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Simultaneous quantification of tumor uptake for targeted and non-targeted liposomes and their encapsulated contents by ICP-MS

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Abstract

Liposomes are intensively being developed for biomedical applications including drug and gene delivery. However, targeted liposomal delivery in cancer treatment is a very complicated multi-step process. Unfavorable liposome biodistribution upon intravenous administration and membrane destabilization in blood circulation could result in only a very small fraction of cargo reaching the tumors. It would therefore be desirable to develop new quantitative strategies to track liposomal delivery systems to improve the therapeutic index and decrease systemic toxicity. Here, we developed a simple and non-radiative method to quantify the tumor uptake of targeted and non-targeted control liposomes as well as their encapsulated contents simultaneously. Specifically, four different chelated lanthanide metals were encapsulated or surface-conjugated onto tumor-targeted and non-targeted liposomes, respectively. The two liposome formulations were then injected into tumor-bearing mice simultaneously and their tumor delivery was determined quantitatively via inductively coupled plasma-mass spectroscopy (ICP-MS), allowing for direct comparisons. Tumor uptake of the liposomes themselves and their encapsulated contents were consistent with targeted and non-targeted liposome formulations that were injected individually.

INTRODUCTION

To date, numerous nanoparticles have been developed as platforms for clinical applications, including drug and gene delivery.^{1–4} Among these many nanoparticulate systems, hollow nanometer-sized vesicles are particularly attractive due to the increased functionality imparted by their amphiphilic structure.^{5–10} So far, a number of liposome-based therapeutics are already in use in the clinic; however, no targeted liposomal system has yet been approved by the US Food and Drug Administration (FDA), reflecting in part the complexity of designing the optimal liposomal carrier for the delivery of therapeutics. This partly stems from the difficulty of acquiring a complete understanding of the pharmacokinetics of liposomes themselves, as well as that of their encapsulated contents.¹¹

Several analytical techniques have already been used for characterization of liposomes following their systemic administration. For example, liposomes are commonly labeled with near-infrared (NIR) fluorochromes and their *in vivo* behavior, including biodistribution, release, and targeting have been studied using fluorescence imaging.^{12–14} However, this method only provides qualitative estimates of biodistribution and thus cannot be assumed to provide sufficiently accurate information on liposome behavior *in vivo*. Further, the disparate tissue penetration and scattering properties of fluorescent dyes with different

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optical properties and the finite width of the optical window for NIR imaging, limits the number of compounds that can be imaged simultaneously, typically to one or two. Another technique that is commonly adopted for acquiring pharmacokinetic data involves the encapsulation and/or incorporation of radioisotopes within liposomes.^{15–19} Compared to fluorescent imaging, radiolabeling possesses several beneficial properties, including the ability to make quantitative and sensitive measurements. As a result of these characteristics, this method has become the “gold standard” for liposome in vivo characterization. However, one major drawback to the use of radioisotopes is that special training, handling, and equipment is required when working with radioactivity. This severely limits the adoption of this technique, particularly in biological labs, which are often not certified for the use of radioactive materials. An additional limitation with using radioisotopes is the inability to simultaneously monitor more than two or three radioisotopes simultaneously due to the poor energy resolution of radioactive material. As a result, it is not possible to perform paired statistics by following multiple liposome formulations and their cargo in single living subjects.

We have recently developed a non-radiative, multiplex approach to track multiple nanoparticle formulations simultaneously by combining lanthanide-doped nanoparticles with inductively coupled plasma mass spectroscopy (ICP-MS).²⁰ Specifically, lanthanide metals were incorporated into a wide range of nanoparticle platforms that were simultaneously injected into a single animal subject. ICP-MS was then used to detect parts-per-trillion (ppt) concentrations of the lanthanide metals, independent of one another, in tissue and blood. Since lanthanide and other heavy metals (e.g. gold, silver, etc.) do not naturally exist within animal subjects, this “ICP-MS multiplex” approach provides a sensitive and straightforward method for quantitatively comparing the biodistribution and blood clearance of numerous formulations simultaneously, without the disadvantages of radioactivity and subject-to-subject variability. It has recently been reported that at least 34 and as many as 100 transition metal isotopes can be uniquely and quantitatively identified by ICP-MS in a single sample.²¹

