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Surface-Engineered Magnetic Nanoparticle Platforms for Cancer Imaging and Therapy

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Conspectus

Enormous efforts have been made toward translating nanotechnology into medical practice, including cancer management. The approaches have generally been classifiable into two categories--those for diagnosis and those for therapy. The targets for diagnostic probes and therapy are often the same, however, and separate approaches to develop diagnostic and therapeutic agents can miss opportunities to improve the efficiency and effectiveness of both. A close and continuous linkage between therapy and diagnosis is also important, because a patient's diagnosis/prognosis will evolve during treatment.

The unique physical properties of nanomaterials enable them to serve as 1) bases for superior imaging probes to locate and report cancerous lesions, and 2) vehicles to deliver therapeutics preferentially to those lesions. These technologies for probes and vehicles have converged in the current efforts to develop nano-theranostics—that is, nanoplatforms with both imaging and therapeutic functionalities. These latest multimodal platforms are highly versatile and valuable components of the emerging beneficial trend toward personalized medicine, which emphasizes tailoring practices to individual needs so as to optimize outcomes. Unlike conventional methods, imaging and therapeutic functions are seamlessly unified in nano-theranostics, thereby permitting updates to diagnosis/prognosis along with treatment, and enabling opportunities to switch to alternative, possibly more suitable, regimens.

Magnetic nanoparticles, especially superparamagnetic iron oxide nanoparticles (hereafter referred to as IONPs), have long been studied as contrast agents for magnetic resonance imaging (MRI). Owing to recent progress in synthesis and surface modification, many new avenues have opened, though, for this class of biomaterials. The idea is to conceptualize the nanoparticles not as merely tiny magnetic crystals, but rather as platforms with large surface-to-volume ratios. By taking advantage of the well developed surface chemistry of these materials, one can load a wide range of functionalities, such as targeting, imaging and therapeutic features, onto their surfaces. This makes magnetic nanoparticles excellent scaffolds to construct theranostic agents and has attracted many efforts toward this goal.

In this account we will summarize the progress made in our recent studies. We will introduce the surface engineering techniques that we and others have developed, with an emphasis on how the techniques affect the role of nanoparticles as imaging or therapeutic agents.

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1. Introduction

Magnetic nanoparticles are an important class of biomaterials and have been made into various functional agents, such as for applications in imaging, cell labeling, drug delivery, gene delivery and hyperthermia.[1] These previous studies have established the foundations for current efforts to construct magnetic nanoparticle-based nano-theranostic agents.[2–6] Nano-theranostics embraces the conventional notion of marriage between therapeutics and diagnostics, but on the foundation of a nanoscale platform. Such an emerging technique adds another piece to the mosaic of personalized medicine and has attracted much attention in the community. The attractiveness of magnetic nanoparticles as building blocks of theranostics is at least two-fold. First, is their prequalification as MR imaging probes. Superparamagnetic iron oxide nanoparticles (IONPs), which show high magnetization in an external magnetic field but none when the magnetic field is removed, have been the most prominent T_2/T_2^* probes for magnetic resonance imaging (MRI); manganese and gadolinium containing particles, on the other hand, are at various stages of development, with the hope that they might replace metal-chelator complexes in a new generation of T_1 contrast agents. Second, is a set of well developed surface chemistry. This includes the capacity to fine tune the physical parameters of a nanoparticle, such as its size, shape, crystallinity, and magnetism[1]. More importantly, this suggests the potential for post-synthetically replacing or modifying the coating materials and, in doing so, tailoring the nanoparticle's surface charge, chemical groups, and overall size.[7]

In this account, we will introduce our work on engineering the surface of magnetic nanoparticles to enhance the nanoparticles' roles as tumor imaging and therapeutic agents. It is our hope that this may help to accelerate further progress in this promising field.

2. Basics of nanoparticle surface engineering

2.1 Surface coating and particle preparation

The synthesis and surface engineering of nanoparticles are closely related. Taking IONPs for instance, a classical paradigm is to co-precipitate Fe(II) and Fe(III) in a basic solution, in the presence of a polymer. The polymer then tangles with the growing nanocrystals, protecting them from overgrowth and aggregation.[1] Feridex, Combidex and Resovist, are products of this paradigm that have been marketed or are in clinical trials. Although these formulas are all coated with dextran (or its derivatives), other hydrophilic polymers have been found to be able to substitute for dextran as the coating material. For example, we have used polyaspartic acid (PASP) to replace dextran as the reaction precursor.[8] PASP bears both carboxyl and amino residues. It is believed that the multiple carboxylates function mainly by passivating the growing nanoparticle surface, while leaving free amine groups available for conjugation.

