Soft Lithography-Based Hydrogel Particles and the Effect of Shape and Size on Biodistribution


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ABSTRACT

Poor solubility and rapid elimination can make small-molecule therapeutics difficult to deliver. In addition, many old therapies are in need of repackaging. In order to overcome these obstacles we have employed a top-down particle fabrication technique called Particle Replication In Non-wetting Templates (PRINT®) to fabricate unique biocompatible particles with distinct therapeutic properties based on their composition, size, shape and cargo. Particles were tagged with a variety of imaging beacons making them effective as contrast agents for magnetic resonance imaging and fluorescence imaging. After tail vein injection balb-c mice were imaged by magnetic resonance imaging and near infrared fluorescence imaging.

Keywords: PRINT, biodistribution, contrast agent, MRI, nanoparticle.

1 INTRODUCTION

Many state of the art small molecule therapeutics are difficult to deliver because of poor solubility, difficulty in tissue and cellular transport, and rapid in vivo elimination by the reticuloendothelial system (RES). In addition, many current therapies have aging patents are in need of repackaging. As a result, researchers are turning to alternative methods to deliver therapeutics. Herein, a top-down particle fabrication technique called Particle Replication In Non-wetting Templates (PRINT®) is being used to fabricate unique biocompatible particles that have distinct therapeutic and materials properties based on their composition, size and shape. This technique is a promising approach that addresses many of the current issues associated with therapeutic delivery. A large part of determining the efficacy of a new delivery approach lies in the ability to visualize the biodistribution and pharmacokinetics of the agent. Along these lines, the PRINT particles can be easily tagged with a variety of beacons making the particles effective as Magnetic Resonance (MR), Positron Emission Tomography (PET), and fluorescence contrast agents. Additionally, radioisotopes common in radiotherapeutics such as Ac-225 and Y-86 can be bound to the surface of the particle, using a DOTA-derivative, making them effective warheads in a variety of cancer therapies. We are using this technique to design particles which are capable of targeting or detargeting specific organs and tissues based on their size and shape.

2 EXPERIMENTAL

2.1 Materials

Unless otherwise noted, all of the reagents were purchased from Sigma Aldrich and used as received without further purification. All water was metal-free (18 MΩ, Milli-Q, Burlington, Mass). The bifunctional ligand, S-2(4-isothiocyanatobenzyl)-1,4,7,10-tetraazocyclododecane-tetraacetic acid (B-205, Macrocycles, Dallas, TX) was used as received. Dylight750-NHS (Fisher Scientific) was used as received.

2.2 Particle Fabrication

Particles were fabricated using a similar approach to that of the Particle Replication In Non-wetting Template (PRINT®) process described elsewhere. Briefly, a monomer solution was prepared with the following general composition: 50-90 wt% PEG-diacrylate, 10-30 wt% aminoethyl methacrylate, and 0.1 wt% 2-hydroxy-4’-(2-hydroxy)-2-methylpropionophenone (Irgacure 2959). The monomer composition was dissolved in water and then...
diluted further with filtered (0.2 µm PTFE Millipore syringe filter) acetone. The first generation (GA) of particles incorporated contrast agent into the monomer solution prior to particle fabrication (see Figure 1). Omniscan™ (GA-1) and iron oxide nanocrystals (GA-2) were added directly to these solutions prior to polymerization.

2.3 Particle Characterization

The particle surface was conjugated with PEG5000-NHS ester (Fluka). For particles that did not contain a MR contrast agent as cargo (Generation B, Figure 1), the bifunctional DOTA ligand was bound to the surface. A typical surface modification involved the competitive reaction of PEG5000-NHS and DOTA-SCN (1:10 mol:mol) in a dilute particle solution (0.3 mg particle/mL) in 3 M ammonium bicarbonate buffer stirred at room temperature for 2 hours. After rinsing twice with metal-free water, the particles were re-suspended in ammonium acetate buffer (pH 5.5) and stirred for 12 hr at 80 °C in one equivalent of GdCl3·6H2O. The particles were then rinsed twice and re-suspended in borate buffer (pH 8.5). Next the remaining primary amines on the particle surface were quenched with Dylight750-NHS. After rinsing an additional 5 times in metal-free water (pH 7.5 adjusted with NaOH), the particles were stored at 4 °C in a dark environment.

2.4 Animal Imaging Studies

The particles were administered to female BALB/c (age 5-7 weeks) by bolus tail vein catheter injection (100 µL of 3.32 mg particles/mL PBS). Coronal scans (GA particles) of the mice were taken in a longitudinal study using a 9.4T Bruker BioSpec MR instrument. Regions of interest in the kidney, liver, and a major blood vessel were monitored over the course of the study. Scans were taken prior to injection, immediately post-injection and every 5 minutes post-injection for 1 hour then at 3, 5, and 24 hrs. The relative intensities of each ROI were compared as a function of time.

