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Protocols

Gadolinium alginate nanogels for theranostic applications

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ABSTRACT

Synthesis of theranostic nanoparticles, which combine both therapeutic and diagnostic capabilities in one platform can be considered as a step forward personalized medicine, since it allows tracing the delivery of the drug to targeted organ. Thus, the aim of this work was to prepare gadolinium alginate gel nanoparticles (gadolinum nanogels – GdNG) by the reverse microemulsions and physical crosslinking method as the vehicles able to carry hydrophilic drugs and to be traced by the Magnetic Resonance Imaging (MRI). The average size of synthesized nanoparticles was about 110 nm and the batch concentration was 10^{10} particles/ml. The morphology of nanogeles was visualized by Cryo-Scanning Electron Microscopy. Surface of nanogels particles was modified by the Layer-by-Layer (LbL) technique using natural polyelectrolytes. The cytotoxicity of non-modified and LbL modified nanogels was evaluated by the cellular viability quantification and cell death assessments using MTT and LDH biochemical tests, respectively. We encapsulated the model compound – fluorescent dye (Rhodamine b) in nanogels networks and proved the possibility of GdNG visualization by MRI.

1. Introduction

Theranostics is a promising field that combines therapeutic and diagnostic capabilities into a single multifunctional platform [1]. This concept is highly relevant to agents that target molecular biomarkers of disease and is expected to contribute to personalized medicine. The majority of the imaging and therapeutic compounds are based on gadolinium complexes, iron oxide nanoparticles, gold nanoparticles or quantum dots combined with anticancer drug [2–7]. The main drawbacks of these kind of small-sized molecules are their not sufficiently long blood circulating times, their cytotoxicity, poor biodistribution, etc. For that reason, it is extremely important, to involve nanotechnology tools, and develop new strategies for simultaneous diagnosis and treatment systems. The most promising approach is based on incorporation of imaging species (e.g. gadolinium ions, iron oxide nanoparticles) into polymeric micro or nanocarriers loaded with therapeutic agents. The most relevant micro/nanocarriers include: dendrimers, vesicles, core-shell structures, hydrogels and carbon nanotubes, which can be functionalized with a targeting moieties, therapeutic, and contrast agents [8–12]. For example, Grange et al. utilized neural cell adhesion molecule-binding peptide-coated liposomes (NCAM) for delivery of doxorubicin and a lipophilic gadolinium (Gd) derivative. Combining NCAM with Gd, allowed the concomitant MRI visualization of the drug delivery in the tumor region [13]. Another approach was proposed by Szczepanowicz et al. [14,15], where magnetically responsive, drug loaded nanocapsules (average diameter 100 nm) were synthesized based on a nanoemulsion core encapsulation by polyelectrolyte (PE) multilayer adsorption. Liu et al. developed multifunctional pH-sensitive polymeric nanoparticle system for simultaneous tumor magnetic resonance imaging (MRI) and therapy, based on gadolinium ions and anti-hepatocellular carcinoma drug Sorafenib [16]. Among many reports, polysaccharide-based gels nanoparticles, appear to be very attractive due to their natural origin, great biocompatibility and biodegradability [17–19]. Na et al. synthesized Gd(III)-encapsulated glycol chitosan nanoparticles (Gd(III)-CNPs) for tumor targeted T1-weighted magnetic resonance (MR) imaging [20]. Similar approach was proposed by Zhang et. al. They formed chitosan nanoparticles (CSNP c.a.150 nm) by ionic gelation method with sodium tripolyphosphate. Gadolinium (III) ions were conjugated to the surface of CSNPs through diethylenetriamine pentaacetic acid (DTPA) and carboiimide crosslinker chemistry [21].

In our previous work [22], we presented the method for calcium alginate and zinc pectin gel nanoparticles synthesis, using reverse

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oil in water microemulsion method, that was a modification of the technique proposed earlier by You et al. [23]. In this paper, we explored the possibility of synthesis of gadolinium alginate (GdNG) nanogels, their characterization and surface modification for theranostic applications.

2. Experimental

2.1. Material

Alginic acid sodium salt (117 kDa), oil soluble surfactant docusate sodium salt AOT ≥99%, sodium chloride, gadolinium chloride (III) hexahydrate, xylenol orange tetrasodium salt, were purchased from Sigma Aldrich, Poland. Additionally, Chitosan – PROTASAN UP CL 114 (50–150 kDa, <90% deacetylation) was obtained from Novamatrix, Norway. Rhodamine B, toluene, acetic acid, calcium chloride, were from Avantor Performance Materials, Poland. For the cytotoxicity tests we used: Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) from Life Technologies Poland. The Cytotoxicity Detection Kit was from Roche Diagnostic, Poland. MTT 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide, dimethyl sulfoxide (DMSO), Triton X-100, and penicillin/streptomycin mixture were from Sigma Aldrich, Poland. All materials were used without further purification. The distilled water used in all experiments was obtained with the three-stage Millipore Direct-Q 5UV purification system.

