Feasibility study of radioluminescence film imaging: an optical way to detect medical radionuclides

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Cerenkov luminescence imaging (CLI), a promising technique to image medical radionuclides, suffers extremely low intensity level and strong attenuation of tissues, making it difficult to apply in clinic. Inspired by radioluminescence phenomenon, we propose an optical way to detect medical radionuclides, named the radioluminescence film imaging (RLFI). With the help of lab-produced film made of radioluminescence microparticles, our RLFI technology enables detection of radionuclides in a living body at a depth much deeper than current CLI method, having great potential applications in preclinical and clinical studies. We first validated the feasibility of our RLFI technology by characterizing the concentration and depth dependency of RLFI signal, and then explored its potential by *in vivo* imaging of radionuclide metabolism in mice.

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1. Introduction

Cerenkov luminescence imaging (CLI) has attracted increased attentions recently for its capacity of imaging nuclear probes in an optical manner [1-3]. By detecting the Cerenkov luminescence (CL), a kind of visible and near-infrared light emitted from radionuclides, CLI has successfully set a bridge between the preclinically-used optical imaging and the clinically-used nuclear imaging [4]. However, CL is a secondary product from radionuclides during the decay process, making its intensity very weak. The acquisition of CL image is time-consuming and requires a light-tight environment, which is not convenient for clinical applications. Additionally, due to strong attenuation of CL in tissues, detecting the radionuclides deep in a living body using CLI is quite difficult and even impossible [5].

Enhancing the intensity level of the emitted CL is one of possible ways to overcome the limitations that CLI has. Several strategies have been reported to enhance the CL intensity, which can be divided to three categories. First, by coupling the radionuclides with some fluorescent dyes, the peak wavelength of the emitted CL can be shifted from a blue to visible or infrared fragments that should have a better tissue penetration ability [5, 6, 7]. Based on the law of conservation of energy, the luminescent intensity is still weak for it is dependent on the CL emitted from radionuclides, making the penetration of thick tissue remain challenge. Second, one can choose radionuclides that irradiate high-energy neutron, having the ability of high throughput of photons [8]. However, this method is hindered by the restricted radionuclides in clinic. Third strategy is to employ lanthanides based radioluminescence microparticles (RLMPs) that can emit radioluminescence (RL) under the irradiation of high-energy rays [9-12]. In this method, the γ-rays produced during the decay of radionuclides, is used as the excitation source to stimulate the RLMPs. The emitted luminescence contains both the CL and RL, so the luminescent intensity can be enhanced. However, the RLMPs is not *in vivo* friend to some degree, making it not suitable for *in vivo* applications.

Here, we experimentally demonstrate the radioluminescence film imaging (RLFI), an optical way to detect medical radionuclides to achieve a higher sensitivity than that of CLI. In the method, a lab-produced radioluminescence film (RLF) is placed on the surface of imaging object and bombarded by high-energy rays that is emitted from radionuclides gathered around a

tumorous target. The stimulated RL would generate a facula on the RLF, indicating the general orientation of tumorous target. It should be noted that this study is an extension of our previous work using the same material [11, 12]. During the preparation of this paper, other groups also did similar studies [13-16]. Compared with their studies, we first used a much more flexible RLF that can cling to mouse skin tightly and then systematacially evaluated the feasibility of such strategy in *in vivo* imaging applications. Based on the radioluminescence phenomenon, our RLFI approach enables detection of radionuclides deep inside a living body with the help of a lab-produced film made of RLMPs. We first validated the feasibility of our RLFI technology by characterizing the concentration and depth dependency of RLFI signal, and then explored its potential by *in vivo* imaging of radionuclide metabolism in mice.

2. Experiment

In this proof-of-concept study, the RLF is made by 2 g terbium doped Gd_2O_2S (Gd_2O_2S:Tb, one kind of RLMPs) evenly sprinkled onto a thin plaster film, forming an effective area of 3 × 3 cm^2 (Fig. 1(a)). The use of thin plaster film makes the RLF soft enough to cling to mouse skin, which indicates that the RLF is much more feasible than that used in previous work.

