Liposome-loaded Microspheres as A Magnetic Susceptibility Agent for pH Sensing

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Introduction

pH sensing has a pivotal role in diagnosis, e.g. tumors or inflammation sites have a lower pH as compared to pH 7.4 of normal tissues. This acidic environment, e.g. 6.5 or lower, has been used for an effective delivery of therapeutic agents, especially using liposomes[1]. Sensitive probes or systems that can detect this change *in vivo* are necessary for disease diagnosis. Liposomes act as R2 probes due to the enclosed paramagnetic materials[2,3], and were reported to have good pH dependence[4]. However, the low sensitivity limits further *in vivo* applications. Here, we have designed liposome-loaded microspheres that has an improved sensitivity in detecting pH changes at 6.8-7.2 by means of R2* and R2 at 7T as a potential susceptibility contrast agent for pH sensing *in vivo*.

Methods

Materials: Microspheres were prepared by double emulsion process using poly(D,L-lactide-*co*-glycolic acid) 50:50 (PLGA; Sigma, St Louis, MO) and rhodamine labeled pH-sensitive liposomes with Dotarem^{TM} core were prepared using thin-film hydration as described previously[5,6]. Liposomes were added to microspheres in 5:1 (low liposome-loaded microsphere, LMS) and 10:1 (high liposome-loaded microsphere, HMS) w:w ratio, respectively, and vortexed for 5 min and then centrifuged. The solutions were place at 4°C before setting into 1% agarose gel for R2 and R2* measurements. R2 and R2* values of LMS and HMS were compared with Liposome1 and Liposome2, which were samples that contained the same amount of liposomes as LMS and HMS, respectively.

In vitro MRI: R2 and R2* (apparent transverse relaxation rate) measurements were performed on a 7T Bruker MRI scanner with a 60 mm RF Tx/Rx quadrature resonator. R2* map was acquired using multi-echo GE sequence with TR/TE = 1000/10 ms, FA = 30°, FOV= 5.12×5.12, slice thickness = 1 mm, acquisition matrix = 128×128, NEX = 2. Similarly, R2 map was acquired using multi-echo RARE sequence with identical parameters, except that TR/TE = 1500/50 ms, FA = 180°, NEX = 1. R2* and R2 values were computed by the echo images versus echo time from GE and RARE sequences respectively on a pixel-by-pixel basis.

Results

Fig. 1 shows the fluorescence images of (a) HMS and (b) LMS, with their corresponding bright field images. A more intense fluorescence was observed in HMS with a larger amount of liposomes. Fig. 1 (c) shows the SEM image of the microspheres with porous surface and particle size of ~ 10 µm. The change in R2 and R2* at pH 6.8-7.2 was 15% and 27% respectively, indicating a better pH-dependence in R2*. Fig. 2 (a) shows the R2* changes of both LMS and HMS at pH 6.8, 7.0 and 7.2 respectively, which had stronger pH dependence as compared to liposome1 and liposome2. We observed a 4-fold increase in the slope of liposome-loaded microspheres. Fig. 3 shows the T2*-weighted images of HMS at (a) pH 7.2 and (b) pH 6.8. The percentage change of R2* at pH 6.8-7.2 was ~30%.

Discussion and Conclusion

Microspheres were able to localize different amount of liposomes. Its porous surface provides a high surface to volume ratio for liposome deposition. Since R2 depends on the compartmentalized contrast agents[2], that is the core not the surroundings of liposomes. R1 change is limited at the physiological pH of interest (6.8-7.2), especially at high field and *in vivo*, owing to circulation of body fluids[4]. Thus, R2 and R2* are a better parameter of choice for assessing the fluidity of liposomes under different pH environments. At low pH, the fluidity of liposomes increases and more contrast agents will be released. Thus a decrease in the transverse relaxation was observed when pH drops. This liposome-loaded microsphere had a R2/R1 = 8.5, which indicated that it was a good T2-agent[3]. We found that the liposome-loaded microspheres resulted in a larger percentage change in R2* than that in R2, and their decrease in R2* at low pH is more pronounce than that of liposomes. The slope of HMS increased ~ 70% as compared with Liposome2. This increase in the slope supported our hypothesis that the lost of paramagnetic complexes led to a significant change in R2* at different pH by localization of liposomes, hence stronger pH dependence. This preliminary *in vitro* data showed an improved pH sensitivity, and microspheres with high liposome loading are favourable for R2* contrast enhancement. Liposomes that enclose a large amount of paramagnetic contrast agents increase R2* enhancement. The amount of contrast agent could be reduced during the process of phantom preparation. To increase the percentage change in R2* further, experiments are underway to increase the amount of contrast agent in liposomes and to prepare more porous microspheres for liposome loading.

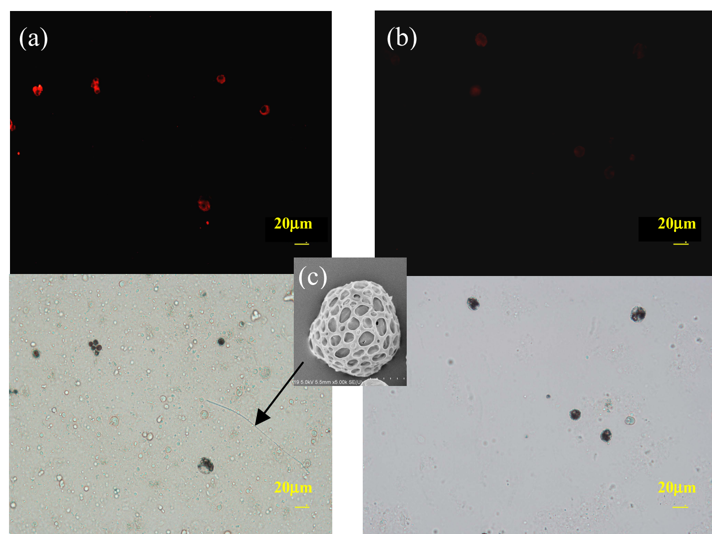

Fig. 1. Fluorescence images show (a) HMS, (b) LMS, and their bright field image, respectively; (c) TEM image of a microsphere (particle size ~ 10µm).

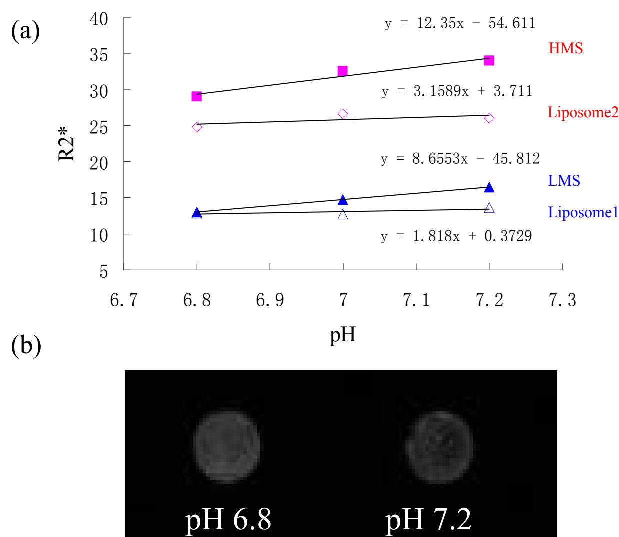

Fig. 2. (a) R2* of HMS and LMS as compared with their respective amount of liposomes, at pH 6.8-7.2. (b) T2*-weighted images of liposome-loaded microspheres at TE = 60 ms.

References [**1**] Hong MS et al. J Pharm Pharm 2002;54:51-58. [**2**] Terreno E et al. Chem Biodivers 2008;5:1901-1912. [**3**] Martina MS et al. J Am Chem Soc 2005; 127: 10676-10685. [**4**] Lokling KE et al. Int J Pharm 2004;274:75-83. [**5**] El-Sherif DM et al. J Biomed Mater Res 2003;66A:347-355. [**6**] Lokling KE et al. Magn Reson Med 2004;51:688-696.