One drawback associated with the co-precipitation method is the suboptimal crystallinity of the products, a limitation that is partially associated with the low reaction temperature. To address this issue, there has been a trend toward replacing the co-precipitation method with a pyrolysis (or thermal decomposition)-based means, where an organic solvent with a high boiling point is used as the reaction medium. For instance, we prepared IONPs from DMF using polyvinylpyrrolidone (PVP) as the coating material.[9–10] The resulting 8–10 nm PVP-IONPs had a magnetization of 110 emu/g Fe, compared to that of 70 emu/g Fe for Feridex.[10] Solvents such as 1-octadecene and benzyl ether, which have even higher boiling points of around 300 °C, are now commonly used as the reaction media. To be compatible with such a change in solvent, the Fe precursors have been changed to compatible analogs--such as $Fe(CO)_5$, $Fe(acac)_3$ or $Fe(oleate)_3$-- and the coatings have been changed to such materials as

oleic acid and oleylamine. The resulting products can provide r_2 relaxivities as high as 300 $mM^{-1}s^{-1}$, almost triple that of Feridex (about 100 $mM^{-1}s^{-1}$).[11] It is worth noting that an improved crystallinity is not the only basis for this increase in magnetization. Rather, the size effect also plays an important role. At the nanoscale, the magnetization of particles increases with the particle size, due to the surface spin canting effect.[12] Unlike the conventional dextran-coated formulas, which have wide core size distributions, the pyrolysis-based preparation can yield products (with accurate size control[12]) up to 50 nm in diameter.

The coating materials (oleic acid and oleylamine), while proven to be better "sculptors" than dextrans, are hydrophobic. As a consequence, many types of nanoparticles made from the pyrolysis methods are not water soluble, and thus unsuitable for bio-applications. To address this issue, many surface engineering techniques have been developed to impart water solubility (as well as various functionalities) to the nanoparticle surface.[1] These aims can be achieved, for instance, through the addition of a second, amphiphilic coating layer. Such a ligand can use its hydrophobic section to interact with the oleic acid/oleylamine layer to get anchored on the particle surface. Meanwhile, its hydrophilic section will be exposed to the surrounding water molecules, affording physiological stability and conjugation-friendly groups (such as amines, carboxyls and thiols). For instance, we have tried to alkylate poly(ethylenimine) (PEI)[13] or a triblock copolymer (Figure 1a)[14] with various lengths of hydrophobic chains. The resulting amphiphilic polymers can self-assemble onto a lipophilic IONP surface and confer water solubility. An alternative approach is to use a ligand which has high affinity toward the IONP surface. When mixed, it can take the place of the original oleic acid/oleylamine coating and lead to hydrophilicity. One representative class of this kind is dopamine and its analogs. With the two adjacent hydroxyl groups, dopamine (or its derivatives) can chelate with the surface Fe on IONPs and, as a consequence, replace the original coating.[11] To improve stability, it is common to pre-conjugate dopamine with a hydrophilic tail, such as poly(ethylene glycol) (PEG).

Alternatively, we have found that proteins, such as human serum albumin (HSA), can be electrostatically adsorbed onto the dopamine-IONP surface to endow the particles with water stability (Figure 1b).[11] Slightly different from the previously mentioned strategies, this approach can be described as two-step engineering. In the first step, we replace the original coating with dopamine in a DMSO/$CHCl_3$ mixed solvent. In the second step, we add the dopamine-coated IONPs in DMSO into an HSA aqueous solution to induce the second coating. Introducing a coating layer via physical adsorption in this way is another common strategy in the surface modification of magnetic nanoparticles. It can be further extended to impart multiple coating layers with alternating charges onto a nanoparticle, the so-called layer-by-layer (LbL) self-assembly approach,[15] and is not limited to IONP modification. For instance, we successfully coupled gadopentetic acid (Gd-DTPA) with PEI (Gd-DTPA-PEI) and coated the conjugate onto silica nanoparticles.[16]

2.2 Surface coating and functionality

The development of favorable pharmacokinetics is an essential criterion in the design of nanoparticles intended for intravenous injection. We and others have identified several factors, including size, charge and hydrophilicity, that can be selected to improve performance. Previously, magnetic nanoparticles, especially IONPs, were used primarily as contrast probes in magnetic resonance (MR) for reticuloendothelial system (RES) imaging. Instead of targeting RES organs, a more advanced avenue is to introduce targeting motifs, either protein-, peptide- or aptamer-based, onto magnetic nanoparticles to create target-specific agents. In most cases, tethering of motifs is achieved through a bioconjugation technique, which uses mediators (such as EDC/NHS) or cross-linkers (such as N-succinimidyl-4-maleimidobutyrate) to form a covalent linkage between the two moieties.[2,7]

Surface engineering again plays a critical role in providing conjugation-friendly chemical groups, such as carboxyl, amine or thiol, on the particle surface. To make efficient coupling and to avoid cross-linking, it is sometimes necessary to pre-covert chemical groups of one side. For instance, we coupled c(RGDyK), a tumor targeting motif, onto amine-terminated, copolymer-coated IONPs. c(RGDyK) affords one amine group from lysine for coupling, so it is possible to use a homodimer linker, such as bissulfosuccinimidyl suberate (BS3), to achieve the coupling. However, such a measure will inevitably cause crosslinking among the same species. A better plan is to thiolate c(RGDyK) with agents such as *N*-succinimidyl *S*-acetylthioacetate (SATA).[14,17] This converts the problem to a conjugation between thiol and amine, which can be achieved via the use of a heterdimer crosslinker such as N-succinimidyl-4-maleimidobutyrate.

