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Sensitivity improvement of Cerenkov luminescence endoscope with terbium doped Gd_2O_2S nanoparticles

Xin Cao,[1] Xueli Chen,[1,a)] Fei Kang,[2] Xu Cao,[1] Yonghua Zhan,[1] Jing Wang,[2] Kaichun Wu,[3] and Jimin Liang[1,a)]
[1]*Engineering Research Center of Molecular and Neuro Imaging of the Ministry of Education and School of Life Science and Technology, Xidian University, Xi'an, Shaanxi 710071, China*
[2]*Department of Nuclear Medicine, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China*
[3]*Department of Digestive Diseases, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China*

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Our previous study showed a great attenuation for the Cerenkov luminescence endoscope (CLE), resulting in relatively low detection sensitivity of radiotracers. Here, a kind of radioluminescence nanoparticles (RLNPs), terbium doped Gd_2O_2S was mixed with the radionuclide ^{68}Ga to enhance the intensity of emitted luminescence, which finally improved the detection sensitivity of the CLE by using the radioluminescence imaging technique. With the *in vitro* and *in vivo* pseudotumor experiments, we showed that the use of RLNPs mixed with the radionuclide ^{68}Ga enabled superior sensitivity compared with the radionuclide ^{68}Ga only, with 50-fold improvement on detection sensitivity, which guaranteed meeting the demands of the clinical diagnosis of gastrointestinal tract tumors. © *2015 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4921858]

Cerenkov luminescence imaging (CLI) is an emerging molecular imaging modality that perfectly bridges optical and nuclear imaging using an optical method to screen the distributions of radiotracers.[1–3] Compared with the traditional nuclear imaging methods, such as the positron emission tomography (PET) and single photon emission computed tomography (SPECT), CLI has the advantages of low cost, high throughput, and relatively short imaging time. In contrast to traditional optical imaging, there are plenty of radiotracers widely available in clinics as probes.[3,4] However, weak intensity and poor penetration of CLI limit its applications in clinical translation.[3–6] To extend the applications in gastrointestinal (GI) tract imaging, a Cerenkov luminescence endoscope (CLE) was developed,[7–9] which integrates traditional CLI technology with an optical fiber based endoscope by using an optical adaptor. Due to a small aperture of endoscope and transmission attenuation of optical fibers, there is great attenuation for the CLE system, resulting in relatively low detection sensitivity of radiotracers.[9]

There are three possible ways to improve the detection sensitivity of the CLE system. One is to develop a fiber endoscope with an enlarged aperture. However, considering the fact that the endoscope contains several channels to meet clinical needs, the size of the endoscope used in clinics is extremely limited. This means that even though the aperture is enlarged, the diameter of the fiber bundle in the endoscope is still very small. Thus, this scheme is not feasible. The second way is to enhance the intensity of detectable luminescence by shifting its peak wavelength from a blue to visible or infrared one,[2,10,11] as the visible or infrared light has better penetration ability in tissues. In the CLE system, the probe of the distal end of the endoscope is very close to imaging target so that the radiotracers can be considered as a surficial source. In this case, the enhancement of the penetration capacity is helpless for sensitivity improvement of the CLE system. Furthermore, the peak wavelength shifting obeys the law of conservation of energy, which implies that the total intensity of emitted luminescence is unchanged even lossy for the wavelength-shift strategy. Thus, it is still not suitable for improving the sensitivity of the CLE. The last way is to choose the radionuclides with high energy and photon production ability, such as ^{90}Y and ^{32}P,[12] which can emit much more photons per nuclear decay. However, the applications of this method are restricted by the adopted radionuclides.

Benefitting from the phenomenon that the lanthanides based radioluminescence nanoparticles (RLNPs) can emit luminescence under the irradiation of high energy rays,[10,13–15] radioluminescence imaging (RLI) emerges and has been applied to biomedical applications. As the γ-rays are steadily generated during the decay of radiotracers and the intensity of emission luminescence bombarded by γ-rays is much higher than that from the Cerenkov phenomenon, the RLI could act as highly sensitive CLI. In this scenario, the RLNPs are mixed with radiotracers to enhance the total intensity of emission luminescence.

