Iron Oxide Nanoparticles and Derivatives for Biomedical Imaging and Application in Cancer Diagnosis and siRNA Therapy

Mehmet V. YIGIT and Zdravka MEDAROVA

*Corresponding author: Tel: 617-643-4889, Fax: Fax: 617-643-4865; Email: zmedarova@partners.org

Molecular Imaging Laboratory, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA

Abstract Our studies have focused on the application of imaging-capable nanoparticulate agents for the delivery of small RNA-based tumor therapy. One example includes magnetic nanoparticles (MN), which have traditionally been utilized as contrast agents for magnetic resonance imaging. The probes typically consist of a dextran-coated superparamagnetic iron oxide core (for magnetic resonance imaging), labeled with Cy5.5 dye (for near-infrared in vivo optical imaging), and conjugated to synthetic small interfering RNA (siRNA) molecules targeting model or therapeutic genes. We have explored the potential of these nanoparticles as delivery modules for small interfering RNA to tumors. Furthermore, we have investigated the feasibility of combining the imaging and delivery capabilities of these nanoparticles for the tracking of siRNA bioavailability. The versatile functionalization potential of MN has allowed us to control properties of the agents, such as uptake mechanism and target organ distribution. The tumoral accumulation of MN-siRNA results in a remarkable level of target-gene down-regulation. Repeated treatment with MN-siBIRC5, targeting the tumor-specific anti-apoptotic gene, birc5, leads to the induction of apoptosis in the tumors and an overall reduction in tumor growth rate. More recently, we have synthesized a second generation of nanoparticles, which combine the capability for high-resolution magnetic resonance imaging with detection by ultrasensitive surface enhanced Raman scattering.

Keywords: MRI, iron oxide, nanoparticle, cancer, therapeutics, diagnosis, nanomedicine, drug delivery, siRNA, magnetic resonance imaging, optical imaging

1. Introduction

Iron oxide nanoparticles have been of interest for biomedical applications due to their functional versatility. Concrete applications include cellular therapy, tissue repair, drug delivery, hyperthermia therapy, MRI, magnetofection *etc* (Gupta and Gupta, 2005). The literature includes numerous reports describing the synthesis of iron oxide nanoparticles via physical and chemical methods. Among them, wet chemistry procedures have been widely used, as size, composition, magnetic properties and shapes are more controllable with this method (Gupta and Wells, 2004). Iron oxides are generally prepared by co-precipitation of Fe^{2+} and Fe^{3+} salts in an aqueous solution. The anionic salt content (chlorides, nitrates, sulphates etc), the Fe^{2+} and Fe^{3+} ratio, pH and the ionic strength in the aqueous solution are key elements in controlling the size of the particles (Sjogren et al., 1994). One important step in the synthesis is to prevent the oxidation of the synthesized nanoparticles and protect their magnetic properties, which is generally established by carrying out the reaction in an oxygen free environment (under N_2) (Gupta and Curtis, 2004). The co-precipitation process is generally done in the presence of a surface coating in order to prevent the agglomeration of the iron oxides into microparticles during the synthesis. There have been several surface coating materials used for stabilizing iron oxide nanoparticles, among which there are synthetic and natural polymers, such as polyethylene glycol (PEG), dextran, polyvinylpyrrolidone (PVP), fatty acids, polypeptides, chitosin, gelatin *etc* (Gupta and Gupta, 2005).

2. Biomedical Applications of Iron Oxide Nanoparticles

The majority of the iron oxide nanoparticles are intended for biomedical applications. Their use as contrast agents for MRI is particularly attractive, due to their high sensitivity of detection (Bulte and Kraitchman, 2004). MRI is a clinically approved noninvasive medical imaging method, which allows the collection of three-dimensional information from inside the body with excellent tissue contrast. Iron oxide nanoparticles have been used to observe different biological events by *in vivo*, MRI including determining the fate of transplanted pancreatic islets (Evgenov et al., 2006; Medarova et al., 2006), tumor progression (Medarova et al., 2009) or lymph node metastasis (Harisinghani et al., 2003), *etc*.

Establishing target specific contrast agents for MRI presents a great opportunity for detecting disease at the initial stages when therapeutic intervention has the highest chances of success. This is possible because molecular targeting of the contrast would accomplish the detection of the early phenotypic changes that define the pathology. Combining diagnosis and therapy would be even more interesting, because it would allow the assessment of therapeutic efficacy as a function of drug delivery. It is possible to impart all of these functionalities to iron oxide nanoparticles by having different ligands attached to the surface of the nanoparticles.