Not all the function loading needs covalent conjugation. The loading of therapeutics, for instance, is often achieved through non-covalent interactions, to ensure an easier release. For instance, it is a common strategy to coat nanoparticles with polycation materials and to use the conjugates as gene delivery vehicles. Due to electrostatic interaction, negatively charged RNA or DNA therapeutics are then able to be loaded onto the nanoparticle surface, and to cross cell membranes, whose lipid bilayer cores are otherwise considered impermeable to polar molecules. Later on, in the endosomes/lysosomes, where the pH is lower, the RNA/DNA cargos are released due to the proton sponge effect. Similarly, it is desirable to be able to load other small molecule-based therapeutics onto nanoparticle surfaces through physical interaction.

Overall, a set of chemistry has been developed, which allows functionality loading to be accomplished in a fast, economic and mild fashion. The means of choice is largely dependent on the chemical structure of the to-be-loaded motifs. However, in general, covalent conjugation is more utilized in the imaging setting, non-covalent loading is more seen in drug loading, and chelation chemistry is largely used in immobilization of radioisotopes.

2.3 Surface engineering and MRI contrast

The impact of surface engineering is not limited to imparting water solubility to nanoparticles; rather, it is also an important factor in modulation of particle r_1/r_2 relaxations. For instance, it was reported that coating thickness can affect protons' physical exclusion from magnetic field and residence time within the coating zone, and therefore, modulate particles' r_2 relaxivities.[18–19] More prominently, the aggregates of nanoparticles (Figure 2) were found to be able to induce more efficient T_2 shortening, a feature that has been harnessed to construct nanoclusters of higher r_2 values (Figure 2).[13,20–21] Taking Alkyl-PEI2k-IONPs as an example,[22] under a magnetic field of 3 T, single-IONP-containing micelles have an r_2 relaxivity of 84 Fe $mM^{-1}s^{-1}$, while multiple-IONP-containing micelles have an r_2 of up to 345 Fe $mM^{-1}s^{-1}$. Such Alkyl-PEI-IONPs also can be self-assembled onto any micro/nano-template pairing with polyelectrolytes and the anchoring density or the inter-particle distance of IONP per template can be controlled by varying the coating conditions such as ionic strength. SiO_2 nanotemplates covered with higher IONP density (Figure 3a) displayed a 70% increase in T_2 relaxivity comparing to the lower density ones (Figure 3b), and about 2.5 times higher than single Alkyl-PEI2k-IONPs.

Surface engineering has also proven useful in determining nanoparticle T_1 relaxivities, although *via* different mechanisms. Unlike T_2 probes, T_1 probes need to have direct contact with the surrounding water molecules to affect the proton relaxation times. The organic coating of the nanoparticles that lies between the two interfaces inevitably interferes with such an interaction. Although it has been reported that PEGylated phospholipid could coat onto pyrolysis-yielded MnO nanoparticles (MONPs) to transform them to T_1 contrast

agents,[23] a potential concern is the hydrophobic zone that surrounds the MONP cores. Such a zone could disallow efficient water exchange and, as a consequence, lead to suboptimal T_1 contrast. Indeed, when switched to the dopamine-plus-HSA coating we observed a 5-fold increase in r_1 (Figure 4).[24]

3. Surface engineered magnetic nanoparticles for MR imaging

As mentioned above, magnetic nanoparticles currently play an active role as probes in MRI. This includes conventional RES-targeting probes, which, after injection, are largely sequestered by immune cells, such as macrophages. While still widely utilized, such an approach has the limitation of only being applicable to RES organs, such as liver, spleen, bone marrow and lymph nodes. There is a growing interest in using surface engineering to develop tumor-targeting nanoparticulate probes that can reach tumors, either primary or metastatic, in a wider range of organs. This can be achieved via the enhanced permeability and retention (EPR) effect, which refers to the increases in endothelial leakage and reductions in lymphatic drainage within tumors that can lead to accumulations of macromolecules or nanoparticles within the tumors. Alternatively, the magnetic nanoparticles can be engineered to display surface biovectors, whose cognate receptors are: 1) aberrantly expressed in tumors and 2) able to serve as target biomarkers to achieve localized probe accumulation.[25–27]

3.1 RES targeting

By studying the relationships between surface properties and *in vivo* behaviors, one can elucidate laws that determine a particle's *in vivo* fate and, as a result, guide the future design of nanoformulas. For instance, we have prepared a series of PVP-IONPs with different hydrodynamic sizes.[9] Both *in vitro* and *in vivo* studies have confirmed a size effect on the particles' RES sequestration. In particular, we have identified one formula, PVP-IO-37 (core size of 37 nm and hydrodynamic size of 100 nm), with a particularly prominent macrophage uptake rate. When injected systematically in a murine orthotropic Huh7 hepatocarcinoma model, we observed, at 1 h post injection, a contrast change (ΔCNR) of 94 ± 6% with PVP-IO-37, compared to that of 81 ± 8% with Feridex (Figure 5). In another study, we tested Mn-doped-iron-oxide (Mn-IO) nanoclusters as contrast probes for liver imaging.[20] The hydrophobic Mn-IO nanoparticles were synthesized in organic phase and then transferred into water with the help of a block copolymer mPEG-b-polycaprolactone (PCL). These Mn-IO nanoparticles self-assembled into small clusters inside micelles with a mean diameter of approximately 80 nm and an r_2 relaxivity of 270 $mM^{-1}(Mn+Fe)s^{-1}$. These nanoclusters induced significant contrast in the liver, resulting in a decrease in signal intensity of 80% within 5 min post-injection.

