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Highly stable multi-anchored magnetic nanoparticles for optical imaging within biofilms

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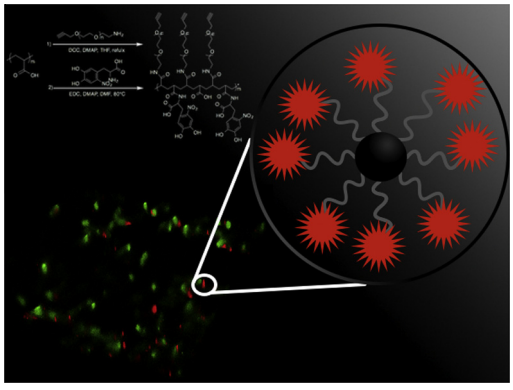
GRAPHICAL ABSTRACT



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ABSTRACT

Magnetic nanoparticles are the next tool in medical diagnoses and treatment in many different biomedical applications, including magnetic hyperthermia as alternative treatment for cancer and bacterial infections, as well as the disruption of biofilms. The colloidal stability of the magnetic nanoparticles in a biological environment is crucial for efficient delivery. A surface that can be easily modifiable can also improve the delivery and imaging properties of the magnetic nanoparticle by adding targeting and imaging moieties, providing a platform for additional modification. The strategy presented in this work includes multiple nitroDOPA anchors for robust binding to the surface tied to the same polymer backbone as multiple poly(ethylene oxide) chains for steric stability. This approach provides biocompatibility and enhanced stability in fetal bovine serum (FBS) and phosphate buffer saline (PBS). As a proof of concept, these polymer-particles complexes were then modified with a near infrared dye and utilized in

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Click chemistry
Biofilms
Imaging
NitroDOPA
Fluorescence

characterizing the integration of magnetic nanoparticles in biofilms. The work presented in this manuscript describes the synthesis and characterization of a nontoxic platform for the labeling of near IR-dyes for bioimaging.

1. Introduction

Functional magnetic nanoparticles have become an important research focus for use in biomedical applications. These applications include magnetic hyperthermia [1–4], magnetic resonance imaging [1,3,5,6], cell separation [5–7], and remediation of biofilms [8]. The small size and manipulation of magnetic properties of these materials also have potential for non-invasive diagnostics and treatments to improve quality of life for patients.

There are a variety of ways to create functional magnetic nanoparticles, but the fundamental designs of these materials stay the same. Generally, in each individual nanoparticle, the core material is magnetic and is coated with a hydrophilic material that is either physically or chemically adsorbed on to the surface. The core can be made up of an assortment of magnetic materials, including iron platinum, cobalt oxide, and iron oxides [7]. Because these materials are generally synthesized using thermal decomposition methods, they have hydrophobic polymer ligands that are only dispersible in organic solvents. They must be modified with hydrophilic materials for biomedical applications. There are a variety of hydrophilic surface designs that have been investigated to create hydrophilic magnetic nanoparticles, including dextran, pluronics, and polyethylene oxide (PEO) [7,9].

Magnetic nanoparticles for biomedical applications, like any pharmaceutical delivery vehicle, have power in numbers. The ultimate goal is to have a high accumulation in the affected area without using high dosages that may result in side effect for the patient. This can be accomplished in magnetic nanoparticle composites by designing polymer ligands that increases the bioavailability of the particles in the circulatory system [7,9], as well as taking advantage of the particles' surface to target certain cells by exploiting over expressed receptors [7,10].

Designing polymer ligands for biological targeting and imaging applications can be exhaustive and the reactions can be inefficient. Functionalization of the nanoparticles generally requires harsh solvents that must be completely removed before any biological interaction [11]. Click chemistry provides a biologically friendly conjugation method to create strong and chemically stable linkages. Originally developed for facile protein modification, this method was later used to efficiently modify polymers [12]. The most common of the click reactions is the Huisgen 1,3-dipolar cycloaddition between an alkyne and an azide [13].