There are several methods to prepare iron oxide nanoparticles but the focus of this paper is on crosslinked dextran coated superparamagnetic iron oxides, whose precursor is a clinically approved contrast agent (Josephson et al., 1999). The iron oxide nanoparticles discussed in this review have a ~5 nm superparamagnetic iron oxide core. They are coated with dextran, which is a biodegradable and biocompatible polymer. The dextran surface is further cross-linked using several chemical processes to introduce amine groups for functionalization. Multiple moieties including targeting peptides (Kumar et al., 2010), aptamers (Yigit et al., 2007; Yigit et al., 2008), NIR fluorescent dyes (Pittet et al., 2006), therapeutic materials (Wang et al., 2011) etc. can be conjugated to iron oxide nanoparticles through amine terminals and bifunctional linkers, Figure 1.

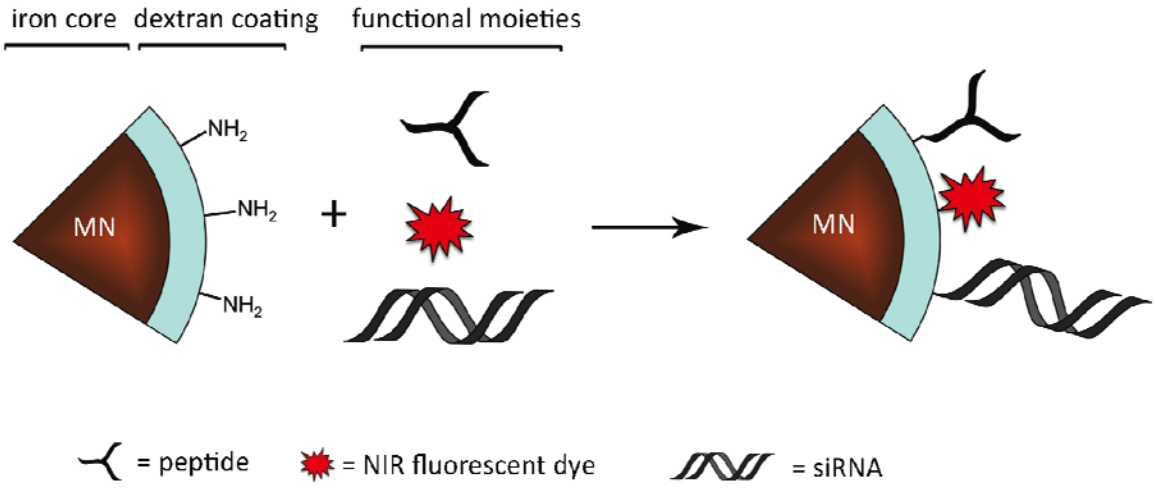

Fig. 1. Superparamagnetic iron oxide nanoparticles coated with dextran serve as a template for conjugation of several molecular moieties. In this example, magnetic nanoparticles are conjugated to a tumor targeting peptide, NIR fluorescent dye (cy5.5) and siRNA.

3. Multifunctional Imaging and Delivery

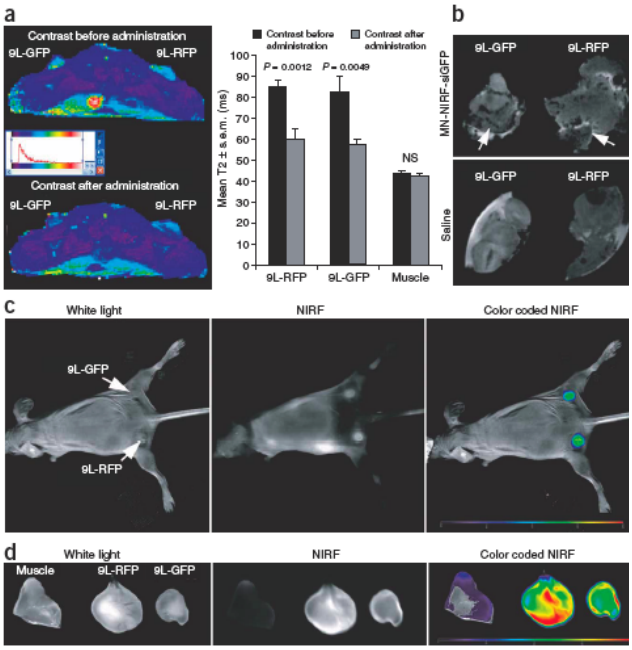

Fig. 2. (a) In vivo MR imaging of mice bearing bilateral 9L-GFP and 9L-RFP tumors before and 24 h after MN-NIRF-siGFP administration. A significant drop in T2 relaxivity was observed in the tumors. (b) Ex vivo high-resolution MR images of excised tumors (78 μm isotropic). (c) In vivo NIRF optical imaging of tumor-bearing mice. The fluorescence signal associated with the tumors confirmed the delivery of the MN-NIRF-siGFP probe to tumor tissues. (d) Ex vivo NIRF optical imaging showed a significantly higher fluorescence in tumors than in muscle tissues. (Reprinted from Medarova et al., 2007 with permission from Nature Publishing Group).

