Application of Ceramic/Polymer Conjugate Materials for Near Infrared Biophotonics

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Over-1000-nm (OTN) near infrared (NIR) wavelength region lying between 1000 and 1700 nm has been known as so-called “biological window” where the optical loss in live body is minimized. Recent development of InGaAs CCD cameras, which can visualize in the above wavelength region, is making it possible to visualize the biological or medical images in the OTN-NIR wavelength region. On the other hand, rare-earth doped ceramic nanophosphors (RED-CNP) is one of the fluorescent nano materials that can be applied for the fluorescence bioimaging in the OTN-NIR wavelength region. This paper will review the developments of the systems and materials for the OTN-NIR fluorescence bioimaging. As an example in the development of imaging system, the mouse brain stem was demonstrated to image by fluorescence imaging without opening the skin or skull of the mouse. As an example of materials’ development, the synthesis and characterization of liposomes which contain Er- and Yb-doped Y\textsubscript{2}O\textsubscript{3} or NaYF\textsubscript{4} are reported along with the OTN-NIR in vivo fluorescence imaging by using them.

Keywords: near infrared, fluorescence, bioimaging, ceramics, rare-earth, liposome

1. Introduction

Currently, most of the in vivo fluorescence imaging are performed by using the fluorescence with a wavelength less than 1000 nm. However, as shown in Figure 1, the transparency of living tissues are mostly maximized in the wavelength range between 1000 and 1700 nm, where both of the optical losses due to scattering and infrared absorption are minimized [1]. The range has been called “biological window” for many years. Great efforts are made to elongate the wavelength of fluorescence by using indocyanine green (ICG) [2] or quantum dots [3]. However, the wavelength is still limited around 900 nm due to the limitation of the observation wavelength of the imaging devices made by semiconductor silicon. In recent years, CCD cameras using InGaAs, which

Received May 12, 2012
Accepted June 5, 2012
can image the light in between 800 and 1700 nm, has come into market. According to the loss spectrum, one can expect ten times more transparency in the wavelength over 1000 nm, over-1000 nm near infrared (OTN-NIR). The observation depth of the current in vivo fluorescence bioimaging is limited to be only several millimeters. However, the use of the OTN-NIR for the imaging can potentially provide an observation depth with several centimeters. The authors group has been developing imaging systems and fluorescent imaging probes for the OTN-NIR fluorescence bioimaging [4-22].

Rare-earth doped ceramic nanophosphors (RED-CNP) are known to emit efficient NIR fluorescence under NIR excitation. Nd-doped yttrium aluminum garnet (YAG) for emitting 1064 nm emission under 800 nm excitation and Er-doped silicate fibers to emit 1550 nm emission under 980 nm can be taken as examples as practically used fluorescent materials in the OTN-NIR wavelength range [22]. Namely, when co-doped with Er and Yb, those ceramics such as Y$_2$O$_3$ [4-17], Gd$_2$O$_3$[20], YPO$_4$ [23], YVO$_4$ [21], LaOCl [24] can emit 1550-nm fluorescence under 980 nm excitation. Namely, Y$_2$O$_3$ is good in size control with a size more than 100 nm by using homogeneous precipitation [7]. Beside those, NaYF$_4$ is one of the good candidates to emit efficient OTN-NIR fluorescence [25-27] and to be controlled with the size around 10 nm [25] by using a hydrothermal processing with a surfactant.

To apply the RED-CNP for biophotonics including bioimaging, conjugation with polymers is imperative to keep the particles dispersed in physiological conditions and to avoid nonspecific interactions with substances in living matters [19, 22, 28]. The introduction of hydrophilic and nontoxic polymer, such as PEG, onto the bio probe is known to be effective to keep the particles dispersed and to avoid the nonspecific interactions. One of smart methods to introduce the PEG (PEGylate) onto the RED-CNP is the use of block copolymers of the PEG with an ionomer. The authors have reported the surface modification by using PEG-b-PAMA [8], PEG-b-PAAc [4, 6] and PEG-b-PVBP [16] for the surface modification of the RED-CNP. On the other hand, liposomes have been popularly used to deliver nano-sized objects to a part of living matter since the structure is essentially the same as that of animal cells and the surface can be artificially modified by using the variety of phospholipid. The authors also have prepared liposome-encapsulated Y$_2$O$_3$ for the OTN-NIR bioimaging with a size of 650 nm [13].