Using 200x200 nm Generation B (GB) particles, a pilot study was recently run in which the particles (200 µL of 9 mg particle/mL) were injected by tail vein catheter. The animal was scanned prior to injection and post-injection. Scans were repeated every five minutes over the course of 2.5 hrs. Afterwards, the animal was removed from the Bruker BioSpec and placed immediately in a Xenogen Imaging instrument for fluorescence imaging. After imaging, the animal was sacrificed and the brain, heart, lung, liver, kidney, spleen, muscle, and blood were harvested for future analysis by ICP-MS.

3 RESULTS AND DISCUSSION

3.1 Passively Encapsulated Contrast Agents in Shape and Size Specific PRINT® Particles (GA)

Particles measuring 200x200 nm and 2x2 µm containing either 10 wt% Omniscan™ or 10 wt% iron oxide nanocrystals were successfully fabricated using the PRINT® technique. Scanning Electron Micrographs (SEMs) of T1 contrast agents containing Omniscan™ are depicted in Figure 2. In a phantom study, the particles exhibited significant contrast enhancement both as T1 and T2 contrast agents. We chose to focus on the Omniscan™ containing particles because of their positive contrast enhancement.

Figure 3 a-b shows a typical pre- and post-injection scan of a mouse with 200x200 nm particles and the corresponding intensity profile. When 200 nm particles were administered, an increase in signal intensity was observed initially for the liver, kidney and blood vessel. The signal intensity increased for 50 minutes and then returned to pre-injection intensity levels over the next three hours. Administration of the 2 µm particles led to a more rapid increase and decline in signal intensity in the liver and blood vessel when compared to the 200 nm particles. Only a small increase was observed in the kidney. It is our belief that the larger particles were rapidly eliminated from circulation by the RES (including the Kupffer cells in the liver) and trapped in the lungs leading to the rapid rise and fall. Unfortunately, because of an inconsistent Omniscan™ release profile, quantifying biodistribution with these particles was not possible. A new particle was designed in
which multiple beacons were covalently bound to the particles eliminating the need to calculate a release profile of the contrast agent from the particle. Passively Encapsulated Contrast Agents in Shape and Size Specific PRINT Particles (GA).

3.2 Shape and Size Specific Particles With Covalently Bound Contrast Agents (GB)

The development of the next generation of particles (GB) is currently under way. GB particles were surface functionalized with covalently bound Gd-DOTA and Dylight750 to prevent leaching of the contrast agents and enable comparisons between imaging modalities. With hopes of increasing the particle circulation time in the blood, the critical diameter of the particle was also reduced in GB particles. Three particle sizes are currently being examined: 80x90 nm, 80x360 nm, 80x2000 nm, and 200x200 nm (see Figure 4). The 200x200 nm particles have a 10,000/1 Gd atom to particle ratio as determined by ICP-MS and appear light blue in color. The 80x360 nm and 80x5000 nm particles are in the process of being characterized. Eight minutes after injection the particles had accumulated in the lungs, liver, spleen, and partially in the kidneys as observed by in vivo fluorescence imaging. After 180 minutes, the particles were barely visible in the lungs or kidney but still had strong signal in the liver and spleen. This was most likely a result of the particles being taken up by the RES. A large change in contrast by MRI was observed in the liver and gall bladder immediately post-injection (see Figure 5) which subsided after about 45 minutes.

We are currently in the process of preparing the 80x90 nm, 80x360 nm and 80x2000 nm particles for animal studies. Once these particles are ready, we will begin analyzing the organ and tissue samples by ICP-MS. This will give us a quantitative understanding of the distribution of the particles in vivo as a function of particle size.

4 CONCLUSIONS

MRI and in vivo fluorescence imaging compliment each other nicely. MRI is capable of obtaining high-resolution soft tissue images but requires large concentrations of contrast agent in order to see significant change in contrast. In vivo fluorescence imaging, on the other hand, has low resolution of soft tissue but is very sensitive even at low concentrations to the presence of contrast agent. Because of this, the design of GB has led to an ideal particle that harnesses the strengths of both imaging modalities. As a result, we can now fabricate particles of any size and shape using the PRINT® technique and carefully monitor their biodistribution. In the near future we plan to load the GB particles with Y-86. This approach promises to lead to an even more quantitative understanding of the distribution of these particles.

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REFERENCES