In this letter, we investigated whether the sensitivity of the CLE system could be improved by using RLNPs. The RLNPs used in the following experiments were the terbium doped Gd_2O_2S (Gd_2O_2S:Tb) nanoparticles with a mean size of 200 nm, which was purchased from the Shanghai Keyan Phosphor Technology Co., Ltd., and synthesized using the complex precipitation method.[16] The RLNPs can emit the luminescence with peaks of 490, 544, 586, and 620 nm due to the $^5D_4$–$^7F_6$, $^5D_4$–$^7F_5$, $^5D_4$–$^7F_4$, and $^5D_4$–$^7F_3$ transitions under irradiation of the high energy rays, respectively.[13] The radiotracer and CLE system used here are the same as in our previous study.[9] The radionuclide ^{68}Ga is in the form of $^{68}GaCl_3$,

a)Authors to whom correspondence should be addressed. Electronic addresses: xlchen@xidian.edu.cn and jimleung@mail.xidian.edu.cn.

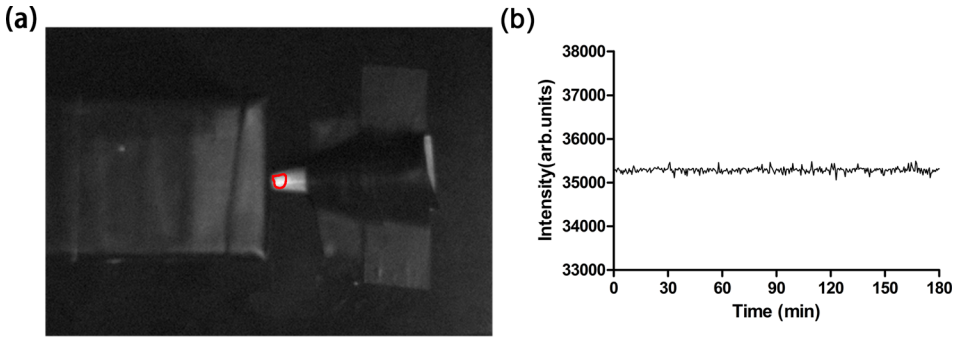

FIG. 1. Investigation of the radiostability of the RLNPs. (a) White-light image of two EP tubes, in which the EP tube containing ^{68}Ga was placed in a black box, and the location of the RLNPs was outlined with red circle and (b) average intensity of luminescence emitted from RLNPs as the time flies.

produced by a ^{68}Ge-^{68}Ga generator (ITG Isotope technologies Garching GmbH, Germany). The CLE system is a seamless combination of an electron multiplying CCD (EMCCD) camera (iXon3 888, Andor), an optical adaptor (Outai Corp), and a fiber endoscope (GIF-XQ40, Olympus), which is used to collect the emitted luminescence from the mixture of ^{68}Ga and Gd_2O_2S:Tb. In all of the experiments, the CLE system was placed in a black light-tight box to mimic a cavity environment of the GI tract, and the imaging object was placed about 5 mm away from the distal end of the endoscope, which is an appropriate distance in a common clinical diagnosis leading to a field of view of about $3 \times 3\,cm^2$.

