Functionalized upconversion nanoparticles for cancer imaging and therapy

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Chapter 5.

Target Labeling of Early-Stage Cancer in Chick Embryo Chorioallantoic Membrane with Upconversion Nanoparticles

In vivo detection of early-stage tumors, i.e. smaller than 2 mm, is still a huge challenge in biomedicine. Upconversion nanomaterials, which can efficiently convert near infrared (NIR) light into visible light, offer a possibility to solve this challenge. In this chapter, detection of cancer at the early stage is demonstrated in chick embryo chorioallantoic membrane (CAM) model, where antibody functionalized upconversion nanoparticles (UCNPs-Ab) are constructed and utilized for targeted labeling of early-stage MCF-7 tumor spheroid (~500 μm) grafted on chick embryo CAM. NaYF₄:Yb³⁺,Er³⁺ UCNPs are covalently functionalized with monoclonal antibody of estrogen receptor alpha (ER-α) via EDC cross-linking method, which show good cellular compatibility and high specificity to human breast cancer MCF-7 cells. In order to study their in vivo targeting, 3-dimensional MCF-7 multicellular tumor spheroid (MCTS) is transplanted on CAM to serve as an early stage tumor model, and systematically administrated with UCNPs-Ab conjugates via intravenously injection. From the intravital imaging, it turns out that the nanoconjugates can extravasate from the blood vessels and specifically label on the xenografted MCTS. The histological study has clearly proved the highly specific labeling of UCNPs-Ab conjugates in the transplanted MCTS. Our research highlights the potential of UCNPs-Ab in in vivo study of cancer at early stage.

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5.1 Introduction

It is axiomatic in clinical oncology that detection of cancer at early-stage, e.g. carcinoma in situ that smaller than 2 mm, is of great importance for improving the cancer cure probability.[1-3] Unfortunately, most of present clinical imaging modalities like ultrasonic (US), computed tomography (CT), and magnetic resonance imaging (MRI) are not sufficient for detecting the early-stage cancers because of their low resolution and poor sensitivity and/or specificity.[4,5] Florescence imaging, an optical detection technique, has recently regained increased attention for cancer diagnosis, because of the new developments in exogenous contrast agents.[6-11] such as rare earth ions doped upconversion nanoparticles (UCNPs) that can convert near infrared (NIR) light into and/or shorter wavelength NIR light. In comparison to traditional ‘down conversion’ fluorescent markers that need ultra-violet or visible (UV-Vis) light for excitation, the UCNPs hold many advantages for biomedical imaging, such as minimized background fluorescence, and no photo bleaching. Furthermore, since UCNPs have large surface area, biofunctional molecules like photosensitizers, doxorubicin (DOX), si-RNA, folic acid, and peptides can be easily conjugated for targeted labeling or therapy. Numerous research studies have been reported in this respect on both in vitro and in vivo tests utilizing UCNPs.[12-25] Xiong et al. reported targeted labeling of 4-6 mm U87MG tumor in mice model utilizing argine-glycine-asparatic (RGD) peptide conjugated NaYF₄:Yb,Er,Tm UCNPs.[13] Zhou et al. achieved tri-mode imaging of upconversion luminescence, magnetic resonance and positron emission tomography (PET) in mouse utilizing fluorine-18-labeled Gd³⁺/Yb³⁺/Er³⁺ co-doped NaYF₄ UCNPs.[22] However, most of these researches are performed on mice model in which the imaging can usually be executed at relatively late stage when tumors reached to 4–6 mm. In vivo target detection of early stage cancer, i.e. smaller than 2 mm, remains a difficult task in biomedicine.

In this chapter, a highly sensitive upconversion luminescence imaging nanoplatform of UCNPs-Ab is constructed by anchoring antibody on UCNPs and used for target labeling of the early stage tumor spheroid that grafted on chick embryo chorioallantoic membrane (CAM). Chick embryo CAM is a well-established model which has already been widely used for cancer and angiogenesis research, drug delivery and immunology.[26-35] Compared with the widely used mice model, chick embryo CAM has unique advantages in
cancer research, including (i) the chick embryo is a naturally immunodeficient system, various types of tumor cells can be transplanted into the CAM without any species-specific restrictions, and (ii) since the chick embryo CAM is an extremely thin membrane layer (~200 μm) that usually lies at the top, it’s very convenient to observe motility process of the injected cancer cells or drug molecules under a microscope with little impact on the host. On top of that, the chick embryo model is simple (without animal manipulation), low cost, easy to maintain, and easily accessible. In our study, multicellular tumor spheroids (MCTS, ~0.5 mm in diameter) of MCF-7 human breast adenocarcinoma cell were cultured and transplanted onto the chick embryo CAM to serve as an early stage tumor model. Since MCF-7 cell line has a high expression level of estrogen receptor alpha (ER-α), the corresponding monoclonal antibodies (Ab) of ER-α were covalently functionalized onto NaYF₄:Yb,Er UCNPs via a simple EDC cross-linking method (shown in Scheme 1). The cytotoxicity and targeting properties of UCNPs-Ab were also assessed in a tumor cell line (MCF-7) and normal cell line (3T3). The *in vivo* microcirculation behavior and targeting property of UCNPs-Ab conjugates were investigated following micro vein injection, which demonstrated that they could efficiently flow through the bloodstream, extravasate from the vasculature, and specifically accumulate at the tumor spheroids. These results indicate that UCNPs have great potential for *in vivo* target labeling and diagnosis of cancer at early stage.

**Scheme 5.1.** Synthesis of UCNPs-Ab nanoplatform.
5.2 Experiments and Methods

5.2.1 Synthesis of carboxyl functioned NaYF₄:Yb,Er, UCNPs

Hydrophobic NaYF₄:Yb(20%),Er(2%) upconversion nanoparticles were synthesized by a modified hyper-thermal decomposition method according to literature. The method involved dissolving 236.54 mg YCl₃·6H₂O (0.78 mmol), 77.48 mg YbCl₃·6H₂O (0.2 mmol), and 7.64 mg ErCl₃·6H₂O (0.02 mmol) in 2 mL methanol before being transferred into a 50 mL three-neck flask containing 3 mL oleic acid (OA) and 7 mL 1-octadecene (ODE). The mixture was stirred at room temperature for 30 min and then slowly heated to 110 °C for 15 min to remove methanol and water under argon atmosphere. After, the solution was heated to 156 °C and maintained at this temperature for 1 h. The received lanthanide precursors were allowed to cool down to room temperature, followed by adding 10 mL methanol solution containing 148.21 mg NH₄F (4 mmol) and 100.02 mg NaOH (2.5 mmol) into the flask and stirring for 1 h. After evaporating methanol at 60 °C, the solution was heated to 300 °C for 90 min followed by a period of cooling down to room temperature. The mixture was precipitated by the addition of 20 mL ethanol, and collected after centrifugation at 5000 r/min for 15 min. Product was re-dispersed with 5 mL hexane and precipitated by adding 15 mL ethanol, then collected after the same centrifugation step. The product was washed three times, and the final product was re-dispersed in 10 mL hexane.

As the OA capped NaYF₄:Yb,Er UCNPs have poor dispersibility in aqueous phase, further surface modification is usually needed to improve their water-solubility and biocompatibility. In our case, a two-step ligand exchange method utilizing polyacrylic acid (PAA, -COOH groups) to replace the OA molecules was used, as is illustrated in Scheme 5.1. To start, 5 mL OA capped UCNPs (50 mg) dispersed in hexane was mixed with 10 mL aqueous solution. After adding 200 μL HCl solution (1 mol/L), the mixture was stirred vigorously for 2 h at room temperature. The oleate ligands were easily protonated and released from UCNPs in the presence of HCl, leaving ligand free UCNPs. These ligand-free UCNPs were then collected after centrifugation and washed with water/acetone for two times to remove the excess HCl and OA, and were then redispersed in 5 mL water. At this stage, 50 mg PAA molecules were added into the ligand free nanoparticles and stirred overnight. Because ligand free UCNPs have very high surface activity, PAA molecules can bond tightly onto UCNPs with strong coordinate interaction between carboxyl groups and rare earth ions. The obtained
carboxyl functioned UCNPs were washed with water for at least two times to remove the free PAA molecules.

