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Organic nanocrystals for nanomedicine and biophotonics

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1. Introduction

In this chapter, we will describe the advanced methodology for opening the new gate of nanomedicine and bioimaging using organic nanocrystals formulation, which is free from organic solvent and nano carrier. The organic nanocrystals aqueous dispersion is prepared by our originally developed technique, the reprecipitation method.

The importance of the contribution of nanotechnology to biomedical application has been greatly increased in few decades. Especially the current scientific endeavours on the researches of nanotechnology based nanomedicine and bioimaging have been giving us the novel and important information of biological events *in vitro* and *in vivo*. When focusing bioimaging and nanomedicine, we definitely treat the fluorescent dyes and drugs. However, currently used numerous organic compounds including drugs and fluorescent dye probes are hydrophobic in nature. Therefore, for their using in drug administration to human and animals or in dye staining imaging in living cells *in vitro* and *in vivo*, organic solvents are widely used for their increasing water solubility. However, organic solvent has not only the problems of causing systemic toxicity and cytotoxic activity, but also disturb obtaining accurately the biological experimental data, which may be modified by the influence of organic solvent. Many researchers have been making the scientific endeavour to clear these problems. Using nano carrier such as liposome, nanocapsule, nanosphere, dendrimer, emulsion, and surfactant have concluded successfully in some part of increasing water solubility of drugs and dyes. However, these carries still suffer from systemic toxicity and cytotoxic activity caused by their own. Additionally, the nano carriers have disadvantage of low amount loading of drugs and dyes in carrier and of their leakage.

To overcome these problems, recently the organic solvent and carrier free method for photodynamic cancer therapy and for bioimaging of living cells *in vitro* using organic nanocrystals are reported including our group (Baba et al., 2007 and 2009). Organic nanocrystals were prepared by the reprecipitation method (details are described in the flowing section 2). Employing these anticancer drug and fluorescent dye nanocrystals are superior in anticancer function and excellent fluorescent cell imaging, which are at least comparable with conventional manner. The crystals are most densely packed structure of molecules and nanocrystals are too. Therefore, the amounts of molecules in nanocrystals, which consist of 100% of molecules, are higher than that of the same size of nano carriers,

and nanocrystals are free from leakage problems. The details of using organic nanocrystals for nanomedicine and bioimaging are described in the section 3. Future perspective of organic nanocrystals in nanomedicine and bioimaging are described in the section 4. Until now, to our knowledge, the scientific researches dealing with pure organic nanocrystals for nanomedicine and bioimaging are quite less. In this chapter, we will introduce recent researches of organic nanocrystals in biomedical applications.

2. The reprecipitation method, which is the preparation method of organic nanocrystals

The reprecipitation method is bottom-up type of preparing technique of organic nanocrystals (Kasai et al., 1992), which is the solvent exchange method from good solvent to poor solvent. For example of the preparation, first the targeting hydrophobic compound for nanocrystallization is dissolved in good solvent acetone (in mM order), then the acetone solution (200 μl) is speedy injected into vigorously stirred poor solvent water (10 ml) using microsyringe. Then the sudden exchanges of solubility from good to poor cause the precipitation, and when the concentration, temperature, and kinds of solvent are adequately selected, nanocrystals are stably dispersed in water, with usually giving the negative charged ζ-potential on the surface of nanocrystals. The ζ-potential helps nanocrystals for preventing their aggregation because of the created balanced stability by negative charged repulsion force of them. The schematic images of the reprecipitation method are shown in Figure 1. The reprecipitation method is excellent for precise controls of their size and morphology compared with that of the other conventional top-down procedure such as milling method. The typical size control range is from several tens nanometres to several micrometers (Fig. 2). The imaging pictures of water dispersion of nanocrystals are shown in Figure 3. The transparent and solution-like appearances are because of the quite low light
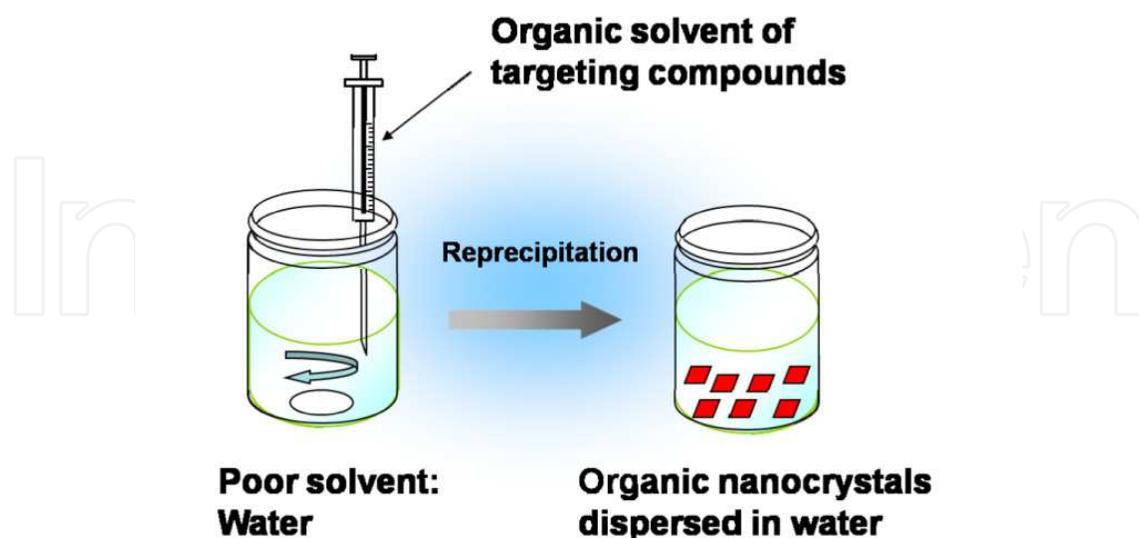