3.2 Tumor targeting

The HSA-coated IONPs discussed above are good examples of achieving tumor targeting *via* passive means. Such a nanostructure can stay long in the circulation, yet extravasate significantly at tumor sites.[28] MRI T_2 maps of a U87MG xenograft murine model showed a drop of 29.9 ± 4.2% in the signal intensity from the tumor area 18 h p.i.[11] Although the main mechanism of tumor-homing was attributed to the EPR effect, the HSA sheath is believed to have played a role, *via* its interaction with glycoprotein (gp60) receptor (albondin) and/or SPARC (secreted protein acid and rich in cysteine), in promoting the particle extravasation and tumor internalization. Likewise, HSA-coated MONPs were found to be able to accumulate in tumor. Also, in the U87MG xenograft model, signal intensity increases of 5.3 ± 0.6%, 13.8 ± 2.0% and 9.7 ± 2.1% at 1, 4, and 24 h p.i. were observed on the T_1-weighted maps.[24]

On the other hand, we have sought ways to conjugate targeting motifs, such as RGD, onto IONPs to create smart probes.[29–30] For instance, we have coupled RGD onto both tri-block copolymer[14] and PASP-coated NPs[8] (TPIO and PASP-IO), and studied the tumor targeting and contrast capabilities of the conjugates. We observed, in both cases, a significant increase in affinity toward integrin $\alpha_v\beta_3$. This was attributable to the presence of multiple RGD peptides on a single nanoparticle surface, the so-called multivalent effect.[25,31] In both cases, we observed strong hypointensities in MRI images in the tumor areas after injection in a U87MG xenograft model. Post-mortal immunohistological studies confirmed that the accumulation of nanoparticles in tumor was mostly mediated through RGD-integrin interaction. Notably, we found that, although many of the particles were able to extravasate and become bound to tumor cells, a large portion of the particles remained trapped in the blood vessel lumen.[8,14] This was because, in such a model, the upregulation of integrin occurs on both tumor cells and tumor endothelial cell surfaces.[32–33]

In most nanoparticle formulas, a great portion of the overall size is contributed by the coating materials. As we mentioned above, the coating materials are intended 1) to stabilize the nanoparticles in the physiological environment and 2) to afford a platform for functionality docking. However, it is generally believed that a smaller nanoparticle size is associated with a greater extravasation and with less immune sequestration. Therefore, it would be advantageous if we could remove the thick polymer coating layer and shift its nanoparticle-suspending job to, for instance, the added functional motifs, which—fortunately—are typically hydrophilic molecules. In one such effort, we conjugated c(RGDyK), via a Mannich reaction, onto catechol-coated IONPs[34] (Figure 6). The resulting nanoparticles had a core size of about 4.5 nm, and could be directly conjugated with c(RGDyK). Such an RGD layer plays a dual role in the nanosystem: 1) it enables integrin to bind to the nanoconjugates, and 2) it confers water solubility to the nanoconjugates. Owing to the lack of a thick polymer coating, such an RGD-conjugated IONP formula has an overall size of only ~8.4 nm, one of the smallest among its category.

3.3 Cell labeling

Aside from uses as systemically injected probes, magnetic nanoparticles are also used as cell labeling reagents. This application is driven by the emergence of cell-based therapeutics and, associated with that, the need to understand the fate of exogenous cells in hosts.[35–36] The idea is to load a sufficient amount of nanoparticles into the cells prior to their administration into the host. Surface engineering again can play a central role in determining the particles' internalization rate and toxicity.[36–37]

A common strategy is to coat the IONP surface with polycation materials to induce endocytosis-mediated particle uptake.[38–39] PEI analogs, especially those with long lengths and branched structures, have been widely utilized. However, high cytotoxicity has been associated with these polymers, which limits the safe incubation dose and, as a result, the cell loading rate. To address this issue, we have been working on developing novel formulas with less cytotoxicity and superior cell internalization efficacy. For instance, we have used alkylated PEI2000 (Alkyl-PEI2k) to encapsulate hydrophobic IONPs made by pyrolysis.[22,40] Alkyl-PEI2k can hold multiple IONPs in a micelle-like nanostructure, leading to higher r_2 values and better labeling efficiency. Moreover, due to not using a long and branched PEI, the resulting nanoclusters were less cytotoxic than the previously used analogs. A second example was somewhat serendipitous, as we found that HSA-coated IONPs (HINPs) could be taken up by a wide range of cell lines at a high rate (Figure 7).[41–42] This was unexpected, since the zeta potential of HINPs is negative (−9.46 mV), which had been thought to be suboptimal in inducing cell endocytosis. Indeed, Feridex has a zeta potential of −21.60 mV, and unless complexed with polycation material, such as PEI or poly-L-lysine (PLL), Feridex is insufficient to label non-phagocytic cells. One explanation

to such a puzzle could be that the HSA sheath does not completely cover the intermediate dopamine coating, and the partially exposed polycation layer contributed to the cell uptake. Nonetheless, unlike the PEI coated formulas, such HINPs have negligible cytotoxicity even at an extremely high concentration and can label a variety of cell lines without use of any excipient.

