Materials Science and Engineering C 33 (2013) 4660–4666

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Synthesis and characterization of PEG-iron oxide core-shell composite nanoparticles for thermal therapy

Robert J. Wydra [a], Anastasia M. Kruse [a], Younsoo Bae [b], Kimberly W. Anderson [a], J. Zach Hilt [a,*]

[a] Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY 40506, USA
[b] Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY 40506, USA

ARTICLE INFO

Article history:
Received 12 September 2012
Received in revised form 29 May 2013
Accepted 17 July 2013
Available online 24 July 2013

Keywords:
Magnetic nanoparticles
Atom transfer radical polymerization
Thermal therapy

ABSTRACT

In this study, core-shell nanoparticles were developed to achieve thermal therapy that can ablate cancer cells in a remotely controlled manner. The core-shell nanoparticles were prepared using atomic transfer radical polymerization (ATRP) to coat iron oxide (Fe_3O_4) nanoparticles with a poly(ethylene glycol) (PEG) based polymer shell. The iron oxide core allows for the remote heating of the particles in an alternating magnetic field (AMF). The coating of iron oxide with PEG was verified through Fourier transform infrared spectroscopy and thermal gravimetric analysis. A thermoablation (55 °C) study was performed on A549 lung carcinoma cells exposed to nanoparticles and over a 10 min AMF exposure. The successful thermoablation of A549 demonstrates the potential use of polymer coated particles for thermal therapy.

