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Amphiphilic protein micelles for targeted in vivo imaging

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ABSTRACT

A variety of polymeric nanoparticles have been developed for bioimaging applications. This study reports on the use of a 50 nm recombinant protein nanoparticle with a multivalent surface as a vehicle for functionalization with a model imaging agent. Multiple fluorescent probes were covalently conjugated to surface amines of crosslinked amphiphilic elastin-mimetic protein micelles using *N*-hydroxysuccinimide ester chemistry. In vivo fluorescence imaging confirmed that protein micelles selectively accumulated at sites of angioplasty induced vessel wall injury, presumably via an enhanced permeability and retention effect. This investigation demonstrates the potential of amphiphilic protein micelles to be used as a vehicle for selective imaging of sites associated with a disrupted or leaky endothelium.

1. Introduction

Fluorescent nanoparticles have been used to localize and assess the extent of a variety of pathological processes [1–4]. In this regard, both encapsulation and covalent conjugation of fluorophores have been used to generate fluorescent nanoparticle [5–7]. Even without surface targeting groups, nanoparticles selectively accumulate at sites of increased vascular permeability, often associated with tumor microcirculation or inflammatory processes via an enhanced permeability and retention (EPR) effect [2,8–10].

Recent strategies to create probes for biomedical imaging have included organic and inorganic nanoparticles, quantum dots (QD), liposomes, proteins and viral particles [2,3,11–16]. Although each approach has unique advantages, none has proved ideal, owing to concerns related to toxicity, biostability and bioavailability. Among these probes, QD are the most interesting class of fluorescent probes for bioimaging, because of their brightness, photostability and narrow and tunable emission spectrum. In addition to QD, magnetic nanoparticles such as iron oxides have been used as contrast enhancing agents for magnetic resonance imaging (MRI) and coupled with fluorescent probes for both MRI and fluorescence imaging. However, under physiological conditions, QD and magnetic nanoparticles are not soluble and tend to aggregate. In addition, Cd, Te, Se or Pb found in QD are cytotoxic [17]. Protein-based nanoparticles may have several advantages over other imaging agents, including limited toxicity and enhanced biocompatibility, particularly for elastin-based micro- or nanoparticles [18–20]. Significantly, such particles display inherent flexibility for functionalization with bioactive molecules either chemically or genetically [21–24]. For example, Simnick et al. [24] recently reported the generation of elastin-like diblock copolymers, engineered with an RGDS peptide sequence at the N terminus and one cysteine residue at the C terminus, which can be chemically conjugated to a fluorescent probe containing maleimide. The present authors' group recently developed recombinant amphiphilic diblock polypeptides (ADP) based on elastin-mimetic sequences consisting of an N-terminal hydrophilic block and a C-terminal hydrophobic block containing glutamic acid and tyrosine residues, respectively. It was demonstrated that these polypeptides formed 50-nm-diameter thermally responsive micellar nanoparticles that exhibited a spherical core–shell structure. By introducing multiple cysteine residues between the amphiphilic blocks, it was possible to obtain stable protein micelles through disulfide bond formation at the core–shell interface [25,26].

This study describes the surface functionalization of elastin-mimetic protein (EMP) micelles via chemical conjugation and the potential to use these particles for in vivo bioimaging. Each diblock polypeptide possesses a single free amine at the N terminus, which is displayed on the surface of the protein micelle owing to self-assembly of C-terminal hydrophobic blocks that occurs above their inverse transition temperature. Thus, it was anticipated that functionalizing EMP micelles with fluorescent dyes via an *N*-hydroxysuccinimide (NHS) ester linker would generate protein nanoparticles with enhanced fluorescent intensity.

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