Biomaterials 33 (2012) 2251–2259

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Long-term theranostic hydrogel system for solid tumors

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ARTICLE INFO

Article history:
Received 8 November 2011
Accepted 26 November 2011
Available online 19 December 2011

Keywords:
Phosphazene
Long-term theragnosis
Contrast agents
Sustained release
Drug delivery system

ABSTRACT

The long-term theranostic hydrogel system for solid tumors was prepared via simple physical mixing, which consisted of three major parts: the thermosensitive/biodegradable poly(organophosphazene) hydrogel, PEGylated cobalt ferrite nanoparticles, and paclitaxel (PTX). The PEGylated cobalt ferrite nanoparticles showed extremely low cytotoxicity due to the surface modification using PEG chains. The long-term theranostic hydrogel system showed adequate properties to be used for long-term MR theragnosis. In particular, the theranostic hydrogel gradually degraded over 28 days, and the PTX was sustainedly released out from the theranostic hydrogel over the same period *in vitro*. Furthermore, the *in vivo* efficacy of long-term MR theragnosis using the theranostic hydrogel system was estimated successfully over 3 weeks by using high field (4.7 T) animal MRI and solid tumor-bearing mice. Based on our results, we expect that this system can supply multiple data regarding a) the progress of therapy and b) the treatment processes via one- or two-time i.t. administration for cases in which surgical approaches are difficult to apply. Meanwhile, cancer patients can be free from the pain of multiple surgical treatments and have the advantage of therapy through a simple i.t. administration.

1. Introduction

Magnetic resonance imaging (MRI) and its contrast agent materials have been used in clinical and biomedical research fields [1–8]. It is known well that MR contrast agents are categorized into two types: a paramagnetic contrast agent, such as Gd-chelates (T_1 contrast agent) and a superparamagnetic contrast agent (T_2 contrast agent), such as ferrite nanoparticles [9–11]. Among them, surfaces of T_2 contrast agents have been modified to be used in clinical applications by using non-toxic materials; these materials have also been commercialized for intravenous (i.v.) administration [12–18]

Recently, the i.v.-type MR T_2 contrast agents have been modified via bioconjugation with specific targeting molecules and drugs for multifunctional theragnosis [19–21]. Thus, a theragnosis in the initial step meant two major functions: the targeted therapy and the imaging diagnosis [22]. However, many studies regarding theragnosis could not solve their primal factors which are an effective concentration in lesion sites, such as tumor tissues, and a sufficiently long circulation time of theranostic agents [20,21]. Though the i.v.-type theranostic agents have 'smart targeting' functions, it is clear that i.v.-type theranostic agents cannot be localized within specific tumor sites over long-term periods. Thus, another alternative approach, such as an injectable platform technology, is required, which uses biodegradable hydrogels and can pass through the circulatory system 'indirectly' using an intratumoral (i.t.) or a subcutaneous (s.c.) administration.

Meanwhile, the delivery pattern of i.v. type theranostic agents depends on the tumor vasculature network [23–25]. Another major problem in i.v. administration might originate from the heterogeneity of distribution within solid tumors. In contrast, local delivery depends on the interstitial space within a tumor. Thus, it is certain that local delivery using injectable platform technology can be an adequate approach for long-term delivery and monitoring of theranostic agents over long periods, which is a 'long-term theragnosis'.

A biodegradable gelation technique within tumor tissues using i.t. administration can make the long-term theranostic system more useful [26–30]. The i.t. administration of this approach can make the blood plasma concentration of theranostic agents minimal and their concentration within tumor tissues maximal [31]. Consequently, the system is able to maintain a minimum level

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doi:10.1016/j.biomaterials.2011.11.083

of side effects or prevent them completely and be one of the alternative approaches for selective treatment with the injection of theranostic substances into the tumor tissues directly.

Herein, we suggest that the thermosensitive/biodegradable poly(organophosphazene) hydrogel system as a method of local drug delivery, can be an adequate biodegradable platform system for long-term MR theragnosis (Scheme 1) [28–30]. It is well known that this system has the following adequate properties: a) thermosensitivity, b) injectability due to a reversible sol-to-gel phase transition near body temperature, c) localizability via rapid gelation, d) the very low cytotoxicity of dissolved elements, e) biodegradability with adjustable periods of biodegradation, and f) sustainability and long-term drug release [28–30]. In this study, we emphasize that our hydrogel system can serve as a long-term theranostic hydrogel system using theranostic substances. Here, multiple characteristics of our theranostic hydrogel system were assessed: basic synthesis and property measurements of materials, *in vivo*/*in vitro* tests, and toxicity tests in each organ.

2. Materials and methods

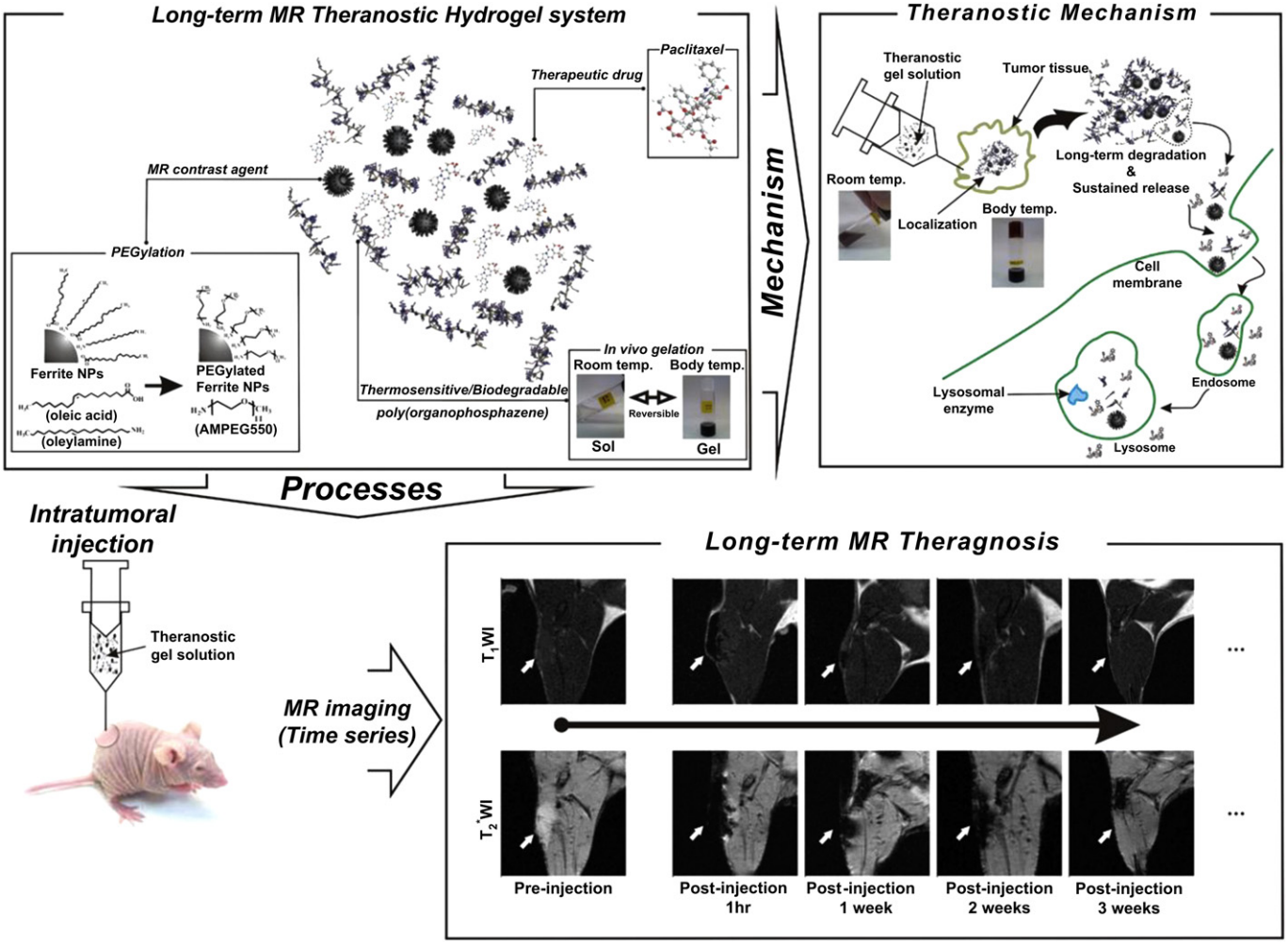
2.1. Instruments and measurements

D/MAX-2500 (Rigaku international Corporation, Japan) was used as small angle X-ray spectroscopy. X-ray type was 1.54056 Å of Cu $\kappa_{\alpha 1}$, 30 kV, and 100 mA. Scan mode was 2Θ/Θ. Scanning range was between 3 and 80°. Scan step was 0.010° and scan speed was 4.000°/min. Tecnai G2 (FEI Hong Kong Co., Ltd.) was used as transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDX) devices. M-ferrite (M = Mn, Fe, Co, and Ni) nanoparticles were dispersed in hexane with 1 wt% solution (except EDX samples (5 wt%)) and PEGylated M-ferrite nanoparticles were dispersed in distilled water. For the measurement of mass magnetization, MPMS5 (Quantum Design Co., USA) was used as superconductor quantum interference device (SQUID) magnetometer at room temperature. The amount of each powder sample was between 80 and 100 