Fig. 1. The scheme of the reprecipitation method.

scattering originated in nano-sized crystals. These dispersions have both liquid and crystal characteristics. Therefore, we can directly measure the physiochemical properties of crystals as the liquid dispersions, or we can fabricate the nanocrystals-layered structure by collecting these nanocrystals from dispersion (Masuhara et al, 2001). Until now, our scientific endeavour revealed the size-controlled and size-dependent optoelectrical properties of functional organic nanocrystals such as nonlinear optical materials (Nakanishi & Katagi, 1998), π-conjugate functional dyes (Kasai et al., 1996), organic pigment for colour filter of liquid crystal display (Miyashita et al., 2008), and fullerene (Masuhara et al., 2009). Recently, numerous researches focusing on the optoelectrical property of organic nanocrystals have been reported by several groups (Zhao et al., 2008, An et al., 2004). However, the applications of organic nanocrystals on biomedical researchers are quite less (Sin et al., 2006, Bwambok et al., 2009), instead of their radical increasing importance. Therefore, recent our interest and challenging topics are applying organic nanocrystals technology toward the applications of drugs for medical treatments and fluorescent probe for bioimaging.
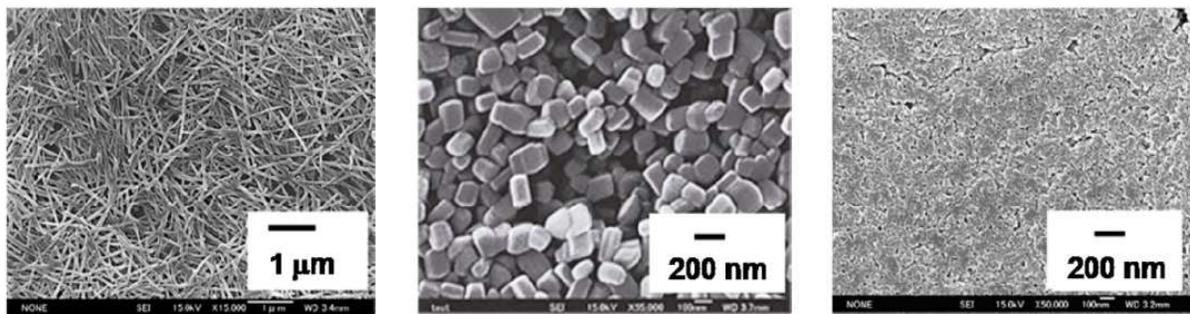

Fig. 2. Size control of organic nanocrystals in same compound.

Fig. 3. Imaging pictures of organic nanocrystals water dispersion.

3. The recent achievements of organic nanocrystals in nanomedicine and biophotonics

3.1 Organic nanocrystals used for nanomedicine

Recently, as with developing the nanoparticles preparation technology, several reports are mentioning about the fabrication technique of drug nanocrystals. However, almost all the reports are concerning about the drug formulation in industrial interest (Kipp, 2004). As our knowledge, quite few reports mentioning about the scientific aspects of using pure organic nanocrystals forms of drugs. Prof. Prasad groups firstly demonstrated the using of photosensitizing anticancer drugs in photodynamic cancer therapy (Baba et al., 2007). The concept of this study based on how to increase the water solubility of hydrophobic drugs without using solubilising agents such as surfactants and nanocarrier, which cause cytotoxicity, and also to exhibit the high efficacy of the drug.

Not only the photosensitizing drugs, but currently used many of pharmaceutical agents including various drugs are hydrophobic in nature (about 60% of drug products). Therefore, special formulations are required to make their aqueous dispersion for delivery of these drugs. Usually, surfactants or nanocarrier based delivery vehicles are used. In the cancer therapy treatment, once systemic administration of drugs are carried out, these nanocarriers including drugs are gradually taken up by tumour tissues according to the concept of the "enhanced permeability and retention effect" (Maeda et al., 2006), which rely on the characteristics of tumour tissues in trapping and retaining circulating nanoparticles because of their leaky stricture. The nanocarriers are member of such as liposomes, polymeric micelles, oil dispersions micelles, polymeric nanoparticles, drug encapsulated polymer complexes. However, such carriers tend to increase the systemic toxicity caused by own. Therefore, there is increasing interest in the developing the novel type of drug formulation and delivery approaches without auditioning any solubilising agents such as surfactants and/or nanocarriers. One novel method proposed for this was using the reprecipitation method. Though the organic nanocrystals formations of hydrophobic compounds have been well studied in optoelectrical materials, there was no report of using this method for drug delivery.

To demonstrate the concept, the nanocrystal formulation of the hydrophobic drug of photodynamic therapy was selected, and its efficacy was compared with the conventional surfactant-supported formulation. Photodynamic therapy is a promising approach for curing several types of cancers as well as some dermatological and ophthalmic diseases such as age meditated macular degeneration. The main advantage of photodynamic therapy compared with the conventional cancer chemotherapy is in the localized treatment using selective light exposure to the tumour tissue. Usually, photodynamic therapy is carried out by systemic administration of photosensitizing drugs, followed by exposing the light to the tumour tissue. Basically, visible or near-infrared light are selected, and after the light is exposed, the photosensitizing molecules transfers their excited state energy to oxygen molecular in the surroundings, and as the result, reactive oxygen are formed, so-called singlet oxygen. The singlet oxygen has ability to distract the structure of cells, and cell deