Fluorescent Polystyrene Latex Nanoparticles for NIR-II in vivo Imaging

Masao Kamimura1,2*, Yuichi Ueya3, Eiji Takamoto3, Kazuhiro Iso3, Moe Yoshida1, Masakazu Umezawa1,2, and Kohei Soga1,2**

1Department of Materials Science and Technology, Tokyo University of Science, 6-3-1 Niijuku, Katsushika-ku, Tokyo 125-8585, Japan
2Imaging Frontier Center (IFC), Research Institute for Science and Technology (RIST), Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
3Tsukuba Research Laboratories, JSR Corporation, 25 Miyakigaoka, Tsukuba, Ibaraki 305-0841, Japan

* masaokamimura@rs.tus.ac.jp
**mail@ksoga.com

In this study, tissue-penetrable near infrared (NIR)-II fluorescent polystyrene latex nanoparticles (NIR-PSt NPs) were designed and prepared as deep tissue in vivo imaging probes. An NIR-II fluorescent dye was successfully loaded into PSt NPs. The resulting NIR-PSt NPs showed strong NIR-II (1100 nm) emission in an aqueous environment. In addition, in vivo imaging of live mice was successfully performed using these NIR-PSt NPs. The prepared NIR-PSt NPs exhibited remarkable properties for in vivo fluorescence imaging.

Keywords: Second biological window, NIR-II, Fluorescence in vivo imaging, Fluorescent beads, Latex nanoparticles

1. Introduction

Fluorescence bioimaging is an important analytical method for observing body interiors, and can be applied for biomedical research and clinical diagnosis [1,2]. However, most current fluorescent probes show visible (VIS) (400 - 700 nm) emission under ultraviolet (UV) (< 400 nm) or VIS light excitation, and hence, these probes are difficult to use for deep tissue imaging. Since various biomolecules such as hemoglobin, melanin, and fat in the body exhibit strong absorption in these short wavelength regions, UV or VIS light cannot penetrate tissues.

On the other hand, near-infrared (NIR) (700 - 1600 nm) light is well-known to show high permeability into the body, due to the low absorption or scattering by biomolecules and water in the body in the NIR wavelength region [1-5]. Current NIR in vivo imaging mostly uses the NIR-I (700 - 900 nm: first biological window) wavelength region; however, compared with the NIR-I region, the NIR-II (1100 - 1350 nm: second biological window) and NIR-III (1550 - 1700 nm: third biological window) wavelength regions undergo lower scattering. Therefore, the NIR-II and NIR-III regions are the most ideal wavelength regions for fluorescence bioimaging. In the past decade, our group reported on NIR-II and NIR-III fluorescence imaging based on various probes such as rare-earth doped ceramic nanoparticles (RED-CNPs) [6-11], single-walled carbon nanotubes (SWCNTs) [12], and organic dyes [13]. These fluorescent probes showed excellent fluorescence properties, and could be used for successful imaging of deep tissues (10 - 20 mm). To date, many researchers have also reported on NIR-II and NIR-III fluorescent probes, including quantum dots, [14-18] RED-CNPs [19-24], SWCNTs [25,26], and organic dyes [27-40].

In particular, organic dyes have received much attention as the most promising candidates for fluorescent probing in biological and clinical applications, due to their excellent optical properties and high biocompatibility. However, organic dyes, such as IR-1061 and IR-26, are difficult to use in
aqueous solutions, and hence, conjugation or incorporation into biocompatible polymers is required.

In this study, we developed novel NIR-II dye–incorporated polystyrene latex nanoparticles (NIR-PSt NPs) for fluorescence in vivo imaging (Fig. 1). The NIR-II dye, IR-1061, was loaded into stable PSt NPs. In addition, to improve the biocompatibility and dispersion stability, the surface of the NIR-PSt NPs was modified using poly(ethylene glycol) (PEG). Furthermore, in vivo imaging of mice, using these NIR-PSt NPs, was also performed.

![Schematic illustration of preparation of NIR-PSt NPs.](image)

**Fig. 1. Schematic illustration of preparation of NIR-PSt NPs.**

2. Materials and methods

NIR-PSt NPs were synthesized using styrene, acrylic acid, and itaconic acid as the monomers, in accordance with a procedure described earlier [41], with slight modification. IR-1061 dye was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. The surface of the NPs were modified with a PEG-derivative blocking agent, Blockmaster® (CE510, JSR Life Sciences Corporation, Tsukuba, Japan). The particle size of the NIR-PSt NPs were measured by dynamic light scattering (DLS; Nanotrac UPA EX150, MicrotracBEL, Osaka, Japan). The emission spectra of the NIR-PSt NPs were measured using a Fluorolog-3 spectrometer (Horiba, Kyoto, Japan). Male ICR mice were purchased from Sankyo Labo Service, Japan. All the experimental procedures using mice were approved by the Animal Experiment Committee of Tokyo University of Science, and carried out in accordance with the guidelines for the care and use of laboratory animals. The NIR fluorescence images of mice were observed by a portable NIR fluorescence in vivo imaging system (SAI-1000, Shimadzu, Kyoto, Japan).

![Particle size distribution of NIR-PSt NPs in aqueous environment.](image)

**Fig. 2. Particle size distribution of NIR-PSt NPs in aqueous environment.**

3. Results and discussion

To functionalize the PSt NP surface, styrene, acrylic acid, and itaconic acid were co-polymerized. Then, the PEGylation reagent, Blockmaster®, was immobilized on the prepared NIR-PSt NP surfaces via amide bonding. The particle size of the NIR-PSt NPs were measured by DLS. Figure 2 shows the particle size of the NIR-PSt NPs in water. According to the results, the prepared NIR-PSt showed a unimodal peak, and the average particle size of the NPs was about 50 nm. Therefore, the prepared NIR-PSt NPs were well-dispersed in water, with no aggregation.

Next, the NIR-II emission of the NIR-PSt NPs was evaluated in water. As shown in Fig. 3, the NIR-PSt NPs exhibit strong 1110 nm emission under 980 nm excitation. This result also indicates that the prepared NIR-PSt NPs can be used in aqueous environments.

![Emission spectrum of NIR-PSt NPs in aqueous environment.](image)

**Fig. 3. Emission spectrum of NIR-PSt NPs in aqueous environment. Excitation wavelength: 980 nm.**
Finally, in vivo imaging of live mice was performed. The NIR-PSt NPs were injected into the mice via the tail vein, and the fluorescence images of the mice were observed. Figure 4 shows an NIR-II fluorescence image of a live mouse just after injection. Strong NIR-II emission of the NIR-PSt NPs was observed from the blood stream. This result indicated the NIR-II emission of the prepared NIR-PSt NPs could be used to visualize the interiors of the bodies of mice without dissection. Therefore, these NIR-PSt NPs have a high potential for use in in vivo imaging of deep tissues.

4. Conclusion

In this paper, we report the preparation and evaluation of novel near infrared (NIR)-II fluorescent polystyrene latex nanoparticles (NIR-PSt NPs) for in vivo imaging. The NIR-PSt NPs emitted strong NIR-II emission in an aqueous environment. Furthermore, in vivo imaging of live mouse was also successfully investigated. The blood stream of a live mouse could be clearly observed from outside the body, using the NIR-II emission of the NIR-PSt NPs. Based on these results, these NIR-PSt NPs would be useful for deep tissue in vivo fluorescence imaging.

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