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Thermally-triggered 'off–on–off' response of gadolinium-hydrogel–lipid hybrid nanoparticles defines a customizable temperature window for non-invasive magnetic resonance imaging thermometry

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ABSTRACT

For effective and safe thermotherapy, real-time, accurate, three-dimensional tissue thermometry is required. Magnetic resonance imaging (MRI)-based thermometry in combination with current temperature responsive contrast agents only provides an 'off–on' signal at a certain temperature, not indicating temperature increases beyond the desired therapeutic levels. To overcome this limitation, a novel Gd-chelated hydrogel–lipid hybrid nanoparticle (HLN) formulation was developed that provides an 'off–on–off' signal defining a thermometric window for MR thermometry. Novel thermally responsive poly(N-isopropylacrylamide-co-acrylamide) (NIPAM–co-AM) hydrogel nanoparticles (<15 nm) with bisallylamidodiethylenetriaminetriacetic acid, a novel crosslinker with Gd^{3+} chelation functionality, were synthesized. The Gd-hydrogel nanoparticles were encapsulated in a solid lipid nanoparticle matrix that prevented T_1-weighted contrast signal enhancement. Melting of the matrix lipid freed the Gd-hydrogel nanoparticles into the bulk water and an 'off–on' contrast signal enhancement occurred. As the temperature was further increased to temperatures greater than, the volume phase transition temperature of the hydrogel nanoparticles, they collapsed and provided an 'on-off' signal diminution. Both the 'off–on' and the 'on–off' transition temperature could be tailored by changing the lipid matrix and altering the NIPAM/AM ratio in the hydrogel, respectively. This allowed MRI thermometry of different temperature windows using the Gd-HLN system.

1. Introduction

The potential of thermotherapy, the deposition of thermal energy, to significantly enhance the efficacy and tolerability of anti-cancer treatments has been demonstrated in several solid tumor types [1–4]. Thermotherapy can be hyperthermic or ablative depending on the applied temperature range. In hyperthermic therapy, temperatures ranging from 41 to 45 °C are produced in a target tissue for periods of minutes to hours in order to achieve cell kill, trigger drug release from drug-loaded temperature-sensitive liposomes [5–9] or induce transcription from heat sensitive transcription factors [10]. In ablative therapy, tissue necrosis is induced through thermal coagulation at temperatures exceeding 50 °C [11].

Precise thermotherapy requires accurate thermometry of tumor and surrounding tissues during the course of heating [1, 11]. Control of spatial and temporal kinetics of heating allows the desired therapeutic effect to be achieved while sparing normal surrounding tissues [11, 12]. Needle or catheter-based thermometry has been used, but only provides a one-dimensional measure at the site of the probe tip [13]. Better temperature control throughout the three-dimensional volume of a tumor or other targeted tissue is important, because variations in the applied thermal dose significantly alter treatment outcome [12, 14], and because tumor and normal tissue are spatially and temporally heterogeneous in perfusion, and thermal and energy absorption [14]. To this end, magnetic resonance imaging (MRI) has been used to provide three-dimensional thermometry during thermotherapy [11,15,16].

MRI can provide simultaneous anatomical and local temperature information [11] through a non-invasive, non-ionizing imaging modality compatible with various heating devices [1,11,13,17,18].

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However, MR thermometry techniques that rely on the temperature dependence of water proton relaxivity or resonance frequency are highly sensitive to motion artifacts or changes in magnetic susceptibility during image acquisition [19–21]. Since these techniques derive tissue temperature from differences in pixel intensity between a baseline image and an image at elevated temperature, any local motion during or between the acquisition of the two images prevents accurate thermometry [11].

To overcome these motion artifacts, small molecule or nanoparticulate temperature-responsive contrast agents (TRCA) are used in MR thermometry [11]. Small molecule agents, including paramagnetic lanthanides [22,23], paramagnetic chemical exchange saturation transfer agents [24], and spin transition molecular materials [25,26] have been applied to MR thermometry with some success, however, all currently suffer from limitations [11,24,27]. Alternatively to small molecules are the nanoparticulate TRCA, the most investigated of which are the temperature sensitive liposomes [27–30]. In a low temperature state, the sparse access of the loaded contrast agent to free water provides minimal contrast enhancement in the MRI [28, 29]. As the local temperature is raised above the melting temperature (T_{melt}) of the lipid, the liposomal membrane becomes leaky, releasing contrast agent, increasing its exchange with bulk water and enhancing the MRI signal. The transition temperature at which this enhanced contrast occurs can be adjusted by changing the lipid composition of the liposomal membrane [27], but the non-linear relationship between contrast enhancement and temperature precludes quantitative thermometry. Moreover, these liposomal TRCA only provide an irreversible 'off–on' signal transition when the T_{melt} is reached [27], and are unable to indicate further temperature increase which may otherwise cause unwanted damage to normal tissue [27].