We have previously reported synthesis of monoanchor systems consisting of a catechol binding group on one end and clickable alkyne on the other end [14,15]. This provides a versatile platform for additional modification, as we and others have demonstrated having multiple anchors (i.e., $f > 3$) prevents salts and proteins from disrupting the steric stability of these systems. To provide this 'multidentate' binding to the surface, we adapt chemistry for the modification of polyacrylic acid (PAA) with catechol amines and PEO, which has been successfully demonstrated by Wang and Na [10,16]. Here, we report the synthesis and characterization of a magnetic nanoparticle composite comprised of an iron oxide core and a functional PEO complex with enhanced colloidal stability in biological environments, due to multi-anchoring of catechol groups to the surface. This polymer-particle complex can be tailored for a variety of biomedical applications using click chemistry. To illustrate the enhanced stability of the aforementioned particle, it was exposed to a variety of simulated biological environments at body temperature and compared to a mono-anchored particle of similar size and composition.

To demonstrate the versatility of our platform, we utilized dye labeled versions of these materials for observing how polymer-coated magnetic nanoparticles interact with *Legionella* biofilms. We have recently reported changes in the morphology and decrease of biomass in *Legionella pneumophila* biofilms after exposure to low concentrations of magnetite nanoparticles (Fe_3O_4 NPs) [8], and sought an effective method for the visualization of the fate of these materials. Previous efforts for the visualization of iron oxide particles in cellular environments have included staining with Prussian blue [17], PET labels [18], and IR dyes [19], MRI, and others. Despite these advances, visualization of the particles within biological samples remains a challenge due to the highly absorptive nature of iron oxide materials. Several different strategies have been proposed throughout the years [20]. Here in, we use a polymer brush linker to extend the dye away from the iron oxide particle. Demonstrating the effectiveness of this technique, the multi-anchored particles were modified with Cy5.5 and imaged in biofilms to observe the fate of these nanoparticles. This dye was selected as excitation and emission are in the near infrared (NIR) region, from 650 to 900 nm. In this region, the absorbance and autofluorescence of biological media is low providing better energy transfer through the tissue [20]. In this work, we show a magnetic nanoparticle system that has enhanced biological stability and has been modified with a NIR dye, which can be used to observe the nanoparticle fate within a biofilm using NIR imaging.

2. Experimental section

2.1. Materials and characterization

Tetrahydrofuran (THF; B.D.H. ACS grade) was purified by reflux over sodium metal (Aldrich Chemistry; sodium lump in kerosene 99%) and benzophenone. Bromophenol blue (Sigma Aldrich), hydrochloric acid (HCl; J.T. Baker, 1 N volumetric solution), ethylene oxide (EO; Aldrich Chemistry), dichloromethane (DCM; EMD Millipore Chemicals, HPLC grade), chloroform (B.D.H.), diethyl ether (DEE; Macron Chemicals), triethyl amine (TEA; Alfa Aesar 99%), 4-(dimethylamino) pyridine (DMAP; Fluka Analytical), succinic anhydride (SA; Alfa Aesar 99%), N,N′-dicyclohexyl carbodiimide (DCC; Thermo Scientific), N-hydroxysuccinimide (NHS; Acros Organics 98+%), Cy5.5 azide (Lumiprobe 95%), poly(acrylic acid) 1800 MW (PAA; Aldrich), copper (II) sulfate (Sigma Aldrich), (+)sodium L-ascorbate (Sigma Aldrich), chloroform-D ($CDCl_3$; D,99.8%, Cambridge Isotope Laboratories Inc.), potassium bis (trimethylsilyl)amide solution (1 M THF, Aldrich), propargyl bromide solution (80% in toluene; Fluka), sodium hydride (NaH; Aldrich), dimethylformamide (DMF; 99.8%, extra dry over molecular sieves; Sigma Aldrich), methanol (absolute ACS reagent grade; Ricca Chemical Company), and Spectrum Spectra/Por® molecular porous membrane tubing, MWCO of 1000 and 12–14,000 were all used as received. Materials used for cell viability studies were as follows: 96 well plates were purchased from Corning, L-929 cells (ATCC® CCL-1™) and Eagle's Minimum Essential Medium (EMEM) were purchased from American Type Culture Collection (ATCC), phosphate buffer solution (PBS) from Sigma Aldrich was