The objective of the present paper is to review the system and materials’ development for the OTN-NIR in vivo fluorescence bioimaging with some new experimental results. As an example in the development of imaging system, brain stem of a mouse is demonstrated to image by fluorescence imaging without opening the skin or skull of the mouse. As an example of materials’ development, the synthesis and characterization of liposomes which contain Er- and Yb-doped Y$_2$O$_3$ or NaYF$_4$ will be reported along with the OTN-NIR in vivo fluorescence imaging by using them.

2. Experimental

2.1 Preparation of Rare-Earth Doped Y$_2$O$_3$ and NaYF$_4$ Nanoparticles

The Y$_2$O$_3$ nanoparticles co-doped with 1 mol% of Yb and 1mol% of Er (Y$_2$O$_3$:Er/Yb) were prepared by a method reported previously [7], where the precursor (Y(OH)(CO$_3$))$_3$ was precipitated by a homogeneous precipitation method by using urea. The precursors were moved for calcination at 1100-1200 °C to convert the precursor into Y$_2$O$_3$. The surface of the Y$_2$O$_3$:Er/Yb was modified with PEG-b-PAAc [4, 6] to be dispersed in water solution with ionic strength which corresponded to that of physiological condition. NaYF$_4$ nanoparticles co-doped with 20 mol% of Yb and 2 mol% of Er (NaYF$_4$:Er/Yb) were prepared by a hydrothermal method with oleic acid reported by Wang et al. [25]. By this method, the NaYF$_4$:Er/Yb are obtained to be mono-dispersed into hydrophobic solutions such as hexane or chloroform by the effect of the surface modification with the oleic acid.

2.2 Preparation of Liposome-Encapsulated Y$_2$O$_3$ and NaYF$_4$ Nanoparticles

Both of the Y$_2$O$_3$:Er/Yb and NaYF$_4$:Er/Yb nanoparticles are encapsulated into PEGylated liposome by different methods. The Y$_2$O$_3$:Er/Yb nanoparticles modified with the PEG-b-PAAc was at first dispersed into HEPES buffer (20 mmol/L, pH=7.4, 150 mmol/LNaCl) and encapsulated into PEGylated liposome (Y$_2$O$_3$-Lipo) by a previously reported micro-encapsulating method [13]. On the other hand, since the obtained NaYF$_4$:Er/Yb nanoparticles are dispensible in hydrophobic solutions, the NaYF$_4$:Er/Yb nanoparticles are at
first dispersed in chloroform. The liposome was prepared by ultrasonic method \[29\] to encapsulate the hydrophobic NaYF\(_4\):Er/Yb nanoparticles in the phospholipid double layer (NaYF\(_4\)-Lipo). For both of the liposomes, the composition was set to be dipalmitoyl-phosphatidylcholine(DPPC): cholesterol: distearoyl-glycerol-PEG(DSG-PEG) = 6: 3: 1.

2.3 OTN-NIR in vivo Fluorescence Bioimaging

NIS-OPT OTN-NIR in vivo fluorescence imaging system (Shimadzu, Kyoto, Japan) is equipped with laser diode excitation at 980 nm and InGaAs CCD with proper filters. The fluorescence wavelength was 1550 nm of Er in both of the phosphors.

3. Results and Discussion

3.1 Brain stem imaging of a mouse by OTN-NIR in vivo fluorescence imaging

The Y\(_2\)O\(_3\):Er/Yb nanoparticles with a size of 150±50 nm dispersed in HEPES buffer at a concentration of 5 mg/mL. The liquid was injected into the mouse brain through palatine. The fluorescence image of the Y\(_2\)O\(_3\):Er/Yb at 1550 nm under an excitation at 980 nm was observed after filtration of the excitation light by using a silicon filter, which could filter the light with a wavelength less than 1100 nm. Figure 2 shows the observed images. The brain stem of the mouse was clearly observed without opening the skin and skull. It was proved that the OTN-NIR fluorescence imaging was more useful than the currently used imaging with fluorescence with a wavelength below 1000 nm especially for a deep tissue imaging.

3.2 Preparation and Properties of Liposome-Encapsulated Y\(_2\)O\(_3\):Er/Yb and NaYF\(_4\):Er/Yb Nanoparticles

Figures 3 and 4 show the FE-SEM images and particle size distribution of the prepared RED-CNP and the liposome-encapsulated RED-CNP. The Figures 3(a) and 3(b) show that Y\(_2\)O\(_3\):Er/Yb and NaYF\(_4\):Er/Yb nanoparticles are successfully prepared with sizes approximately 140 ± 53 and 12 ± 3 nm, respectively, which are consistent with the result of the DLS measurement shown in Figure 4. According to Figures 3(c) and 3(d), the RED-CNP were existing in the liposome. The size of the liposomes were estimated both Figures 3 and 4 to be approximately 450±170 nm for the Y\(_2\)O\(_3\)-Lipo and 110±44 nm for the NaYF\(_4\)-Lipo.

For testing the cell toxicity of the liposome-encapsulated RED-CNP, those particles are added for the incubation of the PK136 and J774 cells as well as a negative control with pure HEPES buffer and a positive one with the HEPES buffer added with carboplatin. The PK136 cell was selected to represent a cancer cell assuming future possibility of cancer imaging. J774 cell was selected to represent a macrophage since objects with a size in several hundreds nm are tend to be
trapped in macrophages in liver or spleen in a mouse body. Also, in case of bare RED-CNP without any surface modification, toxicity to macrophages is reported previously for the RED-CNP due to acidic condition in macrophages [7, 18, 20]. As shown in Figure 5, no obvious toxicity was detected for both of the liposome-encapsulated RED-CNP.

3.3 OTN-NIR in vivo Fluorescence Bioimaging by Using Liposome-Encapsulated RED-CNP

The two kinds of the liposome-encapsulated RED-CNP were injected to mice blood vessels via the tail vein with a concentration of 20 mg/mL in 20 mmol/L HEPES buffer. The amount of the injection was 200 µL. The mice were observed in vivo under the imaging system NIS-OPT after 1 and 24 h after the injection. Fluorescence was mainly observed from the liver of the mouse as shown in Figure 6. Obvious difference between Y2O3–Lipo and NaYF4-Lipo was the transient change of the fluorescence intensity of the liver. Figure 7 shows the comparison of the brightness of the liver fluorescence between the images at 1 and 24 h after the injection. The brightness was calculated by integrating the grayscale value of the liver. The relative brightness was expressed to set the value at 1 h to be 100%. In the case of Y2O3-Lipo injection, the brightness almost did not change between transient times of 1 and 24 h. On the other hand, the brightness of the fluorescence from the liver of the mouse injected with NaYF4-Lipo after 24 h from the injection was 1.5 times more than that after 1 h. The difference may be caused by the trapping rate of the liposomes into the reticular system due to the size difference of the liposome. The Y2O3-Lipo with 450 nm size was quickly trapped in the liver, while the NaYF4-Lipo took more time to be trapped in the liver and circulated longer in the blood vessel.

4. Conclusion

Effectiveness of the OTN-NIR in vivo fluorescence bioimaging was successfully demonstrated by the brain stem imaging of a
mouse. The fluorescence image of the injected Y$_2$O$_3$:Er/Yb nanoparticles gave clear image of brain stem without opening the skin and skull.

As examples of the ceramic/polymer conjugate materials for the OTN-NIR biophotonics, liposome-encapsulated Y$_2$O$_3$:Er/Yb and NaYF$_4$:Er/Yb nanoparticles were prepared under size control. The liposomes were PEGylated and the sizes were controlled to be 450 and 110 nm, respectively for the Y$_2$O$_3$:Lipo and NaYF$_4$:Lipo. Cell toxicities were tested for both of the liposomes and found to be negative for PK316 and J774 cells. Those two kinds of the liposomes were injected into the blood vessel via the tail vein. The OTN-NIR in vivo fluorescence imaging showed that the transient accumulation of the materials into the mouse liver depends on the size of the liposome.

Acknowledgements

This study was supported in part by a grant of Strategic Research Foundation Grant-aided Project for Private Universities from Ministry of Education, Culture, Sport, Science, and Technology, Japan (MEXT), 2009-2013 (S090102).

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