In this study, we explored whether ICP-MS could be used to simultaneously study the tumor uptake of targeted and non-targeted control liposomes, and their encapsulated contents, following a single sample injection into living subjects. The targeted liposomes were functionalized with folic acid to confer specificity for cancer cells. Each liposome had a unique lanthanide metal conjugated to its surface and a second lanthanide encapsulated within its lumen. Analogous to radiolabeled-liposomes, the surface-conjugated lanthanides were used to determine the tumor delivery of the liposomes themselves. Both the targeted and non-targeted liposome formulations were injected into tumor-bearing mice alone or simultaneously, and the tumor delivery of the liposomes themselves and their encapsulated contents were determined quantitatively via ICP-MS, allowing for very accurate and direct comparisons.

EXPERIMENTAL SECTION

Materials

Hydrogenated soy phosphatidylcholine (HSPC), cholesterol (CHOL), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG2000-Amine), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(Lissamine rhodamine B sulfonyl) (Rhod-PE) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-5000] (DSPE-PEG5000-Folate) were obtained from Avanti Polar Lipids (Alabaster, AL). 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono (N-hydroxysuccinimide ester) (DOTA-NHS-ester) and gadolinium (1,4,7,10-tetraazacyclododecane)-1,4,7,10-tetraacetate (DOTA-Gd) were obtained from Macrocylics

(Dallas, TX, USA). Europium chloride (EuCl_3), praseodymium chloride (PrCl_3) and cerium nitrate ($\text{Ce}(\text{NO}_3)_3$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ce-DOTA was synthesized in the lab. All other chemical were used as received. All buffer solutions were prepared with ultrapure grade water.

Liposome Preparation

A 55 mol% HSPC/40 mol% CHOL/2 mol% DSPE-PEG2000-Amine/3 mol% DSPE-PEG2000 mixture was prepared in chloroform in two glass vials. The total amount of HSPC in each glass vial was 20 mg. For the preparation of targeted-liposomes, DSPE-PEG5000-Folate (100 μL , 1 mg/mL in chloroform) was added to one of the mixtures. Rhod-PE (10 μL , 1 mg/mL in chloroform) was also added as a fluorescent indicator for fluorescence microscopy images. The solvent was removed using a direct stream of nitrogen prior to vacuum desiccation for a minimum of 4 h. Nanometer-sized liposomes were prepared using the film hydration technique. Briefly, an aqueous solution (1 mL) was added to the dried lipid film and incubated in a 50 °C water bath for 0.5 h and then sonicated for another 0.5 h at the same temperature. The aqueous solution added to the film containing folate was composed of 0.5 M Gd-DOTA in 0.1 M HEPES, pH 7.4, while the aqueous solution added to the dried lipid film that did not contain folate consisted of 0.5 M Ce-DOTA in 0.1 M HEPES, pH 7.4. Following sonication, samples were subjected to 10 freeze-thaw-vortex cycles in liquid nitrogen and warm H_2O (50 °C), followed by extrusion 21 times through two stacked 100 nm Nuclepore polycarbonate filters using a stainless steel extruder (Avanti Polar Lipids). Non-entrapped Gd-DOTA and Ce-DOTA were removed via size exclusion chromatography using Sepharose CL-4B (Sigma-Aldrich) and rehydration buffer (0.1 M HEPES, pH 7.4) as the eluent.

The two purified liposome samples were reacted with DOTA-NHS-ester at pH 9.0 sodium bicarbonate buffer for 2 h and then purified by centrifugal filter devices (Amicon Ultra-4, 10000 MWCO). For lanthanide surface conjugation, the purified Gd-liposomes were mixed with excess PrCl_3 and the purified Ce-liposomes were mixed with excess EuCl_3 , respectively. Both reactions were performed in 0.1 M citrate buffer (pH 6.0) for 24 hours. Non-conjugated lanthanide ions were removed using PD-10 columns and centrifugal filter devices (Amicon Ultra-4, 10000 MWCO). To ensure complete removal of unencapsulated and unreacted lanthanide, the lanthanide content in the eluent was checked after each centrifugation period until no lanthanide was detectable by ICP-MS.

Cell Culture

KB cells (human nasopharyngeal epidermoid carcinoma cells) were purchased from the America Type Tissue Collection (ATCC) and cultured in RPMI 1640 folic acid free media with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 .

Fluorescence Microscopy

KB cells seeded in 24-well plates were washed once with PBS and then incubated in RPMI (200 μL) containing liposomes for 4 hours at 37 °C. For competitive inhibition experiments, KB cells were also treated with folate-liposomes but in the presence of free folic acid (1 mM) in the RPMI media. The final lipid (HSPC) concentration in the cultured media was 1.0 μM for all imaging experiments. Prior to acquisition of fluorescence images, cells were washed with PBS three times. All microscopy images were acquired with an Olympus IX81 motorized inverted fluorescence microscope equipped with a back-illuminated EMCCD camera (Andor), an X-cite 120 excitation source (EXFO), and Sutter excitation and emission filter wheels. Images of the rhodamine were acquired using the filter set HQ545/30, HQ610/75, Q570LP (Chroma).

Quantitation of Tumor Delivery by ICP-MS

Approximately 6-week-old female Fox Chase SCID mice (Charles River Laboratory, Charles River, MS) were maintained in accordance with the Institutional Animal Care and Use Committee of the University of Pennsylvania. Mice were anesthetized via isoflurane and KB cells were injected subcutaneously into the back left flank (2×10^6 cells in 0.2 mL PBS). Tumors were grown to an approximate size of 100 mm³. The first group (n = 3) was treated with the targeted liposomes (100 μ M HSPC in 0.2 mL PBS). The second group (n = 3) was treated with non-targeted liposomes (100 μ M HSPC in 0.2 mL PBS). The third group (n = 3) was treated with a mixture of targeted liposomes (200 μ M HSPC in 0.1 mL PBS) and non-targeted liposomes (200 μ M HSPC in 0.1 mL PBS). In all cases, the sample in PBS was given to the mice via tail vein injection. The mice were anesthetized prior to sacrifice at 4 h. Tumors were harvested and analyzed by ICP-MS.

For each liposome formulation, the percent injected dose per gram of tumor (% ID/g), was calculated as $[\text{Ln}]_{\text{sample}}/([\text{Ln}]_{\text{inj}} * M_{\text{inj}})$ where $[\text{Ln}]_{\text{sample}}$ is the lanthanide concentration in the sample (tumor), $[\text{Ln}]_{\text{inj}}$ is the lanthanide concentration in the injected liposome solution, and M_{inj} is the mass of liposome solution injected (0.2 g).

Instrumentation

Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano from Malvern Instruments. The scattering angle was held constant at 90°. ICP-MS was performed at the New Bolton Center (University of Pennsylvania).

RESULTS AND DISCUSSION

Preparation and characterization of lanthanide-doped liposomes

To allow the tumor uptake of folate receptor-targeted and non-targeted liposomes and their encapsulated contents to be measured simultaneously via ICP-MS, it was first necessary to create two liposome formulations, each labeled with two unique lanthanides (Figure 1). The folate-targeting formulation, which had DSPE-PEG5K-folate doped within the vesicle bilayer, had the hydrophilic lanthanide-chelate Gd-DOTA encapsulated within its aqueous interior and Pr-DOTA covalently linked to the amine-terminated phospholipids, DSPE-PEG2K-NH₂, on the outer leaflet. The non-targeted liposome had Ce-DOTA encapsulated within its aqueous interior and Eu-DOTA linked to the outer leaflet. Notably, the chemistry used to link lanthanides to the outer leaflet of the liposome membrane was analogous to standard radiochemistry approaches, except the chelated metal was not radioactive in this case.

The ability to incorporate both encapsulated-lanthanides and surface-conjugated lanthanides into one liposome formulation was confirmed by ICP-MS. The molar ratio of encapsulated Gd- to -surface conjugated Pr was 85:15 for targeted-liposomes, while the molar ratio of encapsulated Ce-to-conjugated Eu was 71:29 for non-targeted liposomes. To ensure that the surface-conjugated lanthanides (Pr and Eu) were specifically bound to the liposome surface, analogous liposomes were also prepared in the absence of DSPE-PEG2K-NH₂. In contrast to the aminated liposomes, ICP-MS measurements revealed that these control liposomes did not possess any detectable surface-bound lanthanides (Pr or Eu), confirming that the chelated lanthanides are not simply absorbed to the liposome surface. These results also provided reassurance that the purification strategies that were adopted were effective in removing free lanthanides from the liposome samples.