4. Magnetic nanoparticles for multimodality imaging

Each imaging modality has its own advantages and disadvantages, which justifies the need of developing multimodal imaging techniques that combine the strengths of each modality and synergistically improve diagnostic quality.[43–44] Many research activities are going on at the hardware end. For instance, SPECT/CT and PET/CT have been constructed and are being implemented worldwide. PET/MRI is under active investigation and is about to be implemented. There is, thus, a corresponding urgent need to develop multimodality imaging probes.

With a large surface-to-volume ratio and a sophisticated surface chemistry, magnetic nanoparticles can play a role as nanoplatforms, onto which non-MRI imaging motifs can be easily loaded. This can immediately upgrade the agent from an MRI-only probe to an MRI-plus-X (X= PET, SPECT, NIRF, etc.) probe. For instance, we have coupled c(RGDyK) and Cy5.5 onto TPIOs. The resulting conjugates were able to home to a tumor and to depict its contour on both near infrared fluorescence (NIRF) and MRI images.[14] Similarly, we coupled c(RGDyK) and DOTA--a macrocyclic chelator for metal bounding--onto the surface of PASP-IOs.[8] Prior to the imaging, we loaded ^{64}Cu, a radioisotope that is often used in PET imaging, via chelation with DOTA. The resulting nanoconjugates possessed a tumor targeting feature (due to the c(RGDyK)), as well as dual imaging capabilities via MRI (from the IONP cores) and PET (from the ^{64}Cu).

The advantages of such an MRI + PET or NIRF combination are substantial. The MRI can provide better anatomical information and the PET/NIRF analysis is more sensitive and quantitative or semi-quantitative, allowing better assessment of the probe accumulation in the areas of interest. Such multimodality does not have to be confined to two levels. For instance, we have conjugated both ^{64}Cu-DOTA and Cy5.5 onto the surface of HINPs.[11] The resulting nanoparticles allow tumor targeting (mainly via enhanced permeability and retention) and are MRI/PET/NIRF triple functional (Figure 8). We anticipate that such a nanosystem, capable of integrating the strengths of high anatomical resolution (MRI), quantitative evaluation (PET), *ex vivo* validation (NIRF) and intraoperative potential (NIRF) will have a bright future in theranostics.

5. Magnetic nanoparticles for drug delivery

There have been many efforts to use magnetic nanoparticles as vehicles for drug delivery. This immediately upgrades the nanoconjugates from MRI imaging probes to nano-theranostic agents that combine both therapeutic and diagnostic elements. Unlike other kinds of nanoparticles, such as carbon nanotubes (which are able to load therapeutics through π-π stacking[45]), magnetic nanoparticles, such as iron oxides, do not afford an easy drug loading mechanism. Until now, the drug loading on magnetic nanoparticles has been mainly on the particle coating. This again highlights the importance of surface engineering techniques.

Unlike the tethering of imaging/targeting motifs, where bioconjugation techniques are overwhelmingly used, the loading of therapeutics, although can be accomplished via covalent conjugation,[46–47] is mostly achieved via physical means, such as electrostatic interaction. For instance, magnetic nanoparticle-based nanoplatforms have been intensively studied as gene delivery vehicles.[47–49] The rationale is to shuttle a gene regulator (such as

siRNA/shRNA/antagonist DNA), via nanoparticle vehicles, across the otherwise impermeable cell membrane, where it can subsequently modulate the expression of a certain cancer-related gene. Similar to the scenario of cell labeling, nanoparticles coated with polycation materials have been widely used in such an effort. For instance, we demonstrated that Alkyl-PEI2k-IOs possess many outstanding features that favor siRNA delivery, including good biocompatibility, high siRNA binding capability, protection of siRNA from enzymatic degradation, and ability to release complexed siRNA in the presence of polyanionic heparin. We observed nice gene silencing effects, at both the *in vitro* and *in vivo* levels, with siRNA-loaded Alkyl-PEI2k-IOs.

Magnetic nanoparticles, especially IONPs, have also been used as platforms to load small molecule-based therapeutics. Again, since many therapeutic agents are not amenable to chemical conjugation, there is need for a nanoplatform that is able to formulate, via physical interaction, with a wide range of molecules. Lacking such an attribute themselves, magnetic nanoparticles are commonly loaded, along with therapeutics, into polymer-based matrices. More recently, we have found that albumin can be used as a good matrix material. Particularly, we found that dopamine-coated IONPs can be co-loaded with therapeutics, such as paclitaxel or doxobucin, into HSA matrices. Such a theranostic formulation takes the advantages of the well-documented, excellent ligand binding capability of HSA. Moreover, by replacing the intermediate coating layer of dopamine with caffeic acid or other dopamine analogs it is possible to tailor the surface and facilitate the loading of a broader range of therapeutics (unpublished data).