First of all, the radiostability of the RLNPs was tested under the bombardment with high activity of radionuclide. An epoxy epoxide (EP) tube containing the ^{68}Ga solution with the activity about 1480 MBq (40 mCi) was placed in a black box, to eliminate the interruption of the Cerenkov radiation. The other EP tube that contains about 2 mg RLNPs was placed next to the former one. The two EP tubes were put into the field of view of the CLI system that is configured by an EMCCD camera coupled with a focus lens as well as a light-tight box, as the same one used in our previous study.[9] The white-light image was first taken with an exposure time of 1 s, as shown in Fig. 1(a), which intuitively presented the position of two EP tubes. Subsequently, the luminescent images were taken uninterruptedly for 180 min. Each image was acquired for 30 s and a binning value of 4. After reasonably extracting the region of interests (ROIs), the average intensity of radioluminescence emitted from RLNPs as a function of the passing time was plotted and is shown in Fig. 1(b). We find that the luminescence intensity emitted from RLNPs is almost unchanged, with the mean intensity of 35 and 285.32 and standard deviation of 57.79. It demonstrates that the RLNPs were stable enough for at least 3 h, which is adequate for the following studies. It should be noted that the activity of ^{68}Ga is still as large as about 236.8 MBq (6.4 mCi) at the end of this experiment, which is not a commonly used activity in the CLI studies. Results of this investigation illustrated the stability of the RLNPs under the bombardment with high activity of radionuclide.

Second, an *in vitro* experiment was conducted to evaluate the sensitivity improvement of CLE by using Gd_2O_2S:Tb. Samples containing ^{68}Ga and Gd_2O_2S:Tb were added into a single well of a black 96-well plate with a final volume of 300 μl as: 1.0 mg Gd_2O_2S:Tb and 37 KBq (1 μCi) ^{68}Ga mixed with the adequate amounts of normal saline (NS). After the white-light image was taken with an exposure time of 1 s, the luminescent images were acquired uninterruptedly for 360 min. Each luminescent image was acquired with an exposure time of 5 min and a binning value of 4. Fig. 2(a) shows the sequential images over the decay of the radiotracer. An apparent trend of intensity attenuation was observed with the decay of imaging time. For the quantitative analysis, the same ROIs were extracted

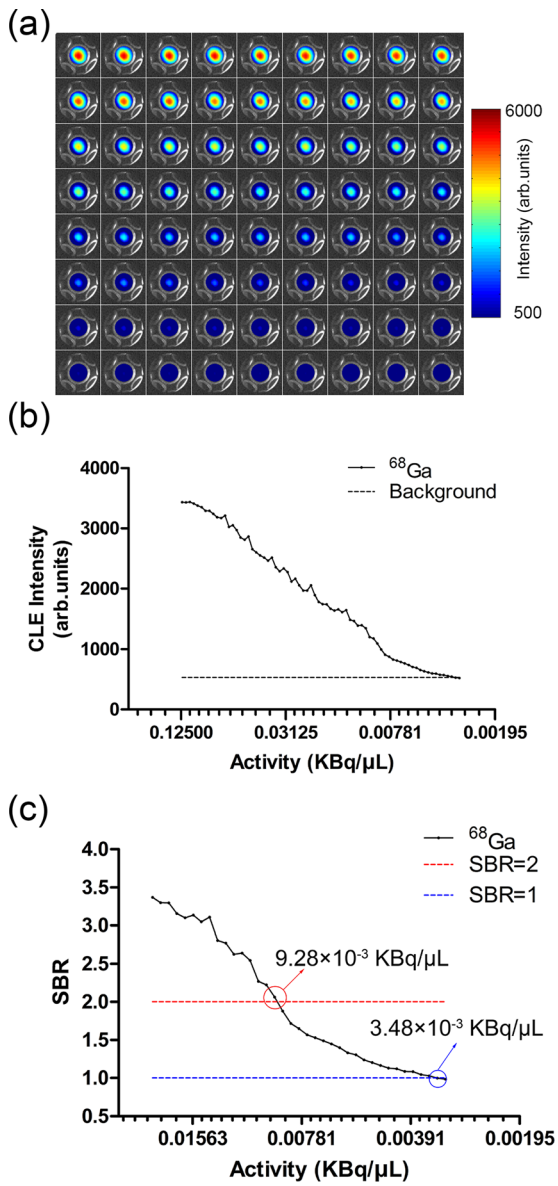

FIG. 2. Results of the *in vitro* experiment. (a) Sequential images over the decay of the radiotracer, (b) fitting relationship between the intensity of emitted luminescence acquired by the CLE and the activity of ^{68}Ga, and (c) ratio of signal to background (SBR) as a function of the activity of ^{68}Ga.

