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# Confocal Viviperception of a Transparent Medaka Fish (*Oryzias latipes*) Using Functionalized Mesoporous Silica Nanoparticles (MSNs)

Wen-Chi Lin,[1] Chun-Wei Chiang,[2] Chen-Yu Hong,[1] Pei-Jen Chen,*[2] and Kevin C.-W. Wu*[1]
[1]*Department of Chemical Engineering, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan*
[2]*Department of Agricultural Chemistry, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan*

(Received March 4, 2011; CL-110185; E-mail: chenpj@ntu.edu.tw, kevinwu@ntu.edu.tw)

With utilization of a transparent medaka fish (*Oryzias latipes*) and fluorescent confocal microscopy, essential knowledge about biodistribution of fluorescein isothiocyanate (FITC)-functionalized mesoporous silica nanoparticles (MSNs) within a living organism becomes accessible without tissue dissection. Data evidences bioaccumulation of FITC-MSNs in liver, gill, and intestine after one month's aqueous exposure. FITC-MSNs were mainly retained in liver for at least a week even after those in intestine had been excreted out.

Mesoporous silica nanoparticles (MSNs) with suitable particle sizes, large surface areas, uniform pore sizes, and abundant silanol groups have been extensively synthesized through inorganic/organic cooperative assembly.[1] More importantly, many papers have demonstrated that MSN is one of the most promising nanodevices in biomedical applications. For examples, the Lin group has demonstrated that MSNs could be used as efficient carriers to deliver gene and protein into live cells.[2] The Mou group has demonstrated that organic dye-functionalized MSNs could be used as imaging probes for long-time cell tracking.[3] The Zink and Stoddart groups and others have demonstrated several MSNs-based stimuli-responsive drug delivery systems that respond to internal stimuli (e.g., pH, temperature, redox potential, and biomolecules)[4] or external stimuli (e.g., radiation and magnetic field).[5]

The in vitro biocompatibility of MSNs has been widely studied using different methods and in a variety of cell lines; however, in vitro studies often show cell-type-specific behavior or toxicity to various NPs or exogenous chemicals because of different metabolic or enzymatic responses among cells.[6] Because of the different toxicokinetics and dynamics regarding adsorption, distribution, metabolism, and excretion (ADME) between cells and an intact organism, full understanding of the in vivo toxic effects of MSNs would greatly facilitate further its clinical developments. To date, the physicochemical nature of MSNs in biological systems has been investigated in mice, which usually involves additionally complicated and time-consuming processes. For instance, either magnetic resonance imaging (MRI) or near-infrared (NIR) agents have to be attached to nanomaterials for visualizing NPs in rodents.[7] The use of mammals (e.g., rodents) as model organisms is often labor-consuming and costly, thus underlining the need of an alternative animal model (e.g., aquatic animals) that fits the requirement of a rapid and cost-effective screening strategy for exploring fundamental knowledge of NPs in vivo.

Medaka fish possesses unique characteristics including a small size (3–4 cm), short generation time (6–8 weeks), asynchronous spawning, easy breeding, and relatively economic husbandry.[8] A new see-through (STII) medaka strain, whose pigments were genetically removed from the skin so its surface body is transparent throughout the entire life, has been created. The main internal organs (heart, spleen, liver, or gut) of STII fish are visible to the naked eye or with a simple stereomicroscope, which enables visualization of effects or interactions of chemicals in a living fish.[9] The STII medaka serves as a superior alternative model for in vivo monitoring of nanomaterial behavior and toxic effects, while combining with fluorometric assays.[10] On the other hand, confocal microscopic technology provides a powerful three-dimensional imaging tool to visualize nanoparticles in cells, however, limited by thickness; conventional applications of confocal microscopy usually require extra processes of tissue dissection after sacrificing animals. Here, for the first time, we report confocal viviperception of STII medaka fish to real time observe the biodistribution of fluorescein isothiocyanate (FITC)-functionalized MSNs in a living intact organism.