Dynamic light scattering (DLS) revealed that the folate receptor-targeted-liposomes had a mean diameter of 103 nm, while control-liposomes had a mean diameter of 100 nm (Figure

2A). To evaluate the ability to store lanthanide doped-liposomes, the hydrodynamic diameter of the liposomes was measured by DLS for one week following suspension in PBS buffer (0.1 mM phosphate, pH 7.4). It was found that both targeted liposomes and non-targeted liposomes were stable over this time frame and did not exhibit any significant change in size or distribution (Figure 2B). To confirm that there were no non-specific interactions between the targeted and non-targeted liposomes, both formulations were mixed for 24 hours and their size measured by DLS (Figure 2A, mixture). The two liposomes acted as independent entities and there was no tendency to fuse or aggregate. It should be noted that identical lipid membrane compositions were used to prepare both formulations, except for the small percent of folate-labeled phospholipids that were incorporated into the targeted-liposomes.

To examine the stability of the liposomes in serum, the targeted and non-targeted liposomes were exposed to 100% serum for 4 h at 37°C. It was found that less than 1% of each lanthanide metal was released from the liposomes, suggesting that the encapsulated lanthanides were not leaked from the lumen and the surface bound lanthanides were not cleaved or transmetalated. This study also further confirmed the high purity of the samples, i.e. there was little to no unbound lanthanides in the mixture.

In vitro binding of targeted lanthanide-doped liposomes

To confirm the targeting capabilities of the lanthanide-doped liposomes functionalized with folic acid, KB cells were incubated with the targeted liposomes for 4 hours and subsequently analyzed by fluorescence microscopy. KB cells were selected as a model cell line since they are known to overexpress the folate receptor.²² Notably, the liposomes had a small percentage of Rhod-PE incorporated into their membrane to serve as a fluorescent tracer. Consistent with cell labeling, all of the KB cells exhibited a bright fluorescent signal (Figure 3). In contrast, little to no fluorescence was observed when KB cells were incubated with control liposomes that did not contain folic acid. To verify that binding of the liposomes was mediated through the folate receptor, competitive inhibition studies were performed by incubating the folate receptor-targeted liposomes with KB cells in the presence of excess free folic acid. Under these conditions, fluorescence was significantly reduced, indicative of receptor-specific binding.

In vivo uptake of targeted lanthanide-doped liposomes

To examine whether tumor uptake of folate receptor-targeted and non-targeted liposomes and their encapsulated contents could be evaluated simultaneously via ICP-MS, a mixture containing both liposomal formulations was administered intravenously into KB tumor-bearing mice. The animals were sacrificed 4 h after sample injection and their tumors were harvested for ICP-MS analysis. A terminal point of only 4 h was selected since it has been widely reported that liposomes can become unstable in circulation.²³ Further, it was expected that an earlier terminal point would better highlight the faster tumor accumulation of targeted liposomes, compared with non-targeted liposomes, since there is less time for enhanced permeability and retention (EPR). In fact, previous studies have shown that non-targeted liposomes can have comparable accumulation within tumors at later time points (e.g. 48 h) since they benefit from longer circulation times and higher EPR compared with folic acid-functionalized liposomes.^{24, 25}

Measurements revealed that at 4 h post-injection the targeted liposomes did exhibit significantly more tumor accumulation compared with the control liposomes, which was reflected by measurements of both the liposome-encapsulated lanthanides and surface-conjugated lanthanides (Figure 4A). Specifically, 0.66% injected Gd/gram tumor was observed for folate-targeted liposomes, while only 0.30% injected Ce/gram tumor was

observed for non-targeted liposomes. Therefore, the presence of targeting ligands on the liposome surface led to a ~2.2-fold improvement in tumor uptake of liposome-encapsulated contents compared with non-targeted liposomes. Much less of a difference was observed when comparing the surface-conjugated lanthanides. Specifically, 0.53% injected Pr/gram tumor was observed for the targeted-liposomes, while 0.43% injected Eu/gram tumor was observed for non-targeted liposomes. Although the improvement mediated by targeting was small when looking at surface bound lanthanides, it was statistically significant ($p < 0.05$).