The Gd_2O_2S:Tb is purchased from Shanghai Keyan Phosphor Technology Co. Ltd and has characterized in our previous study [11]. The radionuclide used in the following experiments is [18F] that is in the form of 2-deoxy-2-[18F]fluoro-D-glucose ([18F]DG) and produced via a cyclotron (GE Industries Inc.) and FDG reagent kit (ABX).

A homebuilt optical imaging system is used to acquire the emitted RL. The system is comprised of a homebuilt light-tight chamber to avoid the interruption of ambient light, a collecting lens, and an electron multiplied charged coupled device (EMCCD) camera (Ixon 897, Andor). All of the configuration of the system is the same as our previous study [3].

3. Results and discussion

3.1. Feasibility of RLFI method

We first demonstrated the feasibility of RLFI method with a preliminary experiment, in which we investigated whether the RLF can be excited by γ-rays and whether the outline of radionuclide can be distinguished. 5.55 MBq (150 μCi) [18F]DG was mixed with normal saline (NS) served as excitation source, forming a total volume of 200 μL in an epoxy epoxide (EP) tube. The EP tube was covered by a piece of black tape to avoid the pollution from CL (Fig. 1(b)). A piece of RLF was then placed on top of the EP tube. The white-light and RLFI images were sequentially acquired with an exposure time of 1 and 30 s respectively. Figure 1(c) shows the fusion image. We found that the RLF can emit RL under the irradiation of γ-rays, and the outline of radionuclide can be easily observed from the RLFI image of RLF. This demonstrated the feasibility of our RLFI method. It should be noted that the color of the other side of RLF is black, to exclude the interference from CL.

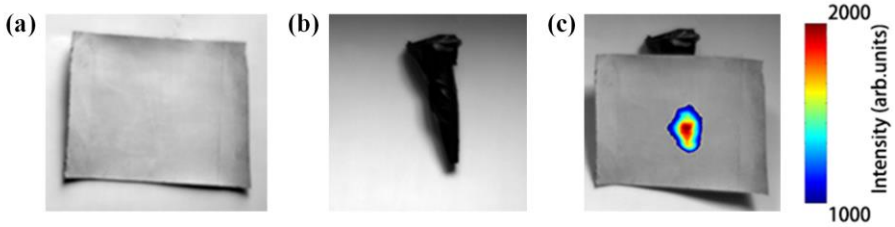

Fig. 1. Feasibility demonstration of the RLFI method. (a) Representative photograph of a RLF; (b) Photograph of an EP tube filled with [18F]DG solution. The covered black tape is used to block the emission of CL; (c) Fusion of the white light and RLFI images

3.2. Characterization of RLFI method

We then characterized the performance of the RLFI method in terms of concentration and depth dependence. To investigate the dependence of RLFI signal intensity on activity of radionuclides, we first prepared five black 96-well plates (300 μL per well), with one well of each plate filled with the mixture of NS and [18F]DG. The activities of [18F] DG are 0.74 MBq (20 μCi), 1.48 MBq (40 μCi), 2.22 MBq (60 μCi), 2.96 MBq (80 μCi) and 3.7 MBq (100 μCi) respectively. An additional plate with one well filled with saline was also prepared as the control group. The RLFs were then placed on the top of each plate and the RLFI images were taken. It should be noted that a black tape was placed between the RL film and the well plate to prevent emission of CL. Each experiment was repeated for 5 times. Fig. 2(a) shows one of the RLFI images of each investigated group. We find that the RLFI signal intensity increased linearly with radionuclide activity (R^2=0.9526), as shown in Fig. 2(b). The signal intensity is calculated as the average intensity in the region of interest (ROI). The

ROI is defined as the area where the intensity is larger than 40% of maximum value.

