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A Facile Coating Strategy to Functionalize Inorganic Nanoparticles for Biosensing

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Abstract

The use of inorganic nanoparticles (NPs) for biosensing requires that they exhibit high colloidal stability under various physiological conditions. Here, we report on a general approach to render hydrophobic NPs into hydrophilic ones, ready for bioconjugation. The method uses pegylated polymers conjugated with multiple dopamines, which results in multidentate coordination. As proof-of-concept, we applied the coating to stabilize ferrite and lanthanide NPs synthesized by thermal decomposition. Both polymer-coated NPs showed excellent water solubility and were stable at high salt concentrations under physiological conditions. We used these NPs as molecular sensing agents to detect exosomes and bacterial nucleic acids.

Inorganic nanoparticles (NPs) often exhibit interesting properties which can be exploited for sensitive and robust biomedical sensing.^{1–3} Examples include superparamagnetic NPs for magnetic sensing,^{4, 5} quantum dots detectable with photoluminescence,^{6–8} phosphorescent nanoparticles,^{9, 10} and noble metal NPs for localized surface plasmon resonance.^{11–13} These unique physical properties exist at room temperature, obviating the need for expensive detectors and specialized systems. Furthermore, chemical and other amplification strategies have been devised to amplify analytical signals for ultra-sensitive and miniaturized detection schemes.^{14, 15}

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Supporting Information

Experiment procedures, Figures S1–S5 and Table S1. This information is available free of charge via the Internet at <http://pubs.acs.org>.

One challenging aspect of rendering NPs truly useful for biosensing has been the need to stabilize NP's surface with biocompatible and non-reactive coating materials. This task is particularly difficult for injectable materials (e.g. superparamagnetic NPs) but also for agents intended for diagnostic *in vitro* use. The surface coating should allow NPs to stay in solution, react with hydrophilic components, provide functional groups to attach affinity ligands, and passivate the metal surface. Without such stabilizing coatings, proteins often absorb non-specifically to the surface, leading to diagnostic interference, high noise levels, or low reactivity. Surface modifications can be particularly challenging for inorganic NPs synthesized via non-hydrolytic thermal decomposition in hydrophobic conditions.^{16, 17} A variety of hydrophilic coating strategies have been described for metal oxides but most of these strategies have drawbacks under rigorous biological conditions or where longterm stability (months-years) is required. Furthermore, under stress conditions (high temperature, high salinity, non-neutral pH) these effects are exacerbated. A simple yet reliable coating strategy for metal oxides is thus needed to convert "pure" core crystals synthesized under thermal conditions.

Here, we report on a generalized coating protocol to convert hydrophobic NPs into hydrophilic ones that can be further modified for molecular sensing. We reasoned that using dopamine as an anchoring group would form stable polymer coated NPs, because dopamine can bind to NP surface with high affinity. We tested the method using two representative particles, namely ferrite NPs and lanthanide NPs. We prepared dopamine-based polyethylene glycol (PEG) polymer and coated hydrophobic NPs with polymer through ligand exchange. The polymer coated NPs displayed excellent colloidal stability at high temperatures and under a wide range of physiological buffer conditions. Furthermore, the conjugates provided facile chemical functionalities to graft various affinity ligands. We used the prepared NPs as sensing agents for two different types of biosensors. The ferrite NPs were used to detect exosomes using a miniaturized nuclear magnetic resonance (NMR) system. Lanthanide NPs maintained their optical properties (i.e., no quenching in luminescence emission), and was used to detect bacterial nucleic acids through time-gated imaging.

Results

Generalized coating scheme

Figure 1 shows the developed NP-coating and bioconjugation strategy. We used dopamine (DOPA) as the affinity ligand, because DOPA can readily attach to metal surface through adsorption. This process, however, is reversible; DOPA can be dissociated from NPs under physiological conditions. To improve coating stability, we synthesized PEG polymers containing multiple DOPAs.¹⁸ Specifically, we grafted DOPA and PEG on a polymer backbone, poly(isobutylene-*alt*-maleic anhydride) (PIMA) through reaction with maleic anhydride, creating DOPA-PIMA-PEG. This method did not need additional crosslinkers, and facilitated creation of DOPA-PIMA-PEG with different PEG length and various functional groups for specific bioconjugation (e.g., EDC coupling, bioorthogonal click reaction).

As proof-of-principle, we synthesized four kinds of polymers with varying PEG lengths (2k, 5k) and functional groups (amine, carboxylic acid). The successful polymer synthesis was confirmed by ¹H-NMR (Figure S1). Different PEG lengths were tested to determine the effect of chain length on long-term colloidal stability. We used DOPA-PIMA-PEG to replace hydrophobic capping agents (e.g., oleic acid, oleylamine) on the NP surface. We then conjugated affinity ligands for molecular recognition. Antibody conjugation was performed with a bioorthogonal reaction using tetrazine and trans-cyclooctene (TCO).¹⁹; oligonucleotides were attached to carboxylic groups using an EDC reaction.

Application to ferrite nanoparticles

We applied the developed coating strategy to stabilize magnetic nanoparticles (MNPs). Among the various NP compositions, we chose Zn-doped ferrite (ZnFe₂O₄) for their high saturation magnetization and chemical stability.²⁰ The particles were first synthesized via nonhydrolytic thermal decomposition (see Experimental procedures for details), which produced monodisperse NPs (core diameter, 16 nm) with high crystallinity (Figure 2a). The measured magnetization (M_s) was 177 emu/g [metal] (Figure S2), close to that of bulk material (200 emu/g).²¹ Following the polymer coating with DOPA-PIMA-PEG_{5k}, the MNPs were well dispersed in aqueous media, and no aggregation was observed (Figure 2b, left). The hydrodynamic diameter (d_H) of polymer-coated MNPs was 41.6 nm, implying ~13 nm of polymer thickness (Figure 2b, right). The hydrodynamic diameter was reduced to 32.2 nm with DOPA-PIMA-PEG_{2k} coating (Figure S3).

We next tested the colloidal stability of polymer-coated MNPs by subjecting them to heat stress or high salt concentrations. The particles maintained colloidal stability against such challenges. No significant changes in particle distribution were observed after continuous heating at 90° C for 2 h (Figure 2c) or emersion in 3 M of NaCl (Figure 2d), which confirmed the stable attachment of polymers to the particle surface. We also assessed long-term stability by storing particles in PBS buffer (Figure 2e). Longer PEG chains resulted in better stability; MNPs coated with PEG_{2k} produced large sized aggregates within 3 weeks, while MNPs with PEG_{5k} showed no aggregation (Figure S4).

Stabilization of lanthanide nanoparticles

Lanthanide NPs can be used as luminescent labels for optical imaging and sensors.¹⁰ The luminescence remains highly stable without photobleaching or blinking, and colors can be tuned by varying dopant ions. We synthesized NaGdF₄ NPs co-doped with Ce³⁺/Tb³⁺ via thermal decomposition (see Experimental procedures). The composition allowed the particles to have long luminescence lifetime, which would benefit time-gated luminescent imaging.²² The synthesized NPs were highly crystalline with a narrow size distribution (core size, 13 nm; Figure 3a). As in MNPs, the polymer-coated lanthanide NPs were well-dispersed in aqueous media without aggregation (Figure 3b). Furthermore, we observed negligible differences in overall particle size and distribution at different NP concentrations (Figure S5).

We next measured the luminescence properties of water-soluble lanthanide NPs. Both particles, either with PEG_{2k} or PEG_{5k} polymers, exhibited characteristic green emission

peaks of Tb³⁺ ions (mainly 546 nm emission assigned due to the transition from ⁵D₄ to ⁷F₅) under 254 nm excitation (Figure 3c). Emission intensity was slightly higher with PEG_{5k} coating, presumably because longer PEGs can better protect Tb³⁺ from vibrational quenching by solvents (Figure S6). The polymer-coated lanthanide NPs showed high colloidal stability under various buffer conditions (HEPES, MES, NaOH) and in high salt concentrations (NaCl, 3 M). However, the particles were found unstable in PBS. Phosphate ions tend to strongly coordinate to the surface of lanthanide NPs, and excess phosphate ions in PBS can remove the polymer by ligand exchange.²³ Although lanthanide NPs are coated with phosphate-polymer, excess phosphate ions from PBS could still hamper their long-term dispersibility. Therefore, the use of PBS should be avoided for lanthanide NPs.

Biosensing applications

We applied the polymer-coated ZnFe₂O₄ MNPs to microNMR-based magnetic sensing. This detection modality is favorable when samples are in complex media (e.g., blood). Because biological samples have negligible magnetic background, microNMR sensing can be highly sensitive in turbid samples. We first measured the transverse relaxivity (r_2) of the MNPs (Figure 4a). Owing to their high magnetic moments, ZnFe₂O₄ MNPs, even with a small core size (13 nm), exhibited higher r_2 values (313 s⁻¹mM⁻¹) than Fe₃O₄ MNPs (core size, 25 nm; 279 s⁻¹mM⁻¹). For the given particle composition, the r_2 values increased with the particle size (574 s⁻¹mM⁻¹ for 17-nm ZnFe₂O₄ MNPs). We used the particles to detect exosomes, nanoscale vesicles secreted from cells. MNPs were conjugated with anti-EGFR antibodies via tetrazine-TCO bioorthogonal chemistry; amine-functionalized MNPs were labeled with tetrazine-NHS, and antibodies were reacted with TCO-NHS. Mixing two agents grafted antibodies on MNP surface through the fast and highly selective click reaction (see Experimental procedures). Exosomes were collected from culture media of a human glioblastoma multiforme cell line (SkMG3), and serially diluted. The titration experiments (Figure 4b) established the detection limit of ~10⁷ exosomes.