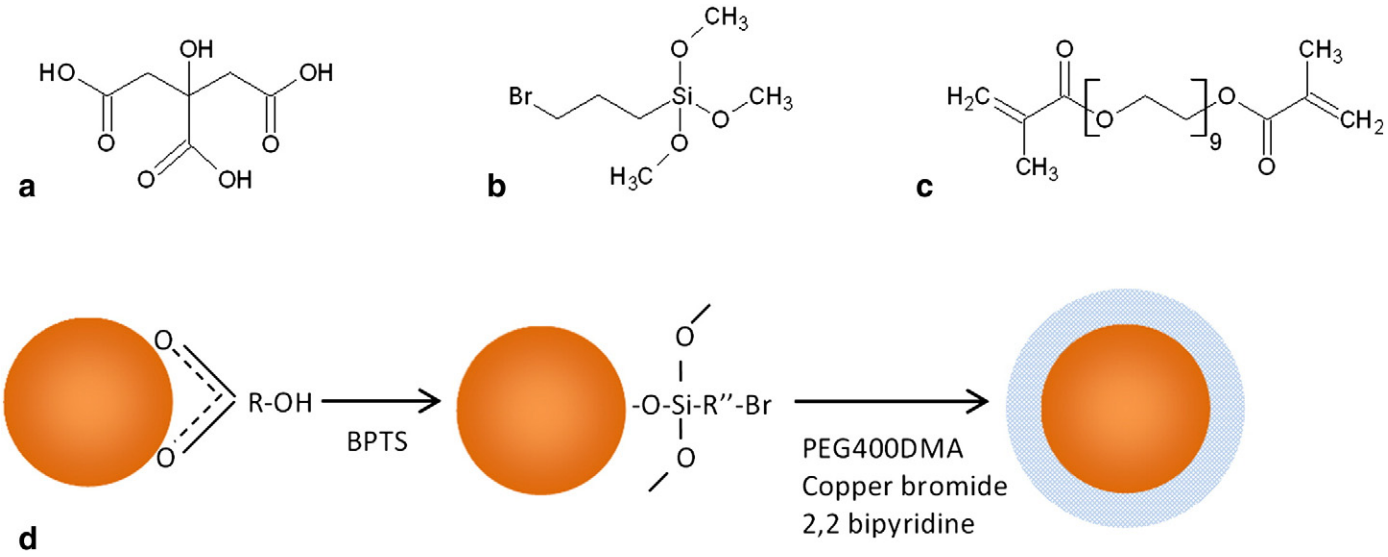
1. Introduction

Due to their unique physical properties, iron oxide nanoparticles are being studied for a wide range of biomedical applications such as imaging, targeted delivery, and thermal therapy of cancer [1–4]. Superparamagnetic nanoparticles remotely heat in an alternating magnetic field primarily due to the Brownian relaxation (physical rotation of the particles) and Neel relaxation (rotation of the magnetic moment) [5–7]. The particles absorb the energy from the magnetic field and convert it into heat through the aforementioned relaxations [8]. Surface modification plays an essential role in determining the success of nanoparticles in their application by improving stability, preventing agglomeration, improving biocompatibility, and providing additional functionalities (e.g. targeting antibodies) [9–13]. PEG-based functionalization is common for biological applications as a means to prevent protein adsorption and thus improve circulation time and minimize host response to the particles [14]. One method of functionalizing the particles is utilizing a surface initiated atom transfer radical polymerization (ATRP) [15–17]. This method first involves attaching an initiator group to the surface that serves as the seed for polymerization. Various polymeric systems can be grafted from the surface making ATRP a very flexible platform. For *in vivo* applications, PEG functionalization would be essential for the stability of the nanoparticles by preventing premature clearance [18]. To date, most surface initiated polymerizations have been utilized to coat iron oxide nanoparticles with a polymer brushes [19]. PEG brushes have been successfully used to prevent rapid clearance by macrophages, resist protein adsorption, and have reduced cytotoxic effects [20–22]. By utilizing a PEG-based hydrogel coating, similar biological properties are expected while having the additional benefit of future applications such as drug loading for controlled delivery. Coating stability is an additional concern to guarantee the long term effectiveness of a nanoparticle system. Miles et al. have demonstrated that carboxylic acid anchors can be displaced by phosphate ions effecting colloidal stability [23,24]. In the case of a crosslinked hydrogel shell, the stability of the coating will not be affected by anchoring group displacement as a continuous shell entraps the core nanoparticle.

Thermal therapy is the process of elevating tumor tissue temperature for therapeutic gains and has been studied for decades, but has yet to gain widespread clinical recognition [25–27]. Two temperature ranges have been identified: hyperthermia, 40–45 °C, and thermoablation, ≥46 °C. Hyperthermia can induce cellular death on its own, but it is better suited for enhancing the effects of chemotherapy and/or radiation therapy [25,28–30]. The exact cause of the increased sensitivity is still under investigation, but it is believed to be a combination of cellular effects: changes in the cell membrane, impaired transport, cytoskeleton damage, and impairment and damage to cellular proteins and DNA; and physiological effects: changes in the vasculature, increased perfusion, and changes in oxygen levels [29–31]. Due to the elevated temperature, thermoablation leads to direct cell necrosis and can be used as an independent treatment [32]. The main issue facing thermal therapy is a clinical means to deliver elevated temperatures to the tumor site. Current methods are characterized by the amount of surrounding tissue heated and subdivided

* Corresponding author at: Department of Chemical and Materials Engineering, University of Kentucky, 177 F. Paul Anderson Tower, Lexington, KY 40506-0046. Tel.: +1 859 257 9844; fax: +1 859 323 1929.
E-mail address: hilt@engr.uky.edu (J.Z. Hilt).

0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.07.019



Scheme 1. Chemical structures of materials utilized in the iron oxide functionalization: (a) citric acid (CA), (b) 3-bromopropyl trimethoxysilane (BPTS), (c) Poly(ethylene glycol) 400 dimethacrylate (PEG400DMA). (d) Schematic of ligand exchange and ATRP reaction on the nanoparticles.

into whole body hyperthermia (water baths or heating chambers), localized hyperthermia (antennas emitting microwaves or ultrasound), and regional hyperthermia (array of antennas) [28,30]. Localizing the heat, tumor targeting, and even temperature distribution across the tumor are some of the shortcomings of the current methods of delivering hyperthermia. Localization of the thermal therapy is necessary to prevent damage to the surrounding tissue and minimize patient discomfort and uniform heating is necessary to guarantee therapy effectiveness. It is of particular interest to utilize the remote heating of the nanoparticles to overcome the barriers of traditional hyperthermia methods [33]. It has recently been demonstrated that hyperthermia induced by magnetic nanoparticles has an advantage over conventional hyperthermia methods in inducing cell death *in vitro* [34]. By passive targeting, nanoparticles can collect at the tumor site and by the application of the alternating magnetic field provide localized heating throughout the tumor.