To provide further evidence that we measured the true tumor accumulation of both the targeted and non-targeted liposomes and their content and that there was no interaction between the two formulations, targeted and non-targeted liposome formulations were also injected into tumor-bearing mice individually. In agreement with results from the single mixed-sample injection, ICP-MS analysis revealed that the targeted liposomes exhibited a statistically significant improvement in tumor uptake compared with non-targeted liposomes, as reflected by both the encapsulated and surface-conjugated lanthanides (Figure 4B). Further, similar trends as the mixed-sample were also observed between surface bound and encapsulated lanthanides for both the targeted and non-targeted liposomes.

Importantly, it should be noted that although ICP-MS can be used to quantitatively track both encapsulated and surface-conjugated lanthanides with high sensitivity, this technique cannot resolve whether or not the encapsulated contents have been released from the liposome within tumor cells. Therefore, liposomes that are capable of transporting larger encapsulated payloads to the tumor site may not always exhibit a better therapeutic effect. Further, similar to radiolabeling, the ICP-MS technique described here does require modification of the liposome surface. While only a small molar percent of phospholipids were labeled with chelated lanthanides (2%), this could be enough to influence the liposome's pharmacokinetics.

CONCLUSIONS

As a proof of concept, we have demonstrated that lanthanide-doped liposomes combined with ICP-MS analysis can be used to evaluate the tumor uptake of liposomes and their encapsulated contents with a single sample injection in living subjects. In the presence of tumor targeting ligands on the liposome surface, liposomes themselves and their encapsulated contents both showed a significant improvement in tumor uptake compared with non-targeted liposomes. In future work, it is expected that the combined approach of ICP-MS and lanthanide-doped liposomes could be a powerful research tool for the high-throughput investigation of a wide range of liposome formulations with diverse physicochemical properties, compositions, and targeting moieties, which are essential for the development of an ideal targeted liposome-based drug carrier.

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References

1. Cho NH, Cheong TC, Min JH, Wu JH, Lee SJ, Kim D, Yang JS, Kim S, Kim YK, Seong SY. *Nat Nanotechnol.* 2011; 6:675–682. [PubMed: 21909083]
2. Lei J, Wang L, Zhang J. *ACS Nano.* 2011; 5:3447–3455. [PubMed: 21446756]
3. Lou X, Schumacher T, Yang H, Ding A. *J Control Release.* 2011; 152:e65–e67. [PubMed: 22195931]

