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Solvent-free atom transfer radical polymerization for the preparation of poly(poly(ethyleneglycol) monomethacrylate)-grafted Fe_3O_4 nanoparticles: Synthesis, characterization and cellular uptake

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Abstract

Poly(poly(ethyleneglycol) monomethacrylate) (P(PEGMA))-grafted magnetic nanoparticles (MNPs) were successfully prepared via a solvent-free atom transfer radical polymerization (ATRP) method. The macroinitiators were immobilized on the surface of 6.4 ± 0.8 nm Fe_3O_4 nanoparticles via effective ligand exchange of oleic acid with 3-chloropropionic acid (CPA), which rendered the nanoparticles soluble in the PEGMA monomer. The so-obtained P(PEGMA)-grafted MNPs have a uniform hydrodynamic particle size of 36.0 ± 1.2 nm. The successful grafting of P(PEGMA) on the MNP surface was ascertained from FTIR and XPS analyses. The uptake of the MNPs by macrophage cells is reduced by two-orders of magnitude to <2 pg Fe/cell after surface grafting with P(PEGMA). Furthermore, the morphology and viability of the macrophage cells cultured in a medium containing 0.2 mg/mL of P(PEGMA)-grafted MNPs were found similar to those of cells cultured without nanoparticles, indicating an absence of significant cytotoxicity effects. T_2-weighted magnetic resonance imaging (MRI) of P(PEGMA)-grafted MNPs showed that the magnetic resonance signal is enhanced significantly with increasing nanoparticle concentration in water. The R_1 and R_2 values per millimole Fe, and R_2/R_1 value of the P(PEGMA)-grafted MNPs were calculated to be 8.8 $mM^{-1}s^{-1}$, 140 $mM^{-1}s^{-1}$, and 16, respectively. These results indicate that the P(PEGMA)-grafted MNPs have great potential for application in MRI of specific biotargets.

Keywords: ATRP; Solvent-free; PEGMA; Magnetic nanoparticle; MRI

1. Introduction

Recently, superparamagnetic nanoparticles of iron oxides have shown great potential in bioapplications, including magnetic resonance imaging (MRI) contrast enhancement [1,2], drug delivery [3,4], bioseparation [5,6], tissue repair [5,6], hyperthermia [1,5], and magnetofection [6]. In these fields, the preparation of the monodispersed magnetic nanoparticles (MNPs) and the introduction of functionalities on the surface of these MNPs through grafting of water soluble, biocompatible groups and targeting ligands are much desired.

Polymer coating of MNPs is now one of the most attractive methods to realize such functionalities because the polymer shell offers flexibility in controlling the chemical composition and functional groups on the nanoparticle surface [6]. Most of such MNP surface modification approaches utilize the "grafting to" strategy, which involves the coating of MNPs with pre-existing polymers via either hydrophobic interaction [7–16] or deposition [17–19] through electrostatic affinity. Although this strategy is flexible, the polymers tend to coat more than one nanoparticle to form nanoparticle clusters. On the other hand, the "grafting from" method, which propagates the polymer chains from the nanoparticle surface, is a good candidate to form small polymer-coated single nanoparticles. Furthermore, with this method, surface modification

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with high graft density and high stability of the polymer shell can be achieved. As such, surface-initiated atom transfer radical polymerization (ATRP) appears a promising "grafting from" technique which offers many advantages including good control over molecular weight and monodispersity, and therefore thickness of the polymer shell [20–24]. Another advantage of ATRP is that the end-functionalized polymers or block copolymers grafted onto the nanoparticle surface can offer a variety of active sites for further multi-biofunctionalization for specific targeting. Despite these advantages, most of the research on MNP surface-initiated ATRP focused on the formation of polystyrene or poly(methyl methacrylate) hydrophobic shells on the MNPs, which have limited potential for bioapplication. Compared with the more widely reported bioapplications of hydrophilic polymer-coated MNPs via the "grafting to" method, the preparation of hydrophilic polymer-grafted MNPs through ATRP and more importantly their bioapplications still need further investigation.

Recently several hydrophilic polymer-coated MNPs through surface-initiated ATRP in polar solvents were successfully developed by our group and Hatton's group [25–27]. In addition, their possible bioapplications were also investigated [25,26]. However, their potential bioapplication for MRI which is one of the most important application fields for MNPs has not been explored. Among the hydrophilic monomers, poly(ethyleneglycol) monomethacrylate) (PEGMA), a poly(ethyleneglycol) (PEG) derivative which contains one pendant hydroxyl group in every monomer, is one of the most attractive monomers for future bioapplication [25]. PEG has been well proven to be nonimmunogenic, nonantigenic, and protein resistant [28–30]. Consequently, PEG has been introduced on the MNPs surface via the "grafting to" method to enhance their biocompatibility [31–34]. Recently grafting poly (PEGMA) or P(PEGMA), instead of PEG, via "grafting from" method showed that it will not only prevent rapid clearing by macrophages, but also provide more sites for further biofunctionalization [25]. However, their further bioapplication was impeded by the poor control over the rate and extent of the ATRP process in solvent due to the formation of flash polymerization when higher monomer concentration is used for ATRP.