In this study, core-shell nanoparticles were prepared using ATRP to coat iron oxide (Fe_3O_4) nanoparticles with a PEG-based polymer shell. Cytotoxicity on two independent cell lines was examined to determine potential systemic effects. Thermal therapy application feasibility was demonstrated *in vitro* with a thermoablation (55 °C) study on A549 lung carcinoma cells.

2. Materials and methods

2.1. Materials

Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$); iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$); 2, 2 bipyridine (Bpy); copper (I) bromide (CuBr); and copper (powder <425 micron) were obtained from Sigma Aldrich (St Louis, MO). Citric acid monohydrate (CA) was obtained from Fisher Scientific and ammonium hydroxide (NH_4OH) from EMD Chemicals (Gibbstown, NJ). 3-bromopropyl trimethoxysilane (BPTS) was obtained from Gelest Inc. (Morrisville, PA). Poly(ethylene glycol) 400 dimethacrylate (PEG400DMA) was obtained from Polysciences Inc. (Warrington, PA). Chemical structures of these materials are shown in Scheme 1. All materials were used as received.

2.2. Iron oxide nanoparticle synthesis

A one-pot co-precipitation method was used to prepare the core citric acid coated iron oxide nanoparticles [35]. Aqueous solutions of $FeCl_3 \cdot 6H_2O$ and $FeCl_2.4H_2O$ were combined in a 2:1 molar ratio in a sealed three-neck flask under vigorous stirring and an inert N_2 environment. Once 85 °C was reached, 5 mL of NH_4OH was injected into the vessel followed by 4 ml of 2 M citric acid. The reaction was

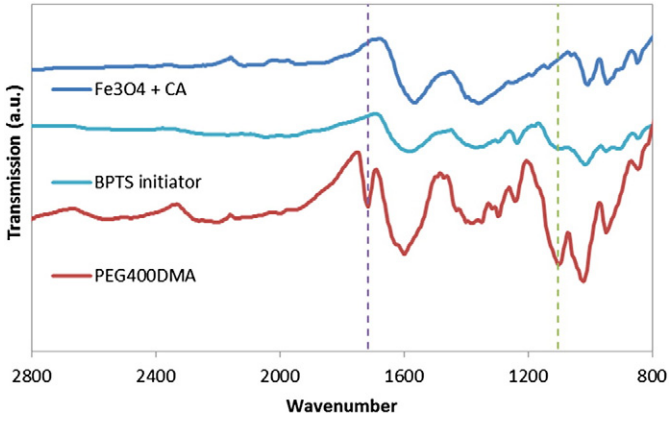


Fig. 1. FTIR spectra of citric acid coated nanoparticles, particles after the BPTS ligand exchange, and particles functionalized with PEG400DMA.

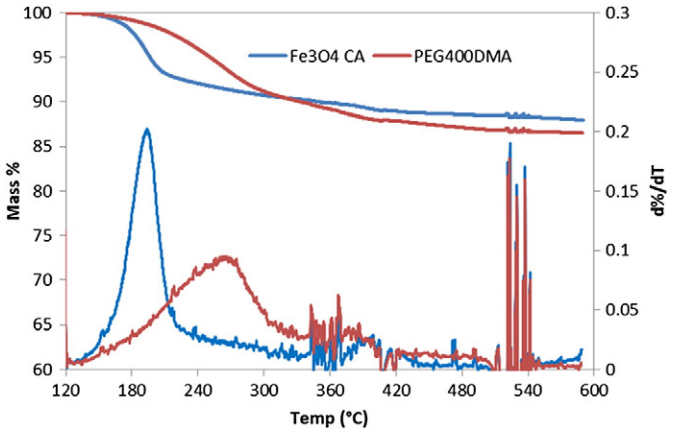


Fig. 2. Mass loss and derivative profile of citrate and PEG400DMA coated iron oxide.