from luminescent images. The average intensity of ROIs was calculated and fitted with the activity of ^{68}Ga. The fitting relationship between the luminescence intensity and the activity of ^{68}Ga is depicted in Fig. 2(b), where the background image was acquired before all of the experiments. To precisely determine the detection sensitivity of the CLE, an evaluation factor of the ratio of signal to background (SBR) was introduced and defined by calculating the ratio of the average intensity of ROIs to that of the background. Fig. 2(c) presents the SBR as a function of the activity of ^{68}Ga, where the activity of ^{68}Ga was selected in the range lower than 0.020 KBq/μl (5.41×10^{-4} μCi/μl) to highlight the minimum activity that could be detected with the CLE system. From Fig. 2(c), we find that a minimum activity of approximating 3.48×10^{-3} KBq/μl (9.40×10^{-5} μCi/μl) could be identified from the background (SBR > 1).

In addition, to investigate the dose effect of Gd_2O_2S:Tb on the detection sensitivity of the CLE, the following tests were conducted. The experimental setting and procedures were the same as the second *in vitro* experiments except that the dose of the RLNPs was changed from 1 mg to 0.8, 0.6, 0.4, and 0.2 mg, respectively. Similarly, the detection sensitivity of the CLE determined by the value of SBR (SBR = 1 or SBR = 2) was calculated for the four doses of RLNPs and is presented in Fig. 3(a), where the red circle line gives the detection sensitivity determined by SBR = 1, and the blue square one shows that determined by SBR = 2. We find that the detection sensitivity of the CLE system remains unchanged as the decrease of the dose of RLNPs. Thus, a hypothesis can be established that the dose of RLNPs is excessive demand for the radionuclide with the activity of 3.48×10^{-3} KBq/μl (9.40×10^{-5} μCi/μl) even that the dose of the RLNPs is as low as 0.2 mg. For a fixed dose of RLNPs, there exists an exact activity of ^{68}Ga to fitly excite it. To validate this hypothesis, additional experiment was conducted. In this experiment, 3.7 MBq (100 μCi) ^{68}Ga and 0.5 mg RLNPs were mixed completely and added into one well of a 96-well black plate to form a final volume of 200 μl. The luminescent images were taken successively with the CLI system for 240 min (about 3.53 half-lives of radionuclide ^{68}Ga), with the exposure time of 30 s and acquisition interval of 1 min. Fig. 3(b) shows the variation of luminescent intensity emitted from the mixture of ^{68}Ga and RLNPs with the decay of ^{68}Ga. We obviously find that the declining trend of luminescent intensity has exacerbated at the demarcation point of 5.35 KBq/μl (0.14 μCi/μl), corresponding to the time period about 119 min (pointed with a red circle in Fig. 3(b)). The measurements are divided into two groups by the demarcation point. Results of this additional test indicate that there is a saturation radioactivity for radionuclide ^{68}Ga to fully excite 0.5 mg RLNPs, being about 5.35 KBq/μl (0.14 μCi/μl). As a result, the radionuclide ^{68}Ga with the activity of 3.48×10^{-3} KBq/μl (9.40×10^{-5} μCi/μl) cannot excite all of the RLNPs with the dose of 0.2 mg. To completely excite the RLNPs with the dose of 0.2 mg, the minimum activity of the radionuclide ^{68}Ga should be about 2.14 KBq/μl (5.78×10^{-2} μCi/μl) in theory.