of Iron Oxide Nanoparticles

There are several examples of using iron oxide nanoparticles *in vitro* (Zhao et al., 2003) and *in vivo* (Weissleder et al., 2005). In one example, the probe consists of magnetic nanoparticles (for MRI) tagged with Cy5.5 dye (for NIRF imaging) and conjugated to a synthetic siRNA duplex targeting green fluorescent protein (gfp). The probe was tested in GFP-expressing tumors as a model system (Medarova et al., 2007). The probe was further conjugated to myristoylated polyarginine peptide (MPAP), which acts as a membrane translocation vector for increased uptake in tumor cells. First, *in vitro* experiments using green fluorescent protein (GFP) or control red fluorescent protein (RFP)-expressing tumor cells confirmed that *gfp* was down-regulated, whereas *rfp* expression remained constant. The *in vivo* studies showed that after systematic intravenous administration of the probe, accumulation was observed in tumors. Tumor uptake was confirmed by MRI and *in vivo* optical imaging (Figure 2). The expression of *gfp* in the GFP expressing tumor cells was reduced, whereas *rfp* expression was not affected in the RFP expressing tumors, indicating that the effect was specific. These results were confirmed by in *vivo* optical imaging and quantitative RT-PCR.

In a more clinically-relevant scenario, the nanoparticles carried siRNA to the antiapoptotic gene *birc5*, which encodes

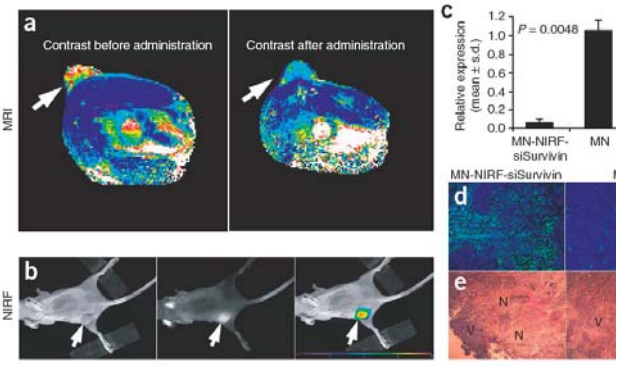

Fig. 3. Therapeutic MN-siSurvivin delivery. (a) *In vivo* MRI of mice bearing subcutaneous LS174T human colorectal adenocarcinoma (arrows). The significant drop in T2 relaxation times observed after administration of the nanoparticles indicated probe delivery. (b) The delivery of the probe to tumors was confirmed by the high-intensity NIRF signal on *in vivo* optical images of mice after injection of nanoparticles (left, white light; middle, NIRF; right,color-coded overlay). (c) Quantitative RT-PCR analysis of survivin expression in LS174T tumors after injection of magnetic nanoparticles conjugated to anti-Survivin siRNA (d) Apoptotic rates in tumor tissues. Note distinct areas with a high density of apoptotic nuclei (green) in tumors treated with probe (left), which were not observed in tumors treated with the control magnetic nanoparticles (right). (e) H&E staining of frozen tumor sections revealed considerable eosinophilic areas of tumor necrosis (N) in tumors treated with probe (left). Tumors treated with magnetic nanoparticles were devoid of necrotic tissue (right). Purple hematoxiphilic regions (V) indicate viable tumor tissues. Scale bar, 50 μm. (Reprinted from Medarova et al., 2007 with permission from Nature Publishing Group).