4. Wu W, Shen J, Gai Z, Hong K, Banerjee P, Zhou S. *Biomaterials*. 2011; 32:9876–9887. [PubMed: 21944827]
5. Cheng ZL, Chen AK, Lee HY, Tsourkas A. *Small*. 2010; 6:1398–1401. [PubMed: 20564486]
6. Chonn A, Cullis PR. *Adv Drug Deliv Rev*. 1998; 30:73–83. [PubMed: 10837603]
7. Ewert KK, Evans HM, Zidovska A, Boussein NF, Ahmad A, Safinya CR. *J Am Chem Soc*. 2006; 128:3998–4006. [PubMed: 16551108]
8. Lasic DD. *Nature*. 1996; 380:561–562. [PubMed: 8606781]
9. Lian T, Ho RJY. *J Pharm Sci*. 2001; 90:667–680. [PubMed: 11357170]
10. Wang HJ, Zhao PQ, Su WY, Wang S, Liao ZY, Niu RF, Chang J. *Biomaterials*. 2010; 31:8741–8748. [PubMed: 20727587]
11. Zhang Y, Cheng CM, Cusick B, LeDuc PR. *Langmuir*. 2007; 23:8129–8134. [PubMed: 17585787]
12. Lowery A, Onishko H, Hallahan DE, Han ZZ. *J Control Release*. 2011; 150:117–124. [PubMed: 21075152]
13. Ghoroghchian PP, Frail PR, Susumu K, Blessington D, Brannan AK, Bates FS, Chance B, Hammer DA, Therien MJ. *Proc Natl Acad Sci U S A*. 2005; 102:2922–2927. [PubMed: 15708979]
14. Li S, Goins B, Zhang L, Bao A. *Bioconjug Chem*. 2012; 23:1322–1332.
15. Chang YJ, Chang CH, Chang TJ, Yu CY, Chen LC, Jan ML, Luo TY, Lee TW, Ting G. *Anticancer Res*. 2007; 27:2217–2225. [PubMed: 17695506]
16. Helbok A, Rangger C, von Guggenberg E, Saba-Lepek M, Radolf T, Thurner G, Andreae F, Prassl R, Decristoforo C. *Nanomedicine*. 2012; 8:112–118. [PubMed: 21645641]
17. Rudolph AS, Klipper RW, Goins B, Phillips WT. *Proc Natl Acad Sci U S A*. 1991; 88:10976–10980. [PubMed: 1961766]
18. Soares DC, de Oliveira MC, de Barros AL, Cardoso VN, Ramaldes GA. *Eur J Pharm Sci*. 2011; 43:290–296. [PubMed: 21605669]
19. Soundararajan A, Bao A, Phillips WT, Perez R 3rd, Goins BA. *Nucl Med Biol*. 2009; 36:515–524. [PubMed: 19520292]
20. Crayton SH, Elias DR, Al Zaki A, Cheng Z, Tsourkas A. *Biomaterials*. 2012; 33:1509–1519. [PubMed: 22100983]
21. Bendall SC, Simonds EF, Qiu P, Amir EAD, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, Balderas RS, Plevritis SK, Sachs K, Pe'er D, Tanner SD, Nolan GP. *Science*. 2011; 332:687–696. [PubMed: 21551058]
22. Lee RJ, Low PS. *J Biol Chem*. 1994; 269:3198–3204. [PubMed: 8106354]
23. Gabizon A, Chisin R, Amselem S, Druckmann S, Cohen R, Goren D, Fromer I, Peretz T, Sulkes A, Barenholz Y. *Br J Cancer*. 1991; 64:1125–1132. [PubMed: 1764376]
24. McNeeley KM, Annapragada A, Bellamkonda RV. *Nanotechnology*. 2007; 18:1–11.
25. Gabizon A, Horowitz AT, Goren D, Tzemach D, Shmeeda H, Zalipsky S. *Clin Cancer Res*. 2003; 9:6551–6559. [PubMed: 14695160]

Figure 1.

Schematic of the ICP-MS based multiplex method for determining tumor uptake of liposomes and their encapsulated contents. Gd-DOTA was encapsulated into the aqueous interior of targeted-liposomes and phospholipids on the outer leaflet were labeled with Pr-DOTA. Similarly, Ce-DOTA was encapsulated into the aqueous interior of non targeted-liposomes and the outer surface was labeled with Eu-DOTA. Folate receptor targeting was achieved by doping the Gd/Pr vesicles with low percentage of DSPE-PEG5000-Folic acid. These two liposome formulations were injected to tumor-bearing mice individually or simultaneously.

Figure 2. Size measurements of liposomes. (A) Number-weighted size distributions of targeted liposomes, non-targeted liposomes and a mixture of the two formulations as measured by dynamic light scattering. Mixtures of targeted and non-targeted liposomes were incubated for 24 h prior to measuring size. (B) Mean hydrodynamic diameter of targeted and non-targeted liposomes at various times following their suspension in PBS buffer.

Figure 3.

Cellular binding of fluorescently labeled liposomes. (A&B) untreated KB cells; (C&D) KB cells following 4 h incubation with folate-targeted liposomes. (E&F) KB cells following 4 h incubation with folate-targeted liposomes in the presence of 1 mM free folic acid. (G&H) KB cells following 4 h incubation with non-targeted liposomes. All liposomes contained encapsulated and surface-conjugated lanthanides. All liposomes were incubated with KB cells for 43h before fluorescence images were acquired. The two rows show phase contrast (top) and fluorescence (bottom) images, respectively.

Figure 4.

Quantitative analysis of tumor uptake. The targeted liposomes and non-targeted liposomes were injected to tumor-bearing mice (A) simultaneously or (B) individually. Asterisk indicates statistical significance ($p < 0.05$) in tumor uptake between targeted liposomes and non-targeted liposomes.