2.2. Synthesis of gadolinium alginate nanogels loaded with fluorescent dye

Gadolinium alginate nanogels were prepared using reverse microemulsion, water in oil method [22]. Briefly, anionic surfactant, docusate sodium salt (AOT) was dissolved in oil phase (toluene) at concentration 1.70% w/v. Next fixed volume of water phase (sodium alginate 0,5% w/v or gadolinium (III) chloride hexahydrate 3,5% w/v) was added under gentle mixing (300 rpm) to oil phases containing surfactant, to form stable microemulsion systems. The composition of microemulsions (ME) in mass percentage were as follows: for sodium alginate (SA) ME:97,83(TOL):1,87(AOT):0,30(SA), for gadolinium (III) ions ME:98,02(TOL):1,87(AOT):0,11(Gd^{3+}). Finally, two microemulsions (with alginate and gadolinium (III) ions) were mixed together and gelation process occurred inside microdroplets (24 h). Then, nanogels stabilized by AOT were extracted from toluene to 0.015 M NaCl. For this purpose 40 ml of sodium chloride solution (0.015 M), was added directly to nanogels suspension in toluene. For removing toxic solvent we used Rotary Vacuum Evaporator or dialysis. GdNG nanogels were stored in 0.015 M NaCl (pH 7) in room temperature.

Analog procedure was performed for synthesis of calcium alginate nanogels (CaNG), which were used as a control sample in MRI measurements [22]. For the encapsulation of fluorescent dye, we followed method based on super-absorption properties of hydrogels networks [24]. Basically, we encapsulated fluorescent dye (Rhodamine B, RodB) in GdNG, by simply mixing of RodB with freshly prepared suspension of nanogels, in ratio GdNG:RodB: 3:1. Electrostatic interactions between Rhodamine B and gadolinium alginate nanogels, provided additional stabilization of substance in hydrogel network. The mixture were incubated for 24 h in the dark, followed by centrifugation to remove free unabsorbed fluorescent dye. Detection of encapsulated RODB in GdNG was performed by spectrofluorimetric measurements with the excitation wavelength 520 nm, on Spectrofluorimeter Fluorolog-3 from Jobin Yvon Inc.

2.3. Modification of GdNG by layer by layer (LbL) technique

GdNG were further modified by sequential adsorption of polyelectrolytes. The saturation method of the LbL technique was used to form a polyelectrolyte multilayer shell according to the procedure described in [25–27]. The pair of natural polymers was used for modification: chitosan (CHI) as the polycation (Zeta Potential +45 mV) and alginate (ALG) as the polyanion (Zeta Potential −35 mV). Fixed volume of negatively charged nanogels suspension was added to the different volumes of polycation solution under mechanical stirring, to form stable first layer. The amount of polyelectrolyte was chosen empirically by analyzing the results of the simultaneous zeta potential measurements. It was optimal when the zeta potential of formed capsules reached the constant value just after overcharging, similar to zeta potential of free polyelectrolyte solution. The process was then repeated to obtain desired number of layers.

2.4. Size distribution, concentration and zeta potential measurements of GdNG

The size distribution and the zeta potential of the GdNG were determined by the Dynamic Light Scattering (DLS) and Laser Doppler Electrophoresis (LDA) methods, respectively, both with the Zetasizer Nano Series from Malvern Instruments. The obtained size values were an average of at least three runs with 10 measurements. For the zeta potential measurements, an average was calculated from the three subsequent runs with 20 measurements. Moreover, size distribution and concentration of GdNG were determined by NTA (Nanoparticle Tracking Analysis) using NanoSight NS500. All measurements were performed at 25 °C in 0.015 M NaCl.

2.5. Stability studies

To evaluate the colloidal stability of GdNG, their freshly prepared suspension were stored in 0.015 M NaCl for 60 days. Size distribution and zeta potential of formed nanogels were measured just after preparation and after appropriate storage time as described above.

2.6. Nanogels visualization

Cryo-SEM imaging was used for visualization of gadolinium alginate nanogels. Briefly, droplet of GdNG suspension was put on the cold holder, which was immediately immersed in liquid nitrogen slush using Quorum PPT2000 cryo-preparation stage (Polaron, Quorum Technologies, United Kingdom). The holder with the frozen sample was raised and cryo-transferred at the temperature of liquid nitrogen vapors to the chamber of the cryo-unit where the sample was subjected to sublimation at −70 °C for 15–30 min. Then the sample was sputter coated with platinum (5 nm thickness) and the specimen was transferred to the cooled stage of Jeol JSM 7600F field emission scanning electron microscope FESEM (Jeol Ltd., Tokyo, Japan).

2.7. Determination of free gadolinum (III) ions in suspension of GdNG

1.2.1.8 We determined concentration of free gadolinium ions in suspensions of GdNG, according to the spectrophotometric quantitation method [28], based on the differences in the UV–vis spectra of free and complexed Xylenol Orange dye. The dye (3 mg) was dissolved in acetate buffer (250 ml, pH 5.8). Then we added 0.2 ml of each of the previously prepared Gd (III) chloride solutions (0 μM–100 μM), directly into the cuvette containing 2 ml of