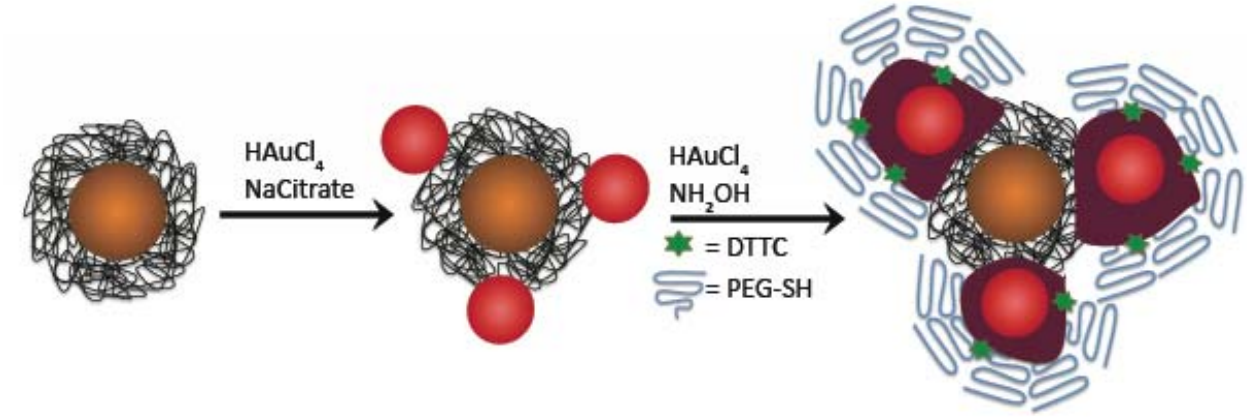

Fig. 4. Schematic representation of the synthesis of a SERS and MRI active contrast agent, AuMN-DTTC. The process involves sequential reduction of gold onto the parental MN, expansion of the gold seeds, and incorporation of DTTC and PEG onto the gold seeds.

survivin. Survivin is an important therapeutic target because it is a member of the inhibitor of apoptosis protein (IAP) family, which shows tumor-restricted expression in most human neoplasms. The synthesized probe was administered intravenously into mice twice a week for two weeks. MRI studies before and after probe administration revealed that the probe accumulated in tumor cells, which was also confirmed by *in vivo* optical imaging.

The specific silencing of *birc5* was confirmed by RT-PCR. As a result, a noticeable increase in tumor-associated levels of apoptosis and necrosis was observed. These results demonstrated the applicability of the siRNA-conjugated nanoparticles as multifunctional therapeutic and imaging agents (Figure 3).

In a similar scenario, in order to improve the bioavailability of the siRNA complex to tumor cells, the nanoparticles were conjugated to the MUC-1 specific EPPT peptide, instead of MPAP. This modification increases the overall nanoparticle availability in tumor cells (Kumar et al., 2010). Studies with the targeted probe revealed that the contrast agents were taken up by tumor cells in vivo resulting in an overall slower tumor growth due to specific silencing of *birc5* by the siRNA-functionalized probe.

This technology has far-reaching implications. By bringing imaging and therapy together, it can be applied to a wide range of diseases, which can be treated using intervention at the level of gene expression.

4. Novel Applications of Iron Oxide Nanoparticles

Although MRI is a valuable imaging tool due to its high resolution, it suffers from low sensitivity. On the other hand, fluorescence is a high-sensitivity detection method, which suffers from low resolution and background auto-fluorescence. Consequently, a multimodal imaging method combining MRI and optical detection with sensitivity equivalent to *in vivo* fluorescence but without background interference represents a unique advantage. To that purpose, we have investigated combining surface enhanced Raman scattering (SERS) with MRI. In SERS, enhanced Raman scattering signal is detected from molecules absorbed on rough metal surfaces such as gold and silver. SERS is particularly valuable, since it is highly sensitive (two orders of magnitude brighter than quantum dots) and the background interference is minimal. The advantage of SERS over other optical methods is reflected in its capability for spectroscopic detection and ultra-high sensitivity suitable for identification of single molecules under certain conditions. It has been shown that in order to obtain an *in vivo* SERS signature,

intravenous or intramuscular administration of as little as 50 femtomoles of SERS active gold nanoparticles is enough to obtain a distinguishing SERS signal from deep tissues or from tumor xenografts (Qian et al., 2008).

In our studies, we have synthesized MRI-active superparamagnetic iron oxide nanoparticles, stably complexed with gold nanoparticles (AuMN-DTTC). The gold nanoparticles serve as a template for a Raman active dye molecule to generate a surface-enhanced Raman scattering (SERS) effect (Figure 4). The synthesized probe was tested for its utility as a T2 weighted MRI contrast agent and a SERS active contrast agent (Yigit et al., 2011).

In vivo MR imaging of mice injected intramuscularly with the probe revealed that AuMN-DTTC indeed could be detected by MRI. SERS measurements using a portable Raman system showed a distinct SERS signal associated with the injection site (Yigit et al., 2011).

This agent presents novel opportunities for noninvasive imaging. By combining the two modalities, it could allow the high-resolution, high-sensitivity in vivo detection of biological processes.

4. Conclusions

Developing multi-functional, multi-modal contrast agents for clinical and pre-clinical *in vivo* imaging and therapy is important. These agents have proven valuable for the delivery of molecular therapy to tumors. Combined with their multimodal image-guidance capabilities, iron oxide nanoparticles could open up new possibilities for disease detection and clinical intervention.

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