We then investigated the dependence of RLFI signal intensity on distance from radionuclides to RLF. In the experiment, 0.74 MBq (20 μCi) of [18F]DG solution was injected into one well of a black 96-well plate that was then covered by a RLF. By moving the RLF away from the plate with a step of 1 cm from 1 to 5 cm, the RLFI images were acquired at each step with an exposure time of 10 s. Each acquisition was repeated for 5 times. Figure 3 shows the RLFI signal intensity as a function of distance between the RLF and radionuclides. An obviously sharp decrease can be observed as the distance increases. We also found that the RLFI still works well even for a large distance of 5 cm, which proved a great potential of the RLFI method in detection of radionuclides deeply inside an imaging subject.

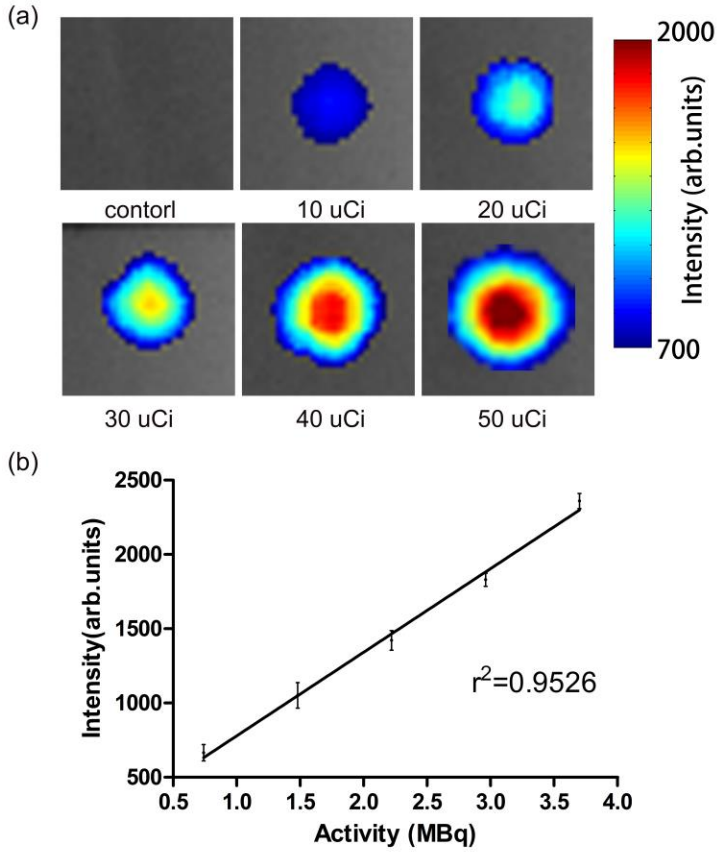

Fig. 2. Dependence of RLFI signal intensity on activity of radionuclides. (a) One of the RLFI images of each investigated group; (b) RLFI signal intensity as a function of radionuclide activity, showing a linear dependence (R^2=0.9526).

3.3. Application potential of RLFI method

Thirdly, to explore the application potential of the RLFI method, we performed *in vivo* RLFI on live adult mice (N=5). The Fourth Military Medical University Animal Studies Committee approved animal care and protocols. All animal procedures were conducted under general anesthesia by inhaling about 1%-2% isoflurane-oxygen mixture. The mice were first received 7.4 MBq (200 μCi) [18F]DG via caudal vein, and then kept warm for 30 min to ensure a relatively large doses of aggregation of [18F]DG in the bladder. Fig. 4(a) shows one of representative images acquired by micro-PET/CT (Mediso Ltd.), demonstrating the aggregation of [18F]DG in the bladder. Then, CLI and RLFI images were sequentially collected with the exposure time of 1, 5, 10, and 30 s respectively. For RLFI images acquisition, a piece of RLF was placed on top of mouse bladder. Figure 4(b) shows one of the representative CLI (left) and RLFI (right) images with the exposure time of 10 s. Quantitative analysis that describes the variations in CLI and RLFI signal intensity with exposure time was shown in Fig. 4(c). We found that the intensity of RLFI was obviously larger than that of CLI. Although the CL signals can be detected, it took a relatively long exposure time to collect a high-quality image. Worse still, such weak CL hardly penetrates

a thick barrier from a deep location due to the tissue attenuation. RLFI, however, would not be hampered by such a limitation because the high energy rays transmit through the tissues in RLFI.