5.2.2 Covalent functionalization of UCNPs-Ab conjugates

PAA stablized NaYF₄:Yb,Er UCNPs were covalently functionalized with monoclonal antibodies of estrogen receptor alpha (ER-α) via a simple EDC cross-linking method in order to acquire target cancer delivering ability. Briefly, 2 mg UCNPs were dispersed in 500 μL MES buffer, 10 mg EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) and 10 mg NHS (N-Hydroxysuccinimide) were added into the solution and shaken continuously for 90 minutes at room temperature. The amino-activated nanoparticles NaYF₄:Yb,Er-NHS were collected by centrifugation and washed twice with MES (2-(N-morpholino)ethanesulfonic acid) buffer, and redispersed in 500 μL MES buffer for further conjugation. Afterwards 100 μg ER-α antibodies was added into UCNPs solution and shaken gently for 1 hour at room temperature. The acquired UCNPs-Ab conjugates were washed with PBS twice and redispersed in 500 μL PBS. Then 3% BSA was added into the UCNPs-Ab conjugates in order to blocking the excess unnecessary bonding sites and stocked at 4 °C for further application.

5.2.3 In vitro experiment

Two different cell lines of human breast adenocarcinoma MCF-7 (ER-α positive cells) and fibroblast NIH 3T3 (ER-α negative cells) were cultured to evaluat the biocompatibility and specificity of UCNPs-Ab conjugates. Both were bought from the American Type Culture Collection (ATCC) and cultured according to standard methods. MCF-7 cells were cultured in the complete Dulbecco’s Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12). The medium was supplemented with 10% fetal bovine serum, 1% MEM Non-Essential Amino Acids Solution (100X), 100 unit/mL penicillin, and 100 μg/mL streptomycin (all from Invitrogen). NIH 3T3 cells were cultured in DMEM medium, supplemented with 10% fetal bovine serum (FBS), 100 μg/mL penicillin and 100 μg/mL streptomycin (all from Invitrogen). Both cells are cultivated in medium at 37 °C in a humidified 95% air and 5% carbon dioxide (CO₂) atmosphere.

The cytotoxicity of UCNPs-Ab conjugates was evaluated by the mitochondrial activity using standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Both 3T3 cells and MCF-7 cells in log phase growing were seeded into 96-well cell-culture plates at a concentration of 5×10⁴ /well and 1×10⁵ /well, respectively, and incubated at 37 °C under 5%
CO₂. After 24 h, different amounts of UCNPs-Ab conjugates were added into the culturing medium at final concentrations of 0, 5, 10, 20, 50 and 100 μg/mL. The cells were incubated for 24 h at 37 °C. Subsequently, 10 μL MTT (5 mg/mL) was added to each well and incubated for an additional 4 h at 37 °C. After removing the medium, the wells were washed by PBS, and the intracellular formazan crystals were extracted into 100 μL iso-propanol. The absorbance of the cell lysate was recorded at 550 nm by a plate reader, and the cellular viability was calculated from the average value of four wells.

To study the specificity of the UCNPs-Ab conjugates for targeted imaging, in vitro experiments were carried out on human breast adenocarcinoma MCF-7 cells (with high expression level of ER-α). Mouse fibroblast cells 3T3 (expressing low levels of ER-α) were used for the control experiments. Both, MCF-7 and 3T3 cells were seeded on a coverslip at a concentration of 10⁴ cells/mL and then treated with UCNPs-Ab conjugates (20 μg/mL) for 8 h at 37 °C. Prior to imaging, the coverslip was washed twice with PBS in order to remove any unbound upconversion conjugates. The cells were then fixed with 4% paraformaldehyde and mounted with 95% glycerol solution. Upconversion luminescence imaging was performed using our previous described confocal microscope system. [38]

5.2.4 Chick embryo preparation and tumor spheroids transplantation

A modified shell-less chick embryo CAM model was developed in order to evaluate the in vivo target labeling property of UCNPs-Ab conjugates. The fertilized white chick eggs (Drost Loosdrecht BV, The Netherlands) were disinfected with 70% alcohol and incubated in a rotating ventilated hatching incubator set at 39 °C and 70% humidity. After 3 embryonic age (EA) days, shell-less chick embryo CAM preparation was conducted by carefully dropping the egg contents into a dry and sterile weighing dish (8 cm) under a clean and light restricted atmosphere. The dish was then covered with parafilm and put back into a static hatching incubator set at 38 °C and 60%. Additional holes were added using an 18G needle to ensure gas exchange.

3-Dimensional multicellular Tumor Spheroids (MCTS) of human breast cancer cells MCF-7 were cultured in vitro for transplantation. To create MCTS, single MCF-7 cell suspensions (0.5×10⁶ cells/mL) in complete DMEM/F-12 cell culture medium were added to 1.5% agar-coated 24 well plates (200 μl/well), and incubated under CO₂ flow. During the 3 days of incubation, tumor cells aggregated with each other to form a spheroid. The spheroids were transferred into regular 24 well plates (non-agar coated) for further incubation.
200 μl of extra DMEM/F-12 was added daily into each well until the tumor spheroids were approximately 0.4 mm in diameter.

The tumor spheroids were implanted in the CAM on day 9 or 10 of embryonic development. Under sterile conditions, a small incision was made, using a 30 gauge hypodermic needle attached to a 1 mL syringe, in the upper surface (ectodermal epithelium) of the CAM in a region away from major blood vessels. A small area of the chorionic layer of the CAM was scratched with the edge of the needle’s bevel. The ectodermal epithelium was successfully removed when the target area appeared watery due to local ischemia. Any resulting sera, blood or debris was gently aspirated with the syringe. At the end, the tumor spheroid was dropped onto the severed area. The dish was then covered with parafilm, and returned to the incubator. Successful implantation was observed when the tumor spheroid was engulfed by the CAM (typically 2 days post-implantation), and the tumor spheroid rested in the highly vascularized mesodermal layer between the allantoic epithelium (endoderm) and the healed chorionic epithelium (ectoderm). At this stage, the model was ready for use for biological and biomedical engineering investigations.

5.2.5 Intravital microscopy fluorescence imaging of MCTS on CAM

On 14 EA days, 50 μL UCNPs-Ab conjugates (1 mg/mL) were systematically administrated into the CAM by microvein injection under a stereomicroscope. The upconversion luminescence imaging from UCNPs were captured by a homemade intravital microscope system which was equipped with a 100 W mercury lamp, a 500 mW 980 nm laser, 890 nm short pass filter to reject the excitation light, a 4x objective and a fast, high resolution, low light performance digital camera (Q Imaging, Retiga-SRV, Canada). The CAM was put on an electrical heating plate to keep the temperature at 37 °C. Both bright field images and fluorescence images of the tumor and surrounding tissue were recorded.

5.2.6 Histological research

On EA day 15, after imaging, areas containing the MCTS were cut out and embedded in tissue fixation gel for cryotom sectioning. The MCTS were cut into 10 μm sections, stained with hematoxylin-eosin (H&E) and further analyzed using a standard white light microscope. In addition, upconversion luminescence confocal imaging was carried out using an inverted Olympus IX71 microscope equipped with an 100× oil immersion objective and a 980 nm Ti:Sapphire laser, as previously described.
5.3 Results and Discussion

5.3.1 Synthesis and characterization of UCNPs

As the NaYF₄:Yb,Er UCNPs synthesized from organic solvent could not be dispersed well in water, a surface modification process was firstly carried out to transfer the hydrophobic UCNPs into hydrophilic ones via a simple two-step ligands exchange method. As illustrated in Scheme 5.1, first the oleic acid (OA) caped outside UCNPs were removed by protonation treatment, to receive ligand free UCNPs. Then the ligand free nanoparticles were treated with poly acrylic acid (PAA), which equipped the UCNPs with carboxylic groups. Figure 5.1 shows the TEM images of ligand free and PAA coated NaYF₄:Yb³⁺,Er³⁺ UCNPs. Both nanoparticles show good dispersibility and uniform size distribution, and the average size of the UCNPs is around 45 nm. Figure 5.1C shows the high resolution TEM image of an individual UCNP, where the lattice fringes with interplanar spacing are about 0.52 nm, corresponding to the (100) plane of hexagonal-phase structured NaYF₄. The insert shows the fast Fourier-transform (FFT) diffractogram, which confirms the hexagonal-phase of the UCNPs.

