Multifunctional Nanoparticles for Dual Imaging

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Multifunctional Nanoparticles for Dual Imaging

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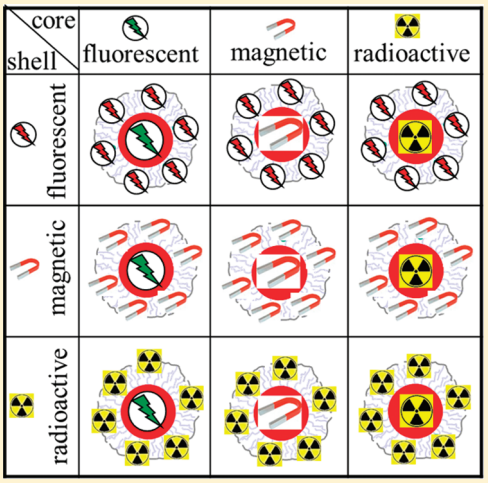
Supporting Information

ABSTRACT: For imaging with different modalities, labels, which provide contrast for all modalities, are required. Colloidal nanoparticles composed out of an inorganic core and a polymer shell offer progress in this direction. Both, the core and the polymer shell, can be synthesized to be fluorescent, magnetic, or radioactive. When different cores are combined with different polymer shells, different types of particles for dual imaging can be obtained, as for example, fluorescent cores with radioactive polymer shells. Properties and perspectives of such nanoparticles for multimodal imaging are discussed.



Most spatially resolved observations of biological processes inside living cells and organisms require labels which allow for reporting the location of the labeled molecules/entities. The purpose of the labels hereby described is to provide contrast so that the labeled entity can be imaged. Several types of labels for imaging are established in life sciences, like fluorescence, magnetic, and radioactive labels, each of which has its advantages and disadvantages.[1,2]

Fluorescence labeling[3−8] is based on fluorophores that emit fluorescence upon optical excitation, which can be detected with an optical microscope (e.g., fluorescence or laser-scanning microscope). Fluorescence microscopy is very popular for imaging of cellular structures, as it allows for high spatial and temporal resolution. Spatial resolution is classically restricted to the diffraction limit of light, which means in practice a few hundreds of nm. Temporal resolution is limited by the brightness of the fluorophore and the performance of the camera (e.g., the data transfer range). Practically ms time resolution can be easily achieved even with standard setups. One problem of fluorescence imaging is the photostability of the fluorophores. Photobleaching results in a loss of fluorescence signal in time. In addition, imaging of tissues deep inside the animal or human bodies is more complicated. Light in the visible region is strongly absorbed by tissue. Though infrared (IR) is less absorbed by tissue, imaging of structures inside tissue is complicated. Multiplexed imaging, i.e., visualization of several structures in parallel with different labels, is possible using fluorophores with different color. We on purpose do not refer here to transmission electron microscopy (TEM), which allows for even better resolution,[9] as TEM is inappropriate for in vivo imaging.

Magnetic labeling[10−14] is based on changes in the relaxation of the magnetization of nuclei such as ^{1}H or ^{13}C induced by the

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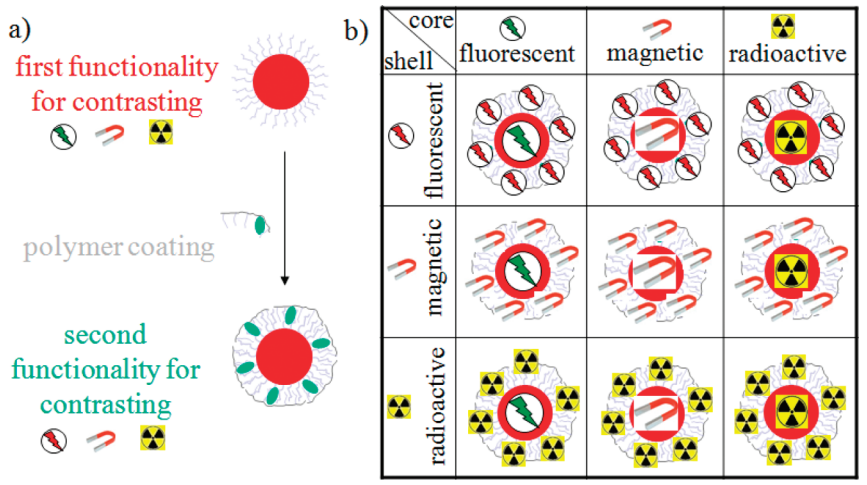