FITC-MSNs were prepared according to a published paper.[11] Typically, 3-aminopropyltrimethoxysilane (APTMS, 0.18 mL) was mixed with FITC (8 mg), and the mixture was stirred for 30 min. Triethanolamine (0.42 g) and cetyltrimethylammonium bromide (CTAB, 2.0 g) were well mixed in distilled water (240 mL) and heated to 80 °C. Then, tetramethoxysilane (TMOS, 1.47 mL) as well as the premixed FITC-APTMS was injected into the CTAB solution, and the mixture was reacted for 2 h at 80 °C. The colloidal FITC-MSN suspension was centrifuged and then dialyzed for 72 h for the removal of CTAB. The final FITC-MSN powder was collected after dried in vacuum. Adult STII medaka fish (3 month-old males; 11 fishes per treatment) were treated with FITC-MSN suspension (10 mg $L^{-1}$) prepared in embryonic rearing solution (ERM) for one month continuously aqueous exposure. The suspension was changed every 2 days. Before taking confocal or fluorescent stereomicroscopic (FSM) images, all fishes were rinsed with clean ERM for half an hour to ensure the surface of the body was free of FITC-MSNs. The fish was then anesthetized for viviperception with FSM.

The SEM image in Figure 1 shows the size of the FITC-MSNs was around 20 nm. The nitrogen adsorption/desorption analysis also indicates that the FITC-MSNs exhibit high BET surface area of ca. 580 $m^2 g^{-1}$ and uniform BJH pore size of 2.5 nm (Figures S1 and S2[12]). Although the particle size measured from dynamic light scattering increased to around 160 nm, indicating some aggregation of the synthesized MSNs (Figure S3[12]), MSNs with such a small particle size still could disperse in solution uniformly, which is very important for the present application using fish. FSM images of the representative STII fish (selected from four fish each treatment) in the presence or absence of FITC-MSN exposure are shown in Figure 2. Strong fluorescence (Figure 2a), showing the aggregation of

  

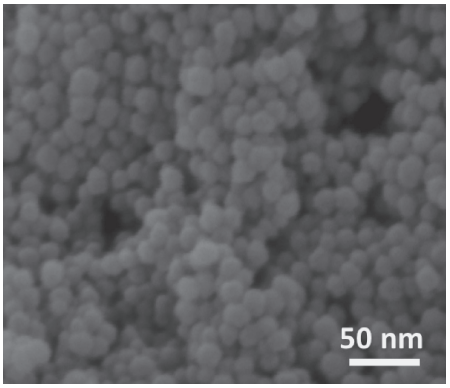


**Figure 1.** A typical SEM image of FITC-MSNs.

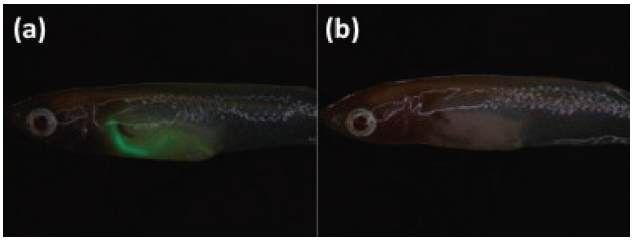


**Figure 2.** Fluorescent stereomicroscopic images of STII medaka fish exposed (a) with FITC-MSNs ($10\,mg\,L^{-1}$) for 2 days and (b) without FITC-MSNs (Photos shown here are representative combined images from fluorescent and light microscopes.).