To prove that PAA molecules were capped on NaYF₄:Yb³⁺,Er³⁺ UCNPs, Fourier transform infrared spectroscopy (FTIR) characterization was performed (spectra are shown in Figure 5.2A). The band around 1422 cm⁻¹ is due to the C–O stretching vibration of the carboxyl groups, and the two strong bands centered at 1580 cm⁻¹ and 1462 cm⁻¹ are associated with the asymmetric and symmetric stretching vibration modes of carboxylate anions, suggesting the effective COO-RE³⁺ complexation on the UCNPs surface. The band at 1728 cm⁻¹ is assigned to the C=O stretching vibration of the free carboxyl groups on the PAA polymer chain.
Figure 5.2B is the upconversion luminescence spectra of ligand free and PAA coated UCNPs of the same concentration (1 mg/mL) in water under 980 nm excitation (400 mW). The upconversion luminescence in visible region has two bands, a green one around 515-560 nm and a red one around 640-675 nm, which are ascribed to transitions of $^4S_{3/2} - ^4I_{15/2}$ and $^4F_{9/2} - ^4I_{15/2}$ from doped Er$^{3+}$ ions, respectively. The two upconversion luminescence spectra are similar, indicating that polymer coating has negligible effect on the UC luminescence of UCNPs.

It is known that the hydrodynamic diameters and surface charges greatly affect cellular endocytosis and toxicity,[39-41] therefore we have measured the hydrodynamic diameters and zeta-potential and the results are shown in Figure 5.2 C and D. Compared with the ligand free nanoparticles, an obvious increase in hydrodynamic diameters is observed in PAA coated nanoparticles, which might be attributed to the dwelling effect of polymer coating at the surface of UCNPs. A significant change was also observed in the surface charges, varying from 45.5 mV (ligand free UCNPs) to -37.9 mV (PAA coated UCNPs), which further confirmed the existence of carboxyl groups at the surface of UCNPs.

![Figure 5.2](image)

**Figure 5.2.** FTIR spectra (A) and upconversion luminescence spectr (B) of ligand free and PAA coated NaYF$_4$:Yb,Er UCNPs under 980 nm excitation (400 mW). (C) and (D) are the hydrodynamic diameter distribution and zeta potential of ligand free UCNPs and PAA coated UCNPs.
5.3.2 Cytotoxicity research of the UCNPs-Ab conjugates

Cytotoxicity was investigated on two different cell lines, human breast cancer MCF-7 and mouse embryo fibroblast 3T3, with different UCNPs-Ab conjugates concentration (0, 5, 10, 20, 50, 100 μg/mL). No significant change was observed in the cell morphology and proliferation of both cell lines after 24 h in the presence of the UCNPs-Ab conjugates. The cellular viability is further evaluated by MTT assay of mitochondrial activity, the results are shown in Figure 5.3. Both cell lines demonstrate good cellular viability, even at the maximum concentration 100 μg/mL the viability maintains higher than 90%. These results indicate that UCNPs-Ab conjugates have good biocompatibility and therefore could be used for \textit{in vivo} imaging.

![Figure 5.3. Cellular toxicity results based on standard MTT assay. Gray column and black column are corresponding to 3T3 and MCF-7.](image)

5.3.3 \textit{In vitro} target labeling of cancer cells with UCNPs-Ab conjugates

Figure 5.4 shows the confocal microscope images of MCF-7 breast adenocarcinoma cells (for positive control) and 3T3 fibroblast cells (for negative control) after treatment with UCNPs-Ab (100 μg/mL) for 8 h. The bright field images show that the cellular morphology is intact, which is consistent with the cytotoxicity results of the UCNPs-Ab conjugates. The dark field images show the upconversion luminescence within the MCF-7 cells (Figure 5.4, top row), whereas little luminescence was observed in the 3T3 cells (Figure 5.4, bottom row). The latter is related with the residual non-specific adsorption of the UCNPs on the 3T3 cell membranes. These results indicate the UCNPs-Ab conjugates can specifically label on the MCF-7 breast cancer cells.
5.3.4 In vivo target labeling of tumor spheroid with UCNPs-Ab conjugates