Figure 1. Inorganic colloidal NPs are synthesized from different materials. (a) After the synthesis, the inorganic cores (drawn in red) are surrounded by organic surfactant molecules (drawn in gray). Depending on the material, the cores can be fluorescent, magnetic, or radioactive. The cores are coated with an amphiphilic polymer (drawn in gray) which renders the NPs water-soluble. Functional organic molecules (drawn in green) have been integrated in the polymer which can render the polymer shell fluorescent, magnetic, or radioactive. (b) When fluorescent, magnetic, or radioactive polymer shells are wrapped around fluorescent, magnetic, or radioactive cores, NPs with two functionalities and similar surface chemistry are obtained.

presence of magnetic particles. Magnetic labels are used as contrast enhancers for magnetic resonance imaging (MRI).[15] Spatial resolution allows for contrasting of organ structures, but subcellular resolution has not been achieved yet. As magnetic fields are essentially not absorbed by tissue, also, imaging of structures deep inside bodies is widely used. Multiplexed imaging would be hard to realize with only magnetic particles. On the other hand, MRI is a noninvasive technique as the used radio-frequency (RF) and static magnetic fields and the magnetic nanoparticles (NPs) are recognized to not damage the tissues. Other techniques for in vivo and clinical imaging are positron-emission-tomography (PET),[16] computer tomography (CT),[17] optical coherence tomography (OCT),[18−20] and surface enhanced Raman scattering (SERS).[21,22]

Radioactive labeling[23] is based on detecting the decay products (most often γ-radiation) of radioactive atoms. Radiation can be detected either with imaging plates which allow for spatial resolution (up to 0.2 mm) or with counters (which do not allow for spatial resolution but have a high sensitivity requiring only pg amounts and have a large dynamic range over 4−5 orders of magnitude of radioactivity).[24−27] In particular, absolute quantification of the number of markers can be done with high precision using radioactivity, as the count rate of each emitter is virtually not affected by the biological environment (for example, pH). As γ-radiation is only weakly absorbed by tissue, also, imaging of organs deep inside the body is possible. On the other hand, γ-radiation counters for animal imaging only have low spatial resolution (hundreds of μm compared to hundreds of nm with optical detection). Problems associated with radiation damage (the tissue is influenced by γ-radiation but not vice versa) automatically reduce the possibilities for the use of these labels for in vivo imaging in humans.[28] Multiplexed imaging is possible using radioactive molecules/NPs with different radiation energies.[29−33]

The above-mentioned restrictions limit the field of applicability of each label available for in vitro and in vivo imaging purposes. Imaging of an organ deep inside the body of an animal would be, for example, complicated with fluorescence detection. On the other hand, imaging of an ex vivo tissue slice taken out of the animal organ with cellular resolution would be complicated with magnetic resonance detection. In an experiment, it could be (for example) interesting to first observe to which organs certain administered molecules go and then afterward to look for their exact cellular distribution with histological techniques on slices taken out from the organs. Here, the first part could be done with MRI in order to avoid loss of the signal by tissue absorption. The second part, on the other hand, could be done with fluorescence detection in order to get high spatial resolution. For this purpose, a label, which is magnetic as well as fluorescent, would be needed. Thus, we note the need for labels which can be detected not only with one but also with several imaging techniques.[34,35]

Labels which can be detected with two techniques have been reported, such as fluorescence + MIR,[36−46] fluorescence + radioactivity,[47] MRI + radioactivity,[48] MRI + CT,[49,50] MRI + PET,[51,52] and PET + CT.[53] In some cases, materials directly provide contrast for more than one imaging technique. Elsewise, hybrid materials can be synthesized which can be imaged with two different techniques. Examples of such hybrid materials are colloidal hybrid NPs with two different domains as labels,[51,54,55] linkage of two different labels by spacer molecules,[56−58] and integration of different labels in a matrix,[42,59,60] as for example, a silica shell[42] or a polyelectrolyte capsule.[61] However, though many different systems for combining two different labels exist, typically, their surface chemistry (as well as their hydrodynamic diameter) and, thus, their interaction with the environment typically varies from system to system.

We feel that colloidal inorganic nanoparticles (NPs) have a good perspective for being used as labels which can be imaged with several modalities. NPs automatically have two different entities: the inorganic core and the surface shell (which on primary purpose provides solubility). In this way, the inorganic core could provide the first label. The second label could be integrated in the surface shell. In case the same surface shell could be used for each inorganic core, all NPs would have highly similar surface chemistry. In this way, they would interact similarly with biological matter. In the following, we will discuss one approach of making such NPs for multimodal imaging.