6. Conclusions and perspectives

In summary, we and others have developed a set of chemistry to prepare magnetic nanoparticles that possess accurate sizes, shapes, compositions, magnetizations, relaxivities and surface charges. These features, in turn, can be harnessed to adjust the toxicity and stability of the nanoparticles, and further, to load functionalities, via various mechanisms, onto the nanoparticle surfaces. These capabilities have greatly expanded the role of magnetic nanoparticles, enabling simultaneous targeting, imaging and therapy. The close coupling of imaging and treatment within a theranostic agent, and the data about the evolving course of an illness that these agents provide, can facilitate informed decisions about modifications to treatment.

While the foresight is clear and exciting, it is fair to admit that we are at a relatively early stage of development. In this manuscript, we weighted more on imaging related work, which is a true reflection of the reality--a trend toward more therapeutic-related formulas but on the basis of chemistry and platforms that have been previously validated in the imaging setting. While multiple loading may no longer be a challenge, a more critical issue now confronting us is how to leverage the capabilities and to translate them into practices. The related investigations, conducted to address questions such as how and to what extent these new formulas can advance the current cancer management, are currently undertaken in our and other labs.

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Biographies

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Xiaoyuan Chen received his Ph.D. in chemistry from the University of Idaho in 1999. After quick postdocs at Syracuse University and Washington University in St. Louis, he joined the University of Southern California as an Assistant Professor of Radiology. He then moved to Stanford University in 2004 and was promoted to Associate Professor in 2008. In the

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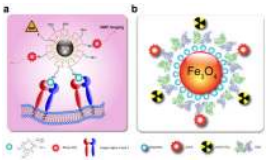

Figure 1.
IONPs coated with a) a tri-block copolymer and b) dopamine-plus-HSA to confer water solubility and functional extendibility.

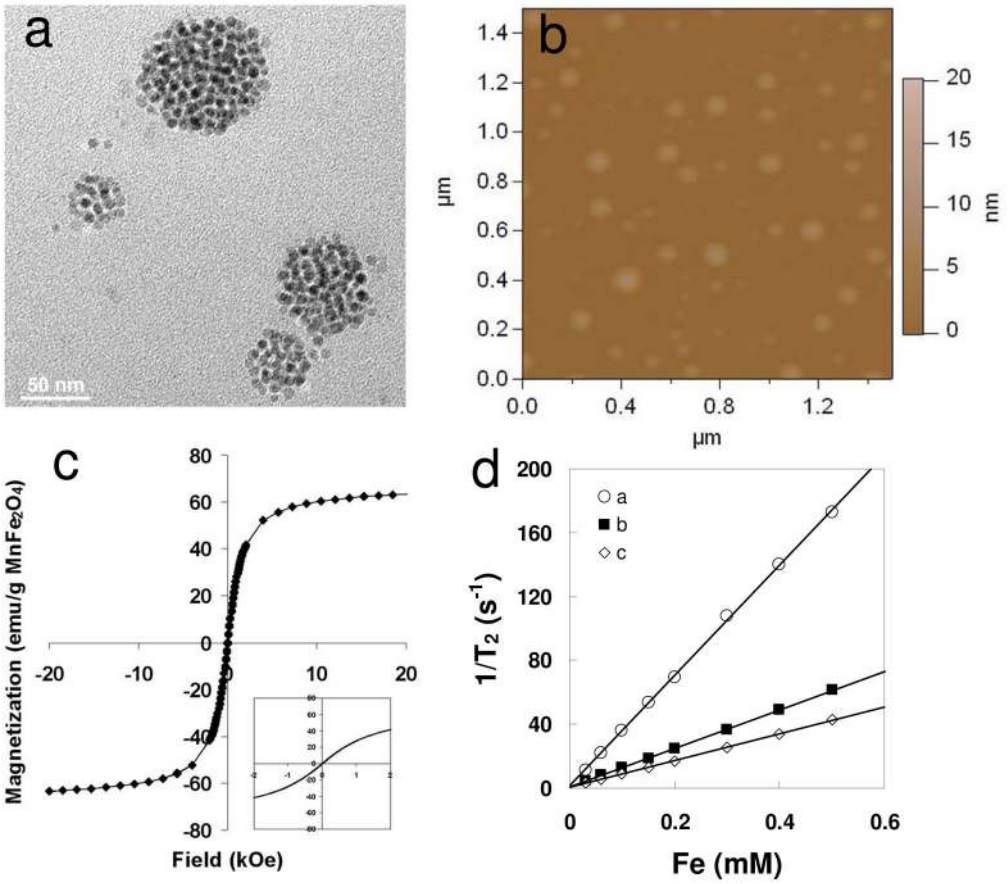

Figure 2.

a) TEM bright field image of mPEG-*b*-PCL/MONP micelles; b) AFM height image of Alkyl-PEI2k-IONPs; c) hysteresis loops of the MONP containing micelles measured at 300 K (inset shows a zoomed-in plot between − 2 kOe and 2 kOe magnetic field); d) T_2 relaxation rates ($1/T_2$, s^{-1}) of Alkyl-PEI2k-IONP nanocomposites as a function of iron concentration (mM) for different polymer/SPIO ratios at (a) 0.6; (b) 1.2; (c) 2.5.

