocompatibility and chemical stability over physiological environments among various magnetic nanoparticles (Lee et al., 2010; Namgung et al., 2011). Magnetic hyperthermia with SPIONs is considered to be an effective method to treat cancer. Magnetic hyperthermia is usually associated with multimodal strategies as it can contribute to chemotherapy, radiotherapy, and immunotherapy (Maeng et al., 2010; Yallapu et al., 2011). Magnetic hyperthermia using SPIONs is the field of treating cancer by heat-generating properties under influence of an alternating magnetic field (AMF) (Kolosnjaj-Tabi et al., 2014). AMF can be defined as a continuous magnetic field that periodically reverses direction, usually sinusoidally. AMF-induced heating ability of the SPIONs is contributed mainly from relaxation loss (including Néel and Brownian relaxation) (Smolkova et al., 2015). During the hyperthermia process, cancerous cells get destroyed or do not grow further as the temperature of cancer tissue increases to around 40–45 °C (Kim, Ebara, & Aoyagi, 2013).

One of the major challenges in magnetic hyperthermia is the local heating surrounding healthy tissues, which deals collateral damage while the tumor region is heated to the desired hyperthermic temperature. AMF is utilized for magnetic nanoparticle-mediated treatment due to the heating ability of SPIONs (Guardia et al., 2012). The AMF-induced heating ability of the SPIONs was mainly due to relaxation loss (Noh et al., 2012).

A previous study demonstrated that HA-coated SPIONs (HA-SPIONs) with a size of 114 nm were further conjugated with doxorubicin for anti-cancer effect (El-Dakdouki et al., 2012). HA-SPION synthesis by functionalization of dopamine-coated SPIONs using EDC/HOBt chemistry was also conducted (Lee et al., 2008), but the use of PEGylated HA-SPIONs and their hyperthermia effect in head and neck cancer have not been previously reported. The purpose of this study is to analyze the effect of HA-SPIONs and HA-PEG10-SPIONs as both MR imaging and AMF-based hyperthermia agents (Scheme 1).

2. Materials and methods

2.1. Materials

Oligo HA (6800 Da) was acquired from Bioland, Republic of Korea. Formamide, pyrene and 3,4-dihydroxy-L-phenylalanine (dopamine) were purchased from Sigma–Aldrich, USA. Methoxy polyethylene glycol-succinimidyl succinate (2000 Da) and methoxy polyethylene glycol-amine (2000 Da) were acquired from Sunbio (Gyeonggi-do, Republic of Korea). N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and dicyclohexylcarbodiimide (DCC) were purchased from Sigma–Aldrich, USA. RPMI-1640 and Dulbecco's modified Eagle's medium (DMEM) were acquired from Thermo Scientific, USA. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) was acquired from Promega, USA. All other reagents were of analytical or chromatographic grade.

2.2. Synthesis of oleic acid-coated SPIONs

A thermal decomposition method was used to make 5-nm monodispersed oleic acid coated SPIONs (Lee et al., 2009; Xu, Shen, Hou, Gao, & Sun, 2009). Iron (III) acetylacetonate (3 mmol), oleylamine (9 mmol), Oleic acid (9 mmol) and 1,2-hexadecanediol (15 mmol) were added and stirred under a nitrogen flow for approximately 10 min in 30 ml phenyl ether in room temperature. After removing the stir bar, the mixture was heated for 30 min to 200 °C under a flow of nitrogen gas, and the temperature was subsequently increased to 265 °C for another 30 min. A black precipitate was formed, which was cooled and dissolved in hexanes in the presence of oleylamine and oleic acid and centrifuged at 6000 rpm for 10 min to remove any undispersed residue. Finally, 4 nm Fe_3O_4 nanoparticles were obtained by precipitating with ethanol.

2.3. Surface modification with HA and mPEG-ss

Oleic acid SPIONs were modified to have a dopamine coat to render them hydrophilic and amine functionalized (Xie et al., 2010). Briefly, 2 mg of oleic acid-coated SPIONs dissolved in 200 μL of chloroform was mixed with 10 mg of dopamine dissolved in DMSO. After the mixture formed a homogenous solution, stirring continued, and then dopamine coated SPIONs were separated by centrifugation and washed with water. For obtaining HA-PEG10-SPIONs, methoxy PEG succinimidyl succinate (mPEG-ss) was added to dopamine SPIONs in D.W. at a 1:0.1 mole ratio (Dopamine:mPEG-ss) and stirred for 24 h; later, HA conjugation was performed by EDC/NHS chemistry. For HA-SPION synthesis, the pegylation step was excluded and HA was added to dopamine SPIONs directly in DW and conjugated by EDC/NHS chemistry.

2.4. Characterization of HA-SPIONs and HA-PEG10-SPIONs

The morphology of HA-SPIONs was analyzed by transmission electron microcopy (TEM JEOL Model JEM Peabody, MA, USA). A sample was prepared by placing a diluted drop of nanoparticles on carbon coated copper grid and allowing it to air dry for 15 min. Thermogravimetric analysis (TGA) was also conducted to quantify the HA conjugate content in the HA-SPIONs (Fig. S1). The size distribution of HA-SPIONs and HA-PEG10-SPIONs was measured using DLS, and the particle surface charge was analyzed by a Zetasizer instrument (Nano-Z590, Malvern Instruments, Worcestershire, UK). Finally, FT-IR analysis was performed to confirm the HA conjugation and PEG functionalization in HA-SPION and HA-PEG10-SPION, respectively (Fig. S2).

2.5. T2 relaxation time of HA-SPIONs

HA-SPIONs were analyzed over four dilutions in a 1.5 ml centrifugation tube using a twofold serial dilution starting from a concentration of 0.25 mM [Fe]. MRI experiments were conducted using a 3.0-T clinical MRI instrument. For the relaxivity measurement, the T2-weigthed scans were performed with 2400 ms of TR (repetition time) and TE (echo time) ranging from 20 to 200 ms. Relaxivity values were calculated through the least-squares curve fitting of relaxation time versus iron concentration.

2.6. In vitro cytotoxicity

NIH3T3 (murine fibroblast cell) cells were cultured in a DMEM medium (Thermo Scientific, Utah, USA) containing 10% FBS and 1% penicillin–streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. Cell viability of HA-SPIONs and HA-PEG10-SPIONs was determined using an MTS assay (Promega, Madison, USA). Briefly, NIH3T3 cells were seeded onto a 96-well plate at a cell density of 10^4 cells per well and incubated overnight in CO_2 at 37 °C. On the next day, HA-SPIONs and HA-PEG10-SPIONs were added into the well containing cells in quadruplicate at a concentration ranging from 0.1 to 100 μg/ml and incubated for 2 h. Triton™ X-100 diluted to 2% solution in PBS was added to cells as positive control