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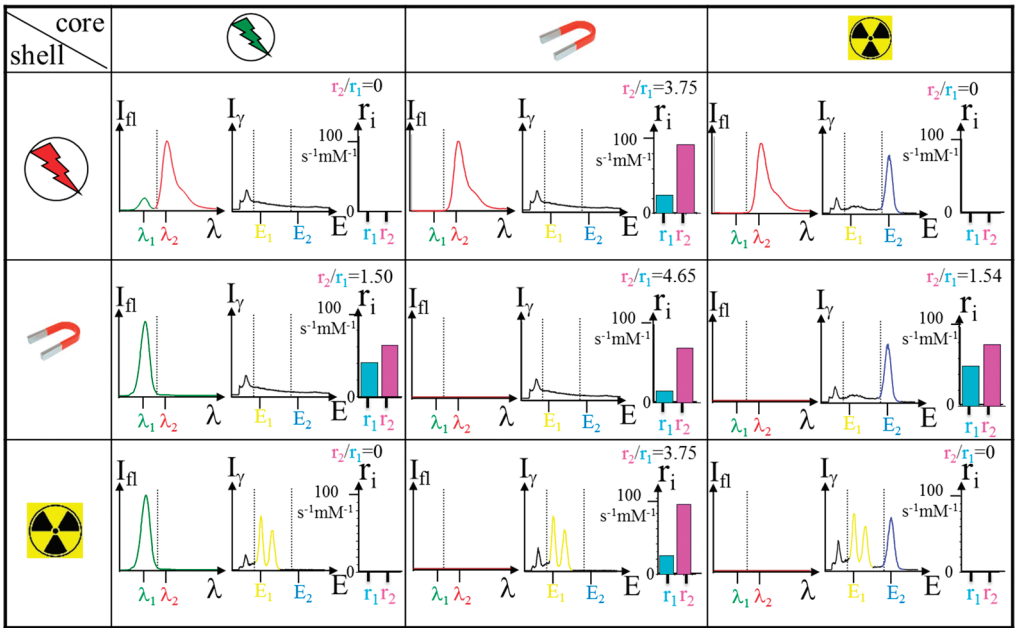

Figure 2. Experimental data obtained for nine different types of NPs which are composed out of fluorescent, magnetic, or radioactive cores with a fluorescent, magnetic, or radioactive polymer shell. For each combination, the performance for imaging with fluorescence microscopy, MRI, and γ-ray spectroscopy is reported. Concerning fluorescence characterization, emission spectra $I_{fl}(\lambda)$ are reported. In all cases of fluorescent core (CdSe/ZnS NPs) and polymer shell (ATTO-590 fluorophore), emission at wavelength λ_1 = 544 nm and λ_2 = 625 nm can be observed. As characterization for MRI, the longitudinal (r_1) and transverse (r_2) relaxivities at 20 MHz are depicted. For characterizing the radioactive properties, intensity I_γ of γ-ray emission is plotted versus the energy E of γ-rays. With energy discrimination, clearly, emission from radioactive cores (^{198}Au NPs, 371 keV < E_2 < 464 keV) can be distinguished from emission of radioactive polymer shells (^{111}In-DOTA, 140 keV < E_1 < 200 keV). Emission at lower energies is due to background radiation. Additional information to all experiments (synthesis and detailed characterization) can be found in the Supporting Information.

NP cores which provide contrast for different imaging techniques can be synthesized by choosing the appropriate material. An example for fluorescent colloidal NPs is the well-established CdSe/ZnS system. Using CdSe/ZnS NPs of different sizes, all required colors of fluorescence ranging from the blue in the visible to the near-infrared (NIR) can be obtained. A typical example for magnetic NPs is Fe_2O_3 NPs,[62] which has been already characterized in detail in literature. Radioactive NPs can be obtained, for example, from Au NPs[63] which are radioactivated by neutron irradiation. These NPs contain the ^{198}Au isotope which is a γ-emitter with a photo peak at 315 and 412 keV.[64] In this way, using three different materials, fluorescent, magnetic, and radioactive, NP cores can be obtained. If the synthesis of the functional cores is performed in organic solvent, the resulting NPs are hydrophobic and have to be rendered hydrophilic before they can be used for biological applications. One possibility in this direction is embedding the hydrophobic NP cores in an amphiphilic polymer shell, which makes the NPs water-soluble.[65−67] This procedure is very general and allows for embedding of inorganic NPs of different materials inside the same type of polymer shell. In order to provide functionality to the polymer shell, the amphiphilic polymers can functionalized with organic molecules.[68,69] For fluorescent, magnetic, and radioactive polymers, organic fluorophores (ATTO-590, ATTO-TEC GmbH), chelator molecules for ions (*S*-2-(4-aminobenzyl)-1,4,7,10-tetraazacyclododecane-tetra-*tert*-butyl acetate, ABz-DOTA, macrocyclics, loaded with Gd^{3+}), and chelator molecules for In^{3+} ions (ABz-DOTA, loaded with In^{3+}) can be used, respectively. In this way, the resulting particles have two functionalities: the one of the inorganic core and the one of the organic molecules which are integrated in the polymer shell; see Figure 1. Combination of the three different cores and polymer shells would lead to the nine different types of NPs as depicted in Figure 1.