We used lanthanide NPs for time-gated imaging-based sensing. In time-gated imaging, particles are first excited with a short light pulse. Detection starts after a short (~1 msec) delay to exclude short-lived fluorescence and to integrate long-lived luminescence only. This scheme removes autofluorescence and interference from excitation light, enhancing overall detection sensitivity and simplifying the optical setup (no optical filters needed). The luminescence decay lifetime of polymer coated lanthanide NPs was ~2.3 msec (Figure 4c). Long decay time in the range of milliseconds allowed for control of time gating with electronics (without mechanical chopper). We devised an assay protocol to detect bacterial DNA using the lanthanide NPs. Carboxylated microbeads (diameter 10 μm) and carboxylated lanthanide NPs were conjugated with oligonucleotides complementary to target DNA (Table S1). The microbeads were used to capture target DNA and subsequently labeled with lanthanide NPs. The resulting luminescence signal from microbeads was then measured via time-gated imaging. Figure 4d shows a proof-of-concept example. Bacterial RNA from *S. aureus* culture was extracted and PCR-amplified to prepare single-stranded DNA. Titration experiments showed that sensitivity was close to reaching single bacteria detection.

In summary, we developed a generalized polymer-coating scheme to prepare hydrophilic NPs for biosensing. The polymer contained multiple dopamine molecules for firm attachment to NP surfaces as well as multiple PEGs to achieve hydrophilicity. When applied to ferrite and lanthanide NPs, polymer coating resulted in excellent colloidal stability. Thus, coated NPs were stable at high temperatures and in physiological buffers. Furthermore, the coating provided various functional groups to further conjugate affinity ligands for molecular sensing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Generalized coating and bioconjugation strategy for nanoparticles

Multiple dopamine containing PEG polymers are used to increase the binding affinity between the polymer and the NPs. The dopamine-based polymers replace the hydrophobic capping agent on the NP surface. To subsequently introduce affinity ligands for molecular recognition, various bioconjugation strategies (e.g., bioorthogonal click reaction, EDC coupling) can be chosen depending on the affinity ligands.

Figure 2. Polymer-coated magnetic nanoparticles (MNPs)

(a) Zn-doped ferrite (ZnFe₂O₄) MNPs were produced via thermal decomposition method. As-synthesized particles were hydrophobic and dispersed in organic solvent. The core size was 16 nm. (b) MNPs were transferred to aqueous phase through the polymer (PEG length 5k) coating. The particles were well-dispersed without aggregation. The mean hydrodynamic diameter (d_H) was 41.6 nm. The insert is photograph of MNP aqueous solution. (c, d, e) Coating stability. The polymer-coated MNPs was challenged either by heating (c), high salt concentrations (d), or long-term storage in PBS (e). No significant changes in particle size were observed, confirming high stability of the polymer coating.

Figure 3. Polymer-coated lanthanide nanoparticles

(a) Lanthanide NPs co-doped with Ce³⁺/Tb³⁺ were synthesized via thermal decomposition and rendered water-soluble through the polymer coating. (b) The initial core size of the lanthanide NPs was 13 nm; following the polymer coating, the hydrodynamic diameter was 32.8 nm, as measured by dynamic light scattering. (c) Emission spectrum from aqueous suspensions of lanthanide NPs. The particles were excited at 254 nm. The main emission peak was at 546 nm, which is characteristic of Tb³⁺. The insert is photograph of colloidal solution under 254 nm UV lamp. (d) The polymer-coated lanthanide NPs were subject to various stress tests. For example, the particle size was unaffected under different buffer conditions. Other test results are available in the Supporting Information.

Figure 4. Biosensing applications of prepared NPs

(a) MicroNMR assays with MNPs. Transverse relaxation rate (R_2) of water-soluble MNPs with different sizes and compositions were measured. Due to their superior magnetic moments, ZnFe₂O₄ MNPs showed higher R_2 than Fe₃O₄ MNPs. Among ZnFe₂O₄ MNPs, the R_2 values increased with the particle size. (b) The MNPs were used to detect exosomes. MNPs were conjugated with antibodies against EGFR to label exosomes from a human glioblastoma multiforme cell line (SkMG3). The detection threshold was approximately $\sim 10^7$ exosomes. (c) Time-gated detection with lanthanide NPs. After the excitation light was turned off, the luminescence from Tb³⁺ persisted with the typical decay time of 2.3 ms. This property was exploited for time-gated detection; UCNPs were pulse-excited, and the resulting luminescence light was detected. The method minimizes background signal coming from autofluorescence. (d) Lanthanide NPs were used to detect bacterial nucleic acids. Microbeads (diameter 10 μm) and NPs were conjugated with oligonucleotides complementary to target bacteria DNA. The limit of detection was close to single bacteria.