mg. Thermo Mattson model infinity gold FTIR (Thermo Fisher Scientific Inc., USA) was used as Fourier transform infrared spectroscopy (FTIR). Potassium bromide (KBr, IR grade, Sigma–Aldrich Co.) pellet was used as the baseline, and the ratio of sample amount versus KBr was 1 mg : 300 mg. In the case of iron content confirmation, SOLAAR M (Thermo Fisher Scientific Inc., USA), a device of flame & flameless analysis, was used as atomic absorption spectroscopy (AAS). All samples were dispersed in distilled water or PBS solution. For PTX release test, OPTIZEN 2021UV (Mecasys Co., Ltd., Korea) was used as UV/vis spectrometer. Quartz sample holders were used and the excitation wavelength of sample was 260 nm. In the case of MTT assay, Spectra MAX 340 (Molecular Devices, Inc., USA) was used with softmax Pro v.5.3. Finally, 4.7 T Bio-Spec® 47/40 USR (Bruker Biospin, Germany) was used for *in vivo* MR imaging.

2.2. Synthesis of ferrite nanoparticles

Magnetite nanoparticles were synthesized by using high temperature thermal decomposition method with reagents as follows: iron(III) acetylacetonate (≥97.0%(RT)), 1,2-hexadecanediol (technical grade, 90%), *cis*-9-Octadecenoic acid (reagent grade, ~99% (GC)), cis-1-Amino-9-octadecene (technical grade, 70%), and dibenzyl ether (99%). All reagents were purchased from Aldrich Chemical Co.

$Fe(acac)_3$ (20 mmol), 1,2-hexadecanediol (100 mmol), *cis*-9-Octadecenoic acid (60 mmol), and cis-1-Amino-9-octadecene (60 mmol) were mixed and magnetically stirred in benzyl ether (250 mL) under an N_2 atmosphere. The mixture was heated to



Scheme 1. Schematic diagram regarding preparation, theranostic mechanism, and process of the long-term MR theranostic hydrogel system for *in vivo* estimation. The system consists of three part: thermosensitive/biodegradable poly(organophosphazene), PEGylated MR contrast agent, and therapeutic drug.