Finally, the *in vivo* sensitivity of the CLE system was measured with a pseudotumor experiment. A nude mouse with a subcutaneous pseudotumor was used to mimic the superficial tumor environment. The pseudotumor mouse model was prepared according to the procedures described in our previous study.[9] Animal care and protocols were approved by the Fourth Military Medical University Animal Studies Committee. All animal operations were performed under general anesthesia by inhalation of 1%–2% isoflurane-oxygen. A mixture made of 40 μl Matrigel (BD Bioscience), 74 KBq (2 μCi) ^{68}Ga, and 1 mg Gd_2O_2S:Tb was obtained to form a final volume of 100 μl in a microfuge tube and was then subcutaneously injected in the right flank of the nude mouse. The mouse was kept warm for 3 min until the Matrigel solidified and then mounted on a warm platform. After that, the mouse was placed in the field of view of the CLE system to acquire white light as well as sequential luminescent images. The parameter settings and procedures for acquisition were the same as those in the *in vitro* experiment. Fig. 4(a) depicts a photograph of the mouse, where the area of the pseudotumor is outlined in red. The white light and luminescent images of the local pseudotumor area were fused together and one of them is shown in Fig. 4(b). To determine the minimum activity that can be detected with the CLE *in vivo*, the relationship between the luminescent intensity of the extracted ROIs and the activity of ^{68}Ga is fitted in Fig. 4(c), while Fig. 4(d) shows the SBR as a function of the activity of ^{68}Ga, where the activity of ^{68}Ga was selected in the range lower than 0.125 KBq/μl (3.38×10^{-3} μCi/μl). Similar to the *in vitro* experiment, we also calculated a minimal activity that could be distinguished from the background, with the value of approximating 2.43×10^{-2} KBq/μl (6.56×10^{-4} μCi/μl) under SBR > 1.

In our previous study,[9] the theoretical minimum activity of ^{68}Ga that can be detected by the CLE system is about 0.186 KBq/μl (5.033×10^{-3} μCi/μl) for the *in vitro* experiment and 1.218 KBq/μl (3.292×10^{-2} μCi/μl) for *in vivo*.

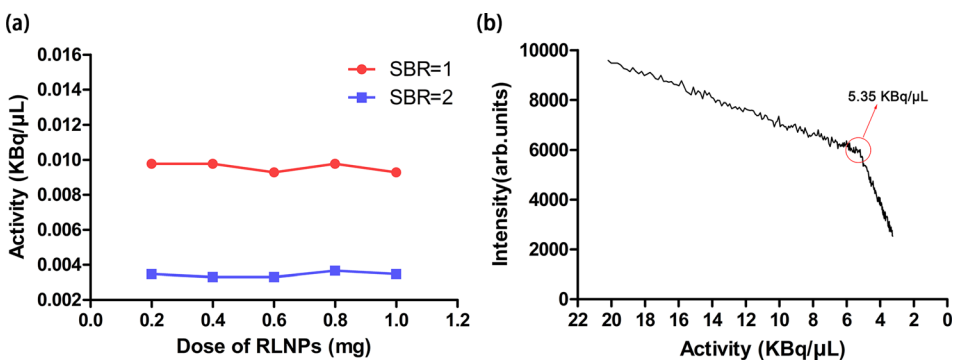

FIG. 3. Investigation of the dose effect on the detection sensitivity of the CLE. (a) Detection sensitivity for different doses of RLNPs, where the red circle line gives the detection sensitivity determined by SBR = 1, and the blue square one shows that determined by SBR = 2 and (b) variation of luminescence intensity emitted from mixture of ^{68}Ga and RLNPs with the decay of ^{68}Ga.