How would the imaging properties of such bimodal NPs be? Will there be interaction and/or crosstalk between the two labels within or on the NP? In order to answer these questions, fluorescence, relaxometric behavior, and radioactivity of the different NPs have to be analyzed and compared.

Fluorescent spectra of all nine different types of NPs are shown in Figure 2. From these spectra, emission of the organic fluorophore in the polymer shell (ATTO-590) can be clearly seen at 625 nm and emission of fluorescent cores (CdSe/ZnS) is at 544 nm. In case the polymer shell with the fluorophore is wrapped around nonfluorescent metal (Au) or metaloxide (Fe_2O_3) cores, part of the fluorescence is, as expected, quenched.[40,70−72] However, as there are several fluorophores around each core,[73] the remaining emission is still enough for imaging. One way to reduce quenching would be the addition of spacers to the fluorophores in order to increase their distance to the surface of cores.[74] In the case of fluorescent cores (CdSe/ZnS), fluorescence resonance energy transfer (FRET) between the cores and the fluorophores in the polymer shell can occur in case emission of one of them matches absorption of the other.[73,75] This effect is also distance dependent.[68] As a result, in case emissions of cores and fluorophores in the polymer shell are enough spectrally separated, emission originating from the core can be clearly distinguished from emission of the polymer shell.

In order to evaluate the contrasting properties of the NPs for MRI, their relaxometric behavior needs to be discussed. Calculations for the longitudinal (r_1) and transverse (r_2) relaxivities are

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given in Figure 2. The relaxometric characterization indicates that when Gd-containing polymer shells were wrapped around nonmagnetic cores (Au or CdSe/ZnS), a significant increase of the relaxation rates can be observed. In fact, both Au and CdSe/ZnS core without Gd in their polymer shell exhibit relaxation rates similar to the ones of water, as expected, whereas after the addition of Gd the relaxation rates (and hence the relaxivities) increase. Particularly, the longitudinal relaxivity r_1 assumes values comparable to (or higher than) the values recorded for positive reference contrast agents such as Omniscan or Dotarem (the commercial name of Gd-DOTA). When the Gd-containing polymer shell is added to magnetic cores (Fe_2O_3), the relaxometric behavior does not vary radically with respect to one of the samples with the original superparamagnetic core. Another useful indicator of the relaxometric properties of a given contrast agent is the ratio between the transverse and longitudinal relaxation rates (r_2/r_1) being 1−2 in the case of traditional paramagnetic (positive) contrast agents and up to 50 in the case of superparamagnetic (negative) contrast agents. The results indicate that the nonmagnetic Au and CdSe/ZnS cores mantled with Gd-containing polymer shells have very similar r_2/r_1 values of around 2, indicative of a positive contrasting effect which can be ascribed only to the Gd in the polymer shell. On the other hand, the NPs based on a magnetic core (Fe_2O_3) have a r_2/r_1 value indicative of a negative contrasting behavior, irrespective to the presence of Gd in the polymer coating, and behave similarly to reference negative contrast agents such as Endorem. Though magnetic cores (Fe_2O_3) and polymer shell (with Gd-loaded DOTA) cannot be imaged independently, the choice of having either the core or the polymer shell with magnetic properties allows one, in principle, to obtain either negative or positive contrasting effects.