In our study shell-less cultured chick embryo was developed as the model to research the in vivo labeling properties of UCNPs-Ab. A typical shell-less chick embryo is shown in Figure 5.5A. The CAM membrane is settled on the top of embryo and yolk, and the blood vessels of CAM can be seen very clearly with naked eyes. In order to assess the in vivo targeting behavior of the UCNPs-Ab conjugates on early stage cancer spheroids, MCTSs were cultured in vitro and transplanted onto the CAM. Compared with the cancer cells cultured in 2-D, the MCTS show a condensed structure in 3-D, and can mimic more closely of the cellular-matrix and cell-cell interactions in vivo. After 3 days of incubation, the MCTS could be embedded into the CAM membrane, and the new grown blood vessels can be clearly seen surrounding the MCTS. Then UCNPs-Ab were systematically administrated into the chick embryo CAM via venule injection under a stereomicroscope. Owing to the depression of autofluorescence during UCL imaging, the microcirculating behavior of the nanoconjugates in blood vessels was able to be neatly investigated with a modified fluorescence intravital microscope that equipped with a 980 nm laser. As shown in Figure 5.5B, left is the white image of a typical CAM blood vessel net, right is the corresponding upconversion luminescence image after 10 min of injection of UCNPs-Ab conjugates. We can distinctly see that the nanoparticles fluently flow with the bloodstream and efficiently extravasate from the main blood vessels into the surrounding tissues. Thus the CAM model provides us a simple approach for real-time
visualizing the in situ interaction of nanoparticles with the vascular networks and also the biotissues, which might be of great value for future Nano-Bio researches.

Figure 5.5 (A) A typical ex-ovo cultured chick embryo. (B) Intravital microscope images of bright field (left) and upconversion luminescence (right) of the chick CAM after 10 min intravenous injection with 100 μg UCNPs-Ab.

The in situ upconversion luminescence imaging of the tumor spheroid was then investigated at different time with intravital microscope. The UCNPs without any antibody functionalization (non-functionalized UCNPs) were also injected for control, data are shown in Figure 5.6. We see the non-functionalized UCNPs were present in both the MCTS and the environment without specific accumulation within the MCTS, both at 1 h and at 24 h after injection. In contrast, the functionalized UCNPs-Ab were accumulated specifically on the MCTS (Figure 5.7). One hour after injection, the UCNPs-Ab were observed mainly in the surrounding tissue of MCTS. Twenty-four hours after injection, strong upconversion luminescence was obviously observed in the MCTS, indicating the good targeted delivery of UCNPs-Ab conjugates.

In order to further demonstrate the selective labeling of UCNPs-Ab in tumor cells, the resected MCTS region was histological examined. Figure 5.8A shows the microscope image of the H&E stained MCTS imbedded into the CAM tissue. Figure 5.8 B and C are the confocal upconversion luminescence images of CAM and MCTS corresponding to the marked areas in Figure 5.8A. As expected, normal CAM regions show very low amount or no luminescence of UCNPs-Ab (Figure 5.8B). Whereas targeted luminescence of UCNPs-Ab was only observed in the transition zone from the CAM into the MCTS (Figure 5.8C). Low fluorescence was detected from surrounding tissue, resulting in a high contrast between targeted MCF-7 cells and surrounding tissue. On the contrary, from histological examination of MCTS administrated with non-functionalized UCNPs, only very little amount of upconversion luminescence was observed in MCTS (data not shown).
**Figure 5.6.** Non-targeted labeling of MCTS transplanted on the CAM with UCNPs at 1 h (top row) and 24 h (bottom row). From left to right are bright field, dark field (980 nm irradiation) and merged intravital microscope images at 4x magnification and 2 min exposure time.

**Figure 5.7.** Target labeling of MCTS transplanted on the chick CAM with UCNPs-Ab conjugates at 1 h (top row) and 24 h (bottom row). From left to right are bright field, dark field (980 nm irradiation) and merged intravital microscope images.
5.4 Conclusion

In conclusion, upconversion nanoparticles have been successfully functionalized and employed in labeling the cancer at early stage in CAM. PAA coated UCNPs were synthesized by a two-step ligand exchange method, and functionalized with ER-α antibody to obtain UCNPs-Ab conjugates. *In vitro* researches reveal that the UCNPs-Ab conjugates have no significant cytotoxicity on mammalian cells, and can specifically label in the MCF-7 breast cancer cells rather than normal cells. The cellular viability was higher than 90% even at relatively high concentration (100 μg/mL) of UCNPs-Ab. The 3-dimensional MCTS (~500 μm) transplanted CAM model has been developed as the early stage tumor model to research the *in vivo* labeling properties of UCNPs-Ab. Intravital microscope imaging demonstrated that intravenously injected UCNPs-Ab conjugates have high specificity in labeling the breast cancer. Our work suggests that UCNP-Ab, in combination with CAM, offers new possibility in early cancer studies.

References