For effective and safe thermotherapy, detection of two temperature thresholds, *i.e.*, the lower and the upper bound, is critical. To this end, a novel thermally responsive hydrogel–lipid-hybrid nanoparticulate (HLN) system has been engineered in this work with a two-point contrast signal change. The high water solubility of the gadolinium-N,N,N′,N″,N″-diethylenetriaminepentaacetic acid (Gd-DTPA) complex would result in rapid release to the aqueous bulk, precluding stable loading of the chelated contrast agent into the hydrogel nanoparticles. In order to achieve stable loading of Gd in hydrogel nanoparticles, a novel cross-linker with metal chelation functionality was synthesized, allowing for the covalent incorporation of a metal chelator into the constituent copolymer of the hydrogel nanoparticle. The novel cross-linker allowed for the synthesis of ultrasmall, thermally-responsive, stable Gd-chelating hydrogel nanoparticles. These Gd-loaded hydrogels were then loaded into larger solid lipid nanoparticles to form the HLN. Upon heating, the melting of the lipid component of the HLN and release of the Gd^{3+}-loaded hydrogels into the surrounding bulk fluid resulted in an 'off-on' contrast enhancement transition. With further heating beyond the volume phase transition temperature (T_{tr}) of the hydrogel nanoparticles, an 'on–off' diminution of contrast signal enhancement occurred. Since both the hydrogels and the solid lipid nanoparticles possess customizable T_{tr} and T_{melt}, respectively, a temperature window can be tailored to the specific thermal regimen necessary for individual patient thermotherapy.

2. Experimental

2.1. Chemicals and reagents

DTPA, allylamine, eicosanoic acid, xylenol orange, gadolinium (III) chloride, acrylamide (AM), poly(ethylene glycol)-100-stearate (Myrj59), poly(ethylene glycol)-40-stearate (Myrj 52), cupric acetate, potassium persulfate, sodium dodecyl sulfate (SDS), myristic acid, ethanol (EtOH), methanol, isopropanol, acetonitrile, pyridine (Py), and acetic anhydride (AcAn) were purchased from Sigma-Aldrich Inc. (Oakville, Ontario, Canada) and used without further purification. N-isopropyl acrylamide (NIPAM) was purchased from Monomer-Polymer & Dajac Labs Inc. (Feasterville, PA, USA). Pluronic F68 was purchased from BASF (Mississauga, Ontario, Canada). Deuterated nuclear magnetic resonance (NMR) solvents, including deuterated dimethylsulfoxide ($DMSO\text{-}d_6$) and deuterium oxide (D_2O) were purchased from Cambridge Isotopes Laboratories, Inc. (Andover, MA, USA).

2.2. Synthesis and characterization of N,N′-bisallylamidodiethylenetriamine-N,N′,N″-triacetic acid (BADTTA)

Using a similar method as previously described [31–33], DTPA was dehydrated to form DTPA bisanhydride by reaction with pyridine and acetic anhydride in acetonitrile at 60 °C for 12 h (Fig. 1A). After the recovery of DTPA bisanhydride and the verification of its purity with a standard ^{1}H-NMR pulse sequence using a Varian Mercury 300 MHz instrument (Agilent Technologies Canada, Inc., Mississauga, ON, Canada) (data not shown), DTPA bisanhydride was mixed with acetonitrile and isopropanol. Following the addition of allylamine in two-fold molar excess to DTPA bisanhydride, the mixture was stirred overnight at 45 °C. BADTTA was isolated and the structure was confirmed by standard proton (^{1}H), carbon (^{13}C), and ^{1}H-^{1}H gradient correlations spectroscopy (GCOSy) NMR pulse sequences on a Varian Mercury 300 MHz instrument. BADTTA and DTPA were titrated in 0.1 mM potassium chloride with 0.1 M potassium hydroxide. Acid dissociation constant (pK_a) values were calculated from the generated curves using CurTiPot© Software (Gutz, I., University of São Paolo, São Palo, Brazil).

2.3. Synthesis and characterization of hydrogel nanoparticles

Hydrogel nanoparticles of poly(NIPAM–co-AM) with well-characterized thermoresponsive properties and biocompatibility were synthesized by emulsion polymerization in aqueous surfactant solution, with modification of methods previously published [34, 35]. NIPAM and AM monomer were added to each reaction to a total of 0.05 mol. SDS (0.24 g) and BADTTA (0.31 g) were added, followed by 400 mL of distilled, deionized water heated to 60 °C and bubbled with nitrogen gas. After 30 min, polymerization was initiated with potassium persulfate and allowed to continue for 60 min at 60 °C. Then the suspension of

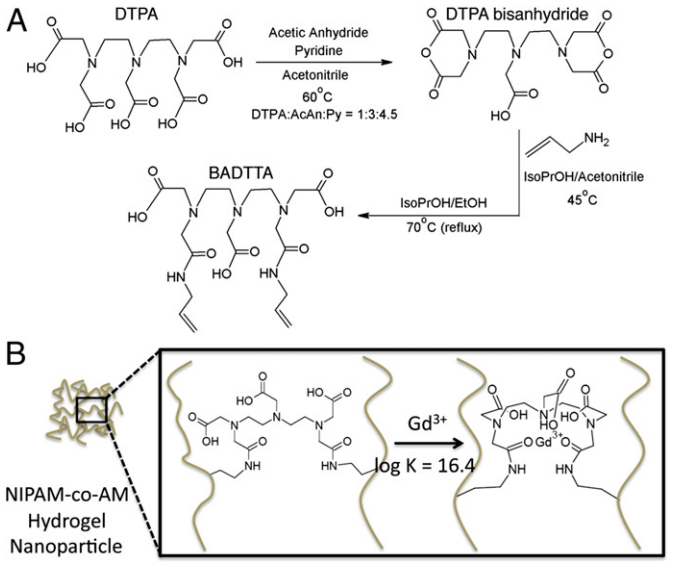

Fig. 1. Synthesis of the novel chelator-cross linker BADTTA. (A) The novel chelator-cross linker BADTTA was synthesized through the dehydration of DTPA, and subsequent reaction with allylamine. (B) A schematic diagram of the cross-linking of copolymer chains (brown) by BADTTA, and the putative structure of Gd^{3+} bound to the chelator. The dissociation constant of $\log K_D = 16.4$ is given.