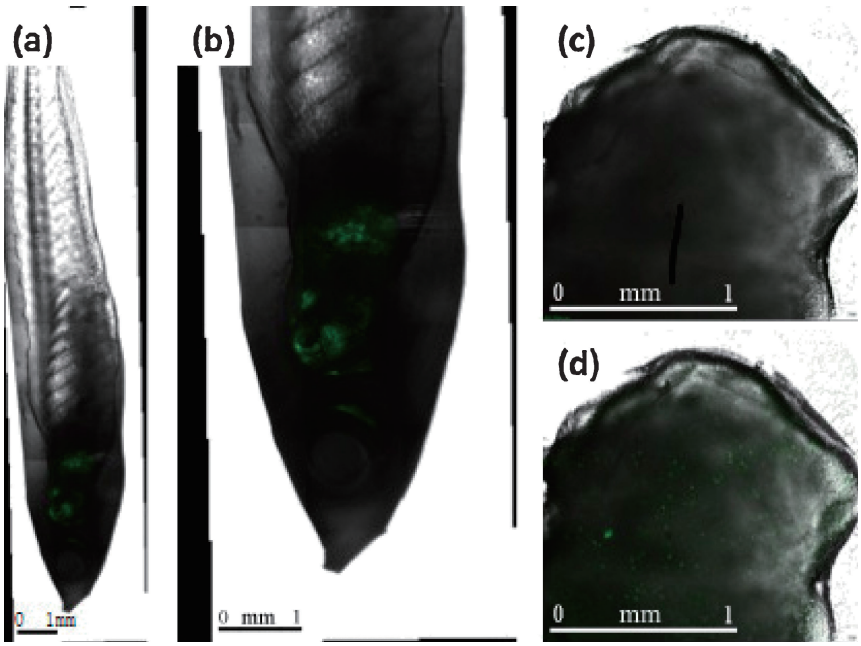


**Figure 3.** Confocal images of STII medaka fish following one month's aqueous exposure of FITC-MSNs and a one week elimination process. Whole fish image (a) and enlarged image between fish head and abdomen (b) of STII after 2 days elimination process; the surface (c) and middle (d) portion of the liver in STII after 7 days elimination process (Photos shown here are representative combined images from fluorescent and light microscopes.).

FITC-MSNs, was only observed in the fish intestine, indicating the MSN nanomaterials were mainly taken by the alimentary tract during aqueous exposure. On the contrary, the image of the control fish (no MSN exposure) (Figure 2b) shows that the autofluorescence of medaka organs was minute enough to be neglected.

The fish treated with FITC-MSNs was then reared in clean ERMs for a week to eliminate FITC-MSNs in the intestine. The confocal imaging of the above fish was performed in vivo, and the image is shown in Figure 3. It is clearly seen that FITC-MSNs had been eliminated from the fish intestine after 2 days excretion (Figures 3a and 3b). However, FITC-MSNs were still observed in several organs including gills and liver (Figure 3b). Particularly, FITC-MSNs were retained widespread at the middle portions of the liver in STII fish (Figure 3d), while FITC-MSNs were barely seen on the surface of the liver (Figure 3c). These results evidence that FITC-MSNs were uptaken not only by the alimentary tract (main adsorption route) but also through the gill during aqueous exposure. Also, the adsorbed MSNs were capable of penetrating cell membranes of several organs and then eventually bioaccumulated in the liver after one month aqueous exposure.

Individual variation between in vivo models usually hinders viviperception of organisms, so sometimes individual tracing of labeled NPs (e.g., fluorescent MSNs) is necessary to obtain real time information of cell imaging, drug delivery, and release or other clinical biomedical messages. By using FSM, confocal microscopy, FITC-MSNs and STII medaka, we can visualize nanomaterial distribution in vivo without sacrificing animals and thus make individual tracing possible. The bioaccmulative potency of MSNs in fish live could facilitate the development of medical therapy that can use MSNs as a promising carrier to

  

specifically deliver drugs, genes, or protein to liver of living animals. Future studies include the investigation of toxic potency of MSNs in medaka fish by using specific biomarker assays that reflect in vivo response from molecular, biochemical to physiological changes as well as elucidation of correlation between ADME of different types of functionalized MSNs and observed toxic effects.

In conclusion, we have successfully utilized FITC-MSNs as a fluorescent probe for in vivo visualizing its distribution and accumulation in the STII medaka fish. This research provides a cost-effective in vivo system that can facilitate real-time observation of dynamic behavior of nanomaterials in the living organism and a high throughput screening method of selecting effective and environmental benign biomedical NMs. In addition, MSNs with high surface area of ca. 580 $m^2 g^{-1}$ and uniform pore size of 2.5 nm would also provide potential applications in drug/biomolecules delivery into medaka fish for fundamental biological study.

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12 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

  