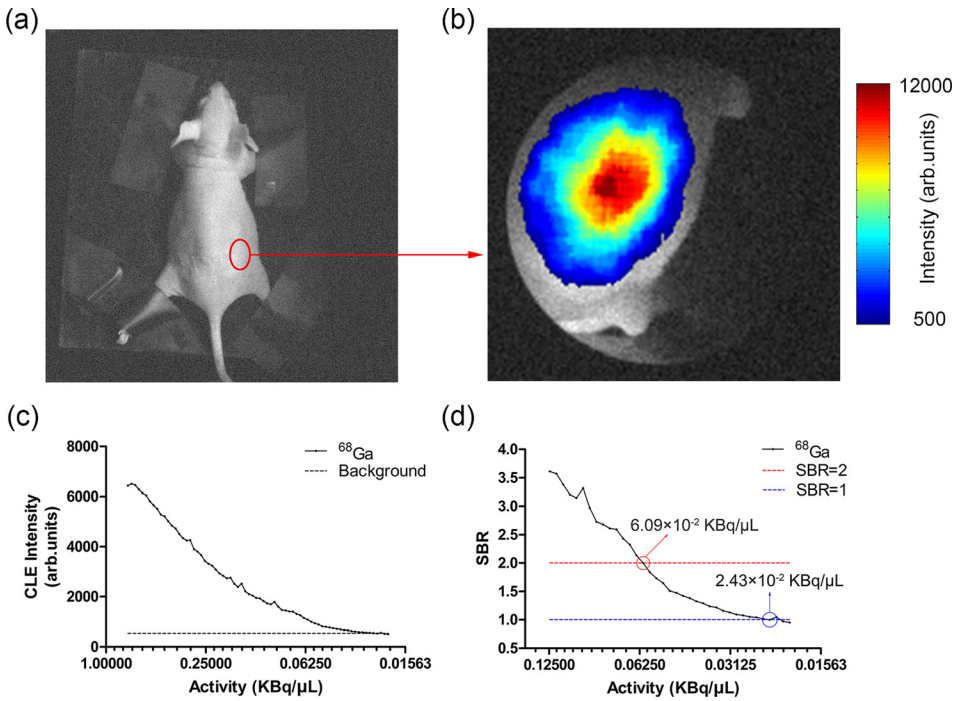

FIG. 4. Results of the *in vivo* experiment. (a) Photograph of nude mouse, (b) fusion image of white light and luminescence for the local pseudotumor area acquired by CLE, (c) fitting relationship between the intensity of emitted luminescence acquired by the CLE and the activity of ^{68}Ga, and (d) ratio of signal to background (SBR) as a function of the activity of ^{68}Ga.

Compared with using the radionuclide ^{68}Ga only, utilizing the RLNPs mixed with the radionuclide ^{68}Ga enabled much superior sensitivity with the improvement of 53.4-fold for *in vitro* and 50.1-fold for *in vivo,* respectively. The sensitivity can also be further improved by using the radiotracer of ^{90}Y, as 207-fold improvement was obtained compared with the radiotracer of ^{18}F.[12] Furthermore, considering the mean activity of the radiotracer that gathered around the GI tract tumors in a clinical diagnosis, with a value of 5.55×10^{-2} KBq/μl (1.5×10^{-3} μCi/μl),[17] our *in vivo* sensitivity can guarantee to meet the demands of clinical use. It should be noted that theoretical values of sensitivity mentioned above were determined under the value of SBR being larger than 1. Even with the value of SBR being larger than 2, which is regarded as a relatively good SBR for optical imaging, the minimum detectable activity for the *in vivo* pseudotumor experiment was about 6.09×10^{-2} KBq/μl (1.65×10^{-3} μCi/μl), which was still comparable to the clinical value.

In conclusion, an effective strategy was presented to improve the detection sensitivity of our developed CLE system by using a kind of RLNPs, terbium doped Gd_2O_2S. By mixing the radionuclide ^{68}Ga with Gd_2O_2S:Tb and utilizing radioluminescence imaging technique, the detection sensitivity of CLE was greatly improved, with a larger than 50-fold improvement. *In vivo* results demonstrated that using RLNPs enabled superior sensitivity which guaranteed meeting the demand of clinical diagnosis of GI tract tumors. Although cytotoxicity and delivery of the nanoparticles would hinder the wide applications of this strategy, which makes its clinical translation take a long time, the RLNPs would be more *in vivo* friendly by changing its size and shape as well as using the biological materials modification technology.[13] Future studies will concentrate on the development of the RLNPs based targeted probe with the acceptable biocompatibility and cytotoxicity and their biomedical applications.

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