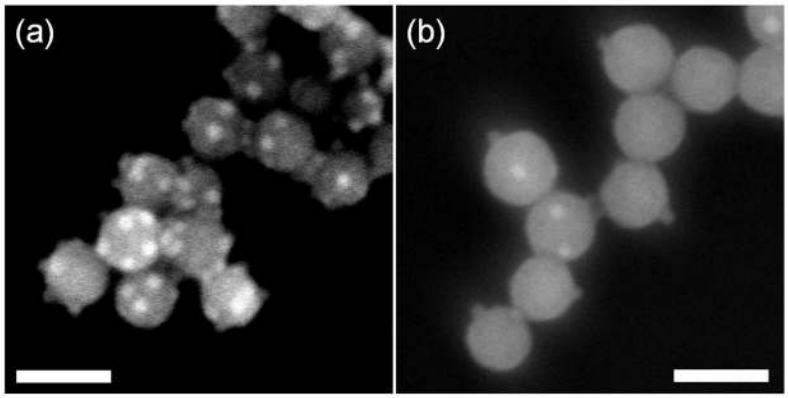

Figure 3.
Alkyl-PEI2k-IONP nanocomposites adsorbed on polyelectrolyte covered SiO_2 nanotemplates with a) higher and b) lower anchoring density. (Scale bar = 100 nm)

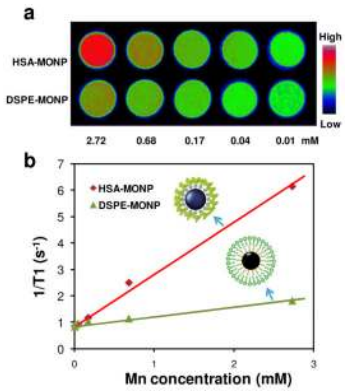

Figure 4.

a) Phantom studies with HSA and phospholipid-coated MONPs at the same concentrations. Due to existence of a hydrophobic coating zone between the particle surface and surrounding water molecules, phospholipid coated MONPs tend to have a less prominent T_1 reducing effect. b) r_1 relaxivity evaluation from the results of a).

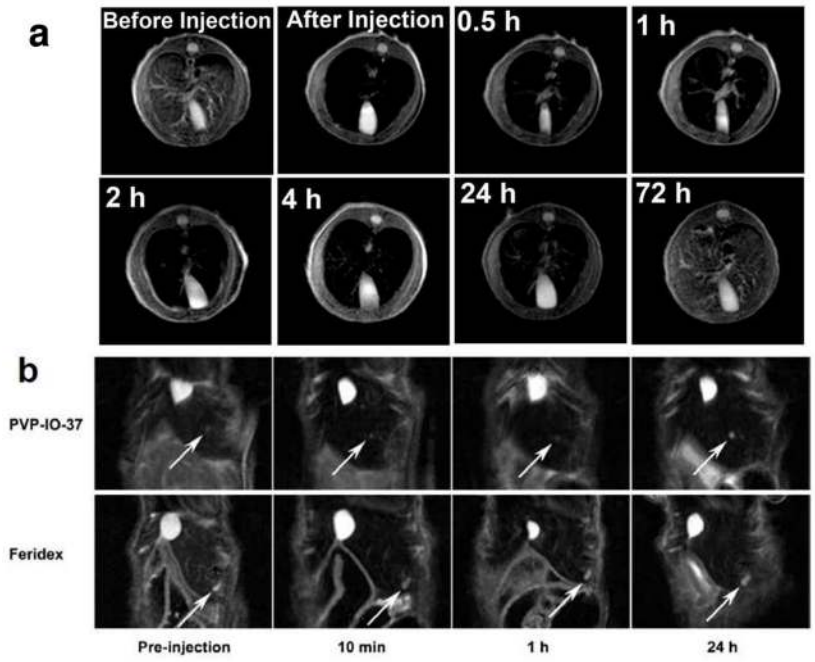

Figure 5.
In vivo MR imaging with a) normal mice and b) an orthotropic Huh7 hepatocarcinoma model after injection with PVP-IO-37 and Feridex. Arrow points to tumor.