In Figure 2, the radioactivity of the NPs as detected by γ-ray counters is shown. Energy discrimination hereby allows for distinguishing between different emitters which in the described case were ^{198}Au (as present in neutron-activated Au cores, emitting at 315 and 412 keV) and ^{111}In (as present in In-loaded DOTA in the polymer shell, emitting at 171 and 245 keV). γ-Emission of ^{198}Au and ^{111}In is not correlated in any way, which enables parallel detection of both emitters and, in this way, also discrimination of Au cores and polymer shells containing In. In this way, two types of NPs can be imaged in parallel: NPs with ^{198}Au core and regular polymer shell and other cores with ^{111}In-containing polymer shell. In addition, the possibility of recording absolute count rates would, for example, facilitate experiments which probe whether core and polymer shell are always colocalized or if they are separated, for example, when NPs have been taken up by cells.

Combining the properties of inorganic NPs with a modified polymer-coating procedure, in which an additional marker for imaging is integrated, promises to be a straightforward way for the creation of a toolkit of NPs which can be simultaneously imaged by two different techniques. As polymer-coating of NPs has become an established technique, we want to point out that with little effort, by incorporation functionalities in the polymer, additional functionalities can be achieved, while retaining the simplicity of the coating procedure. The generality of this approach allows for combination of the properties of a multitude of different cores with polymer shells with different integrated functionalities. In particular, these particles allow for dual imaging with two different techniques in parallel. Due to similar surface chemistry, even different NP solutions could be easily mixed.

How are the potential perspectives of the use of such bifunctional NPs for in vivo imaging studies? One general prerequisite is high colloidal stability and purity of the NP sample. Polymer coated NPs purified with gel electrophoresis and size exclusion chromatography have been demonstrated to fulfill these criteria.[76] The resulting NPs are also reasonably small with hydrodynamic diameters around 10 nm.[76,77] However, for in vivo imaging experiments, also, high retention times would be required. With PEGylation of the polymer shell of the NPs,[78] acceptable retention times (20% of the NPs are still in the blood circulation 24 h after injection) can be obtained.[79] We, however, have to critically state that so far no data are available about the integrity of the NPs in in vivo studies. In principle, as well, the inorganic cores, as the polymer shell, could be effected, possibly leading to a detrimental effect both on the biocompatibility and on the imaging effectiveness of the NPs. Corrosion of NP cores is well-known.[80−82] In the worst case, significant amounts of core material could be released as ions to solution. In case of fluorescent and magnetic NPs, this would involve only negligible contamination of the label, as for example Cd and Se ions released from fluorescent CdSe NPs are not fluorescent, and the relaxivity of Fe ions is much lower than the one of Fe_2O_3 NPs. In case of radioactivated Au NPs, the distribution of radioactive Au isotopes within the Au core should be homogeneous and, thus, release of Au ions to solution would result in radioactive Au ions in solution, which eventually would no longer colocalize with the Au NPs. A few percent losses in Au atoms from the Au cores would, thus, result in a few percent contamination of the label by noncolocalized Au atoms. As Au NPs are quite stable in vitro,[83] we do not foresee a big impact. Also, the label of the polymer shell could be lost. The organic fluorophores as fluorescence labels and the chelators for the magnetic and radioactive labels are covalently bound to the polymer, and the polymer-coated NPs have been extensively purified with HPLC and gel electrophoresis. Chelators were loaded with Gd and In in case of magnetic and radioactive labels, respectively. Ions might escape from the chelators, which would involve free Gd or In ions in solution. In both cases, the magnetic and radioactive label of the NPs would be contaminated due to the relaxivity of the Gd ions and the radioactivity of the In ions, respectively. As there are only a few hundred Gd or In ions in the polymer shell around each NP core (as probed with elemental analysis, in particular with inductively coupled plasma mass spectrometry (ICPMS)), loss of only few ions would already contaminate the label. The same potential problem applies to all chelator-based labels. However, as explained above, loosely attached ions can be removed with stringent purification with HPLC and gel electrophoresis and, therefore, the occurrence of the worst scenario described above can reasonably be ruled out.

In conclusion, we have pointed out perspectives of polymer-coated NPs for in vivo imaging studies, in which the NPs can be imaged with different techniques. An advanced toolbox such as the described nine different NPs will also help to investigate potential disintegration of the labels, as separate tracing of the destiny of cores and organic surface on NPs is possible. As different types of NPs can be mixed due to their similar surface chemistry, biodistribution studies correlating the colocalization of the different components could be performed. We have discussed in one example how nanotechnology helps combining different functional building blocks to one hybrid material. We predict that such hybrid materials will have significant impact on in vivo imaging in the future, as they allow for combining

functionalities, as for example, integration of two contrast providing labels in one single entity.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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