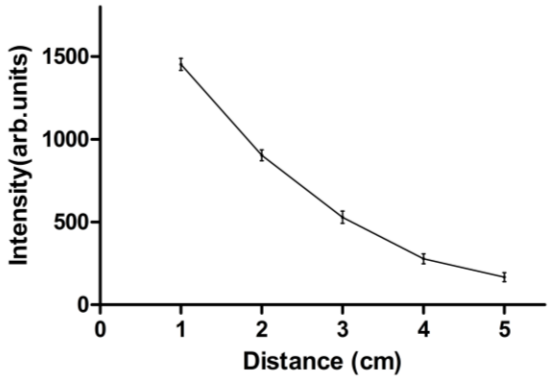

Fig. 3. Radioluminescence film imaging signal intensity as a function of distance between the radioluminescence film and radionuclides

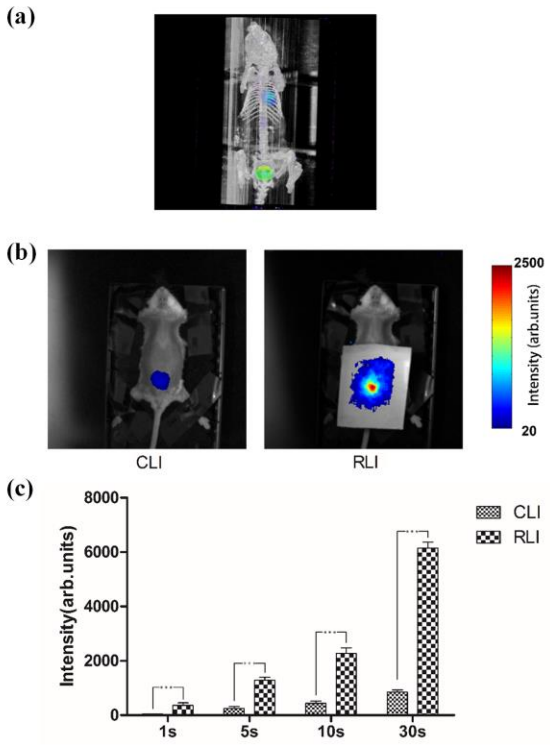

Fig. 4. Results of in vivo small animal experiment. (a) One of the representative Micro-PET/CT images; (b) One of the representative CLI (left) and RLFI (right) images; (c) Quantitative analysis that describes the variations in CLI and RLFI signal intensity with exposure time

4. Conclusion

In summary, we presented the radioluminescence film imaging (RLFI) for detecting radionuclides in an optical manner. Using the lab-made RLFs, we demonstrated the feasibility and potential of the RLFI method with both *in vitro* and *in vivo* experiments. Compared with current CLI method, our RLFI can provide two advantages. First, it can provide a relatively large imaging depth. The high energy rays can penetrate tissues almost without any limit, but the luminescent light cannot. Second, it can significantly reduce data acquisition time. It takes several minutes for CLI to acquire a high-quality image; whereas, only a few seconds may be adequate for RLFI. Moreover, the RLFI method can also answer the *in vivo* unfriendly issue of RLMPs, a big obstacle for clinical applications existed in the RLMPs based intensity enhanced CLI method. However, there is a problem that cannot be ignored for the RLFI method. The resolution is dependent on the distance between the source of radionuclides and the film. This can be improved by adding a collimator before detection. The other approach is to make the film close to the imaging body as much as possible. Future concerns will focus on both the preclinical and clinical applications of the RLFI method.

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