We herein present a relative simple and scalable approach for preparing P(PEGMA)-grafted Fe_3O_4 core–shell nanoparticles with well-controlled properties using a solvent-free ATRP. Yang and co-workers first reported the solvent-free ATRP of styrene on Fe_3O_4 nanoparticles and showed that with this method the desorption of the initiator molecules from the particle surfaces could be reduced, and the polymerization occurred favorably on the macroinitiator surfaces [21], giving rise to monodispersed polymer-coated nanoparticles. In our present work, the initiator, 3-chloropropionic acid (CPA), used for ATRP [20] at inorganic surfaces was introduced through ligand exchange [20–23,27] with the oleic acid on the surface of the pristine nanoparticles prepared from the high-temperature decomposition of iron acetylacetonate [35]. The ligand exchange method enables the solubility of the MNP to be changed to match the requirement of solvent-free ATRP since surface capping agents can be exchanged in a controllable fashion, depending on the functional groups and concentrations of the surfactants [20–23]. The physical properties, cytotoxicity and MRI of the P(PEGMA)-grafted MNPs from solvent-free ATRP were investigated and the results showed that these MNPs may be a good candidate for bioapplications utilizing MRI.

2. Experimental

2.1. Materials

Benzyl ether, 1,2-hexadecanediol, oleic acid, oleylamine, iron(III) acetylacetonate, 3-chloropropionic acid, 2,2′-bipyridyl (Bpy), and copper(I) chloride were purchased from Aldrich Chemical Co. and used as received. Poly(ethyleneglycol) monomethacrylate macromonomer (Mn~360) was passed through a silica gel column to remove the inhibitor and stored under an argon atmosphere at −10 °C. Mouse macrophage cells (RAW 264.7) and 3T3 fibroblasts were purchased from ATCC. RPMI-1640 medium, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, L-glutamine, penicillin, and streptomycin were purchased from Sigma. All other solvents and chemicals were purchased from either Fisher Scientific or Aldrich and used as received.

2.2. Preparation of magnetic nanoparticles

The Fe_3O_4 MNPs were prepared according to a previously reported method [35]: iron(III) acetylacetonate (1.766 g, 0.337 mL, 5 mmol), 1,2-hexadecanediol (6.461 g, 25 mmol), oleic acid (4.237 g, 4.761 mL, 15 mmol), oleylamine (4.012 g, 4.935 mL, 15 mmol), and benzyl ether (50 mL) were mixed and magnetically stirred under a flow of nitrogen. The mixture was heated to 200 °C for 2 h and then, under a blanket of nitrogen, heated to reflux (300 °C) for another 1 h. The black mixture was cooled to room temperature after removal of the heat source. Under ambient conditions, ethanol (100 mL) was added to the mixture, and a black material was precipitated and separated via centrifugation. The black product was dissolved in 40 mL of hexane in the presence of oleic acid (1 mL) and oleylamine (1 mL). Centrifugation (6000 rpm, 10 min) was applied to remove any undispersed residue. The product, Fe_3O_4 nanoparticles, was then precipitated with ethanol, and collected by centrifugation (6000 rpm, 10 min). The nanoparticles were then dried under reduced pressure and stored at 0–4 °C.

2.3. Ligand exchange of magnetic nanoparticles

A 100 mg of oleic acid-stabilized nanoparticles were dispersed in 100 mL of 0.25 M CPA in hexane and stirred for 24 h at room temperature under the protection of argon. The resulting black precipitate was separated using a centrifuge at 6000 rpm for 4 min and washed three times with hexane to remove the excess initiator. The nanoparticles were then dried under reduced pressure and stored at 0–4 °C.

2.4. Solvent-free atom transfer radical polymerization (Fig. 1(a))

The CPA-stabilized nanoparticles (60.0 mg) were first dissolved in PEGMA monomer (6 mL in a Pyrex tube containing a magnetic stir bar) to form a transparent brownish solution. The solution was purged with argon for 15 min, and CuCl (5.1 mg) and Bpy (24 mg) were then added. After purging with argon for another 10 min, the Pyrex tube was sealed and kept in a 30 °C water bath for 18 h under stirring. After the reaction was complete, the mixture was diluted with tetrahydrofuran (THF) at a