Selectively Dissolvable Manganese/Alginate Microcapsules For Novel Drug Delivery Strategies and Positive Contrast Imaging at 3T

P. Hota[1,2], B. P. Barnett[1,2], Y. Har-el[1], P. Walczak[1,2], D. Qian[1], H. B. Na[3], J. H. Lee[4], K. An[3], T. Hyeon[3], G. Sgouros[1], J. W. Bulte[1,2], P. Gailloud[1], and A. Arepally[1]

[1]Radiology, The Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, United States, [3]National Creative Research Initiative Center for Oxide Nanocrystalline Materials, Seoul National University, Korea, Republic of, [4]Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Korea, Republic of

Introduction: Here we describe the preparation and characterization of a novel strategy for delivery of therapeutic agents from liposomes-in-alginate microcapsules (LIAMs) that can be selectively dissolved *in vivo*. LIAMs were prepared by first entrapping a therapeutic factor within liposomes. These liposomes were then encapsulated within alginate hydrogel microcapsules. The release profile from LIAMs was assessed for small MW fluorescent molecule calcein and the lipophillic sclerosing agent, sodium morrhuate encapsulated within liposomes. Furthermore, the release profile of these agents from LIAMs was examined after the addition of EmboClear, a mixture of alginate lyase and EDTA that rapidly dissolves alginate biomaterials[1]. To enable MR tracking, thereby providing a method of assessing targeted delivery, LIAMs were labeled with manganese oxide (MnO) nanoparticles (incorporated in the alginate matrix) that provide a positive contrast on T_1-weighted MR images.

Methods: To create MR-trackable LIAMs, liposomes were first prepared by standard methods. Briefly, mixtures of phosphatidylcholine (PtdCho): cholesterol (1:1 molar ratio) and fluorescently-labeled lipids (<1-mol percentage of total lipid) in $CHCl_3$ were dried in a rotary evaporator. The lipids were resuspended in phosphate-buffered saline (PBS) containing either calcein or sodium morrhuate and then annealed at 55°C for 2 hr[2]. This lipid suspension was then taken through 21 cycles of extrusion (Liposo-Fast; Avestin) through two stacked polycarbonate filters with a 100-nm filter pore diameter. Unencapsulated calcein or sodium morrhuate was removed by size exclusion chromatography (Sephadex G-50). Next, uniform-sized MnO nanoparticles were synthesized by the thermal decomposition of Mn-oleate complex[3] and were made water soluble and biocompatible by methods previously described[4]. Liposomes and MnO nanoparticles were then suspended in 2% w/v Protanal HD alginate and extruded through an electrostatic droplet generator. The resulting microcapsules were collected in a 100mM $CaCl_2$ to enable gelation. Release profiles from LIAMs were assessed by fluorometric or spectrophometric detection of respectively calcein or sodium morrhuate in the supernatant of LIAM suspensions. MnO-labeled LIAMs were imaged in a gelatin phantom with a clinical carotid coil on a Phillips Achieva 3T using an FFE sequence with TR/TE 25/3.2. Following transplantation of MnO-labeled LIAMs into the peritoneal cavity of a mouse, images were obtained on a Bruker 9.4 T with MSME (Multi Spin Multi Echo).

Results: Assays conducted *in vitro* indicate that LIAMs provide a sustained release of calcein over a period of 25 days in the absence of EmboClear (Fig. 1a). With addition of EmboClear, calcein is rapidly released (Fig. 1b) and LIAMs are safely dissolved into non-toxic liquid by-products that can freely pass through the microvasculature and are rapidly cleared from the body[1]. A similar rapid release of sodium morrhuate was demonstrated with addition of EmboClear (Fig. 1c). Microscopic analysis of fully formed LIAMs (Fig. 1d) further confirmed that with the addition of EmboClear, capsules rapidly dissolve (≈ 30 sec.) into liquid components. Imaging of MnO LIAMs in a gelatin phantom on a clinical 3T scanner demonstrated the ability to image individual 200 μm capsules on a clinical grade scanner (Fig. 2a). Furthermore, a qualitative increase in signal was demonstrated from larger aggregates of capsules at 3T (Fig. 2b). Imaging in the peritoneal cavity of a mouse demonstrated the ability to clearly distinguish capsules from the underlying organs with single capsule resolution *in vivo* at 9.4T.