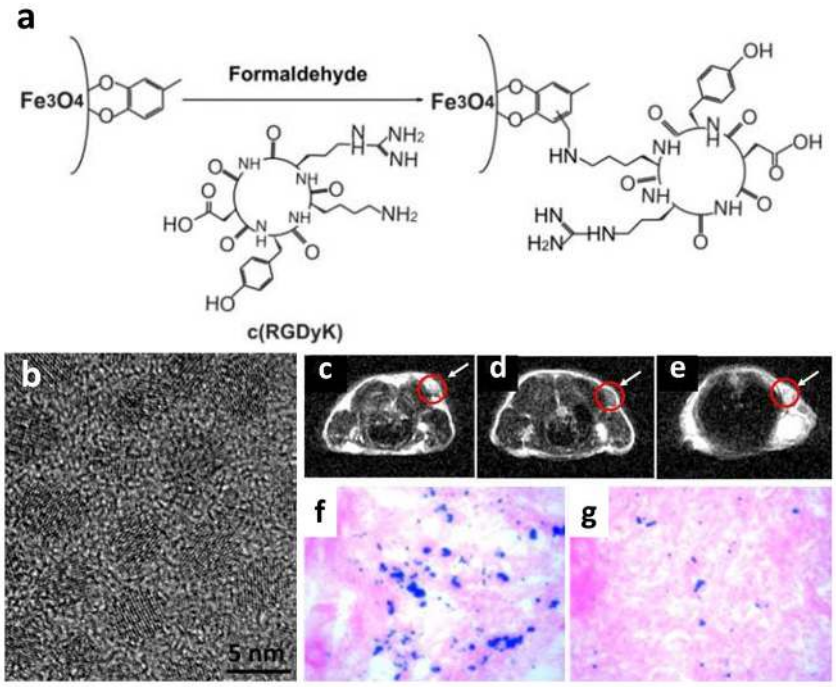

Figure 6.

a) Conjugating RGD onto 4-methylcatechol coated IONP surface. b) High resolution TEM images of the IONPs. c–e) MR images taken after IONP injection on a U87MG xenograft model. c) without NPs, d) with c(RGDyK)-IONPs, and e) with c(RGDyK)-IONPs and with blocking dosage of c(RGDyK). f–g) Prussian blue staining on tumor tissue samples from d) and e). Arrow points to tumor.

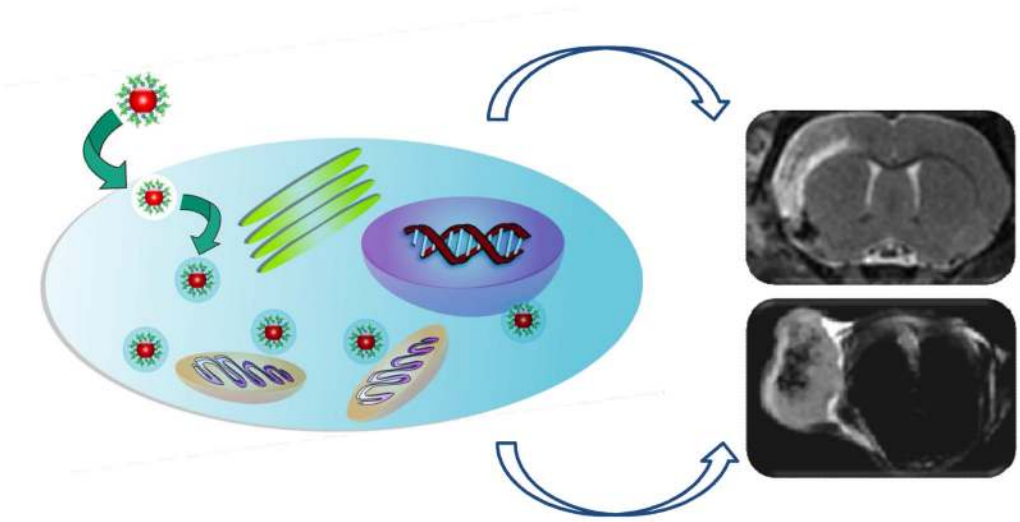

Figure 7.
HINP-loaded macrophages were injected into a stroke model (upper right) and xenograft tumor model (lower right). Such exogenous macrophages accumulated in the areas of diseases and were detected by MRI.

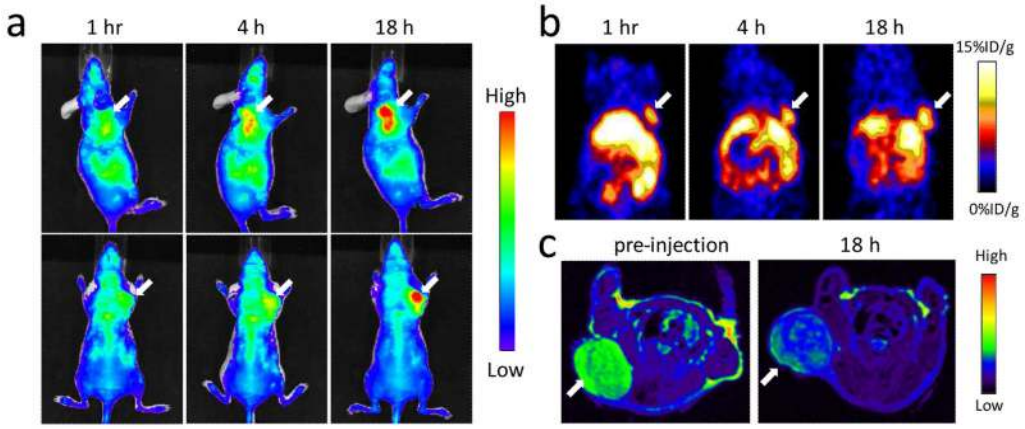

Figure 8.
MRI/NIRF/PET tri-modal imaging (a, NIRF; b, PET; c, MRI) with HINPs that were conjugated with both ^{64}Cu-DOTA and Cy5.5.