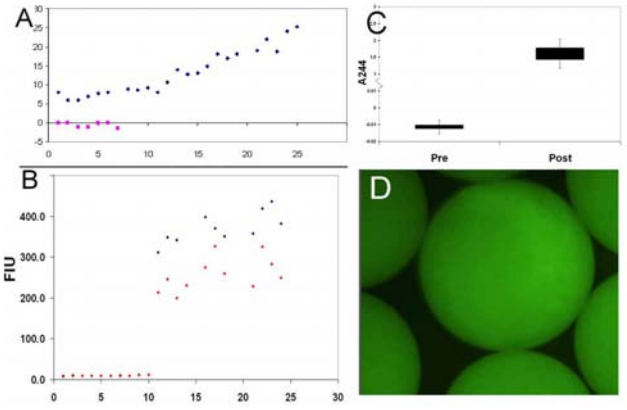

Figure 1: Drug release profile from LIAMs with and without EmboClear. (A) Release profile of calcein from LIAMs. (B) Release profile of calcein from LIAMs with addition of EmboClear at day 10. (C) Release of sodium morrhuate from LIAMs with addition of EmboClear. (D) Microscopic image of 200 μm LIAMs containing calcein loaded liposomes.

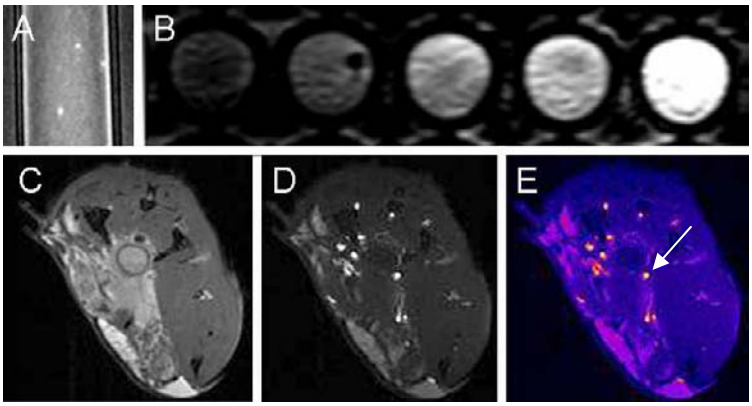

Figure 2: ^{1}H MRI of MnO LIAMs in (A) gelatin phantom revealing detection of single capsules on a clinical 3T scanner. (B) Image of 0, 10, 25, 50, and 100 MangCaps in multiwell plate at 3T. Image of MnO LIAMs in peritoneal cavity of mouse at 9.4T; (C) T_1 axial (TR/TE: 2010/20), (D) T_2 axial (TR/TE: 163/6.7) , (E) T_2 axial with color map (LIAMs in orange). Note the ability to detect single capsules.

Discussion:

MnO-labeled LIAMs could prove ideal for MR-guided targeted delivery of a broad range of therapeutic agents such as sodium morrhuate or other sclerosing agents, chemotherapeutic agents, radioisotopes, angio-active agents, and vectors for gene therapy. This delivery method is particularly attractive for intravascular delivery strategies as microcapsules can be used as embolic agents to create a reversible stasis thereby allowing a high payload of therapeutic agent to be delivered to a relatively well targeted area. Initial imaging studies appear promising, with the ability to detect single 200μm capsules on a clinical grade 3T scanner. Further studies exploring the use of MnO capsules for MR-targeted delivery of therapeutic agents and encapsulated cellular therapeutics is warranted.

References: 1. B.P. Barnett & P. Gailloud. 6th Asian & Oceanian Congress of Neuroradiology and Head & Neck Radiology. Singapore, 2006. **2.** Castile JD et al. Int J Pharm 188, 87 (1999). **3.** J. Park, et al., Nat. Mater. 2004, 3, 891. **4.** Dubertret, B. et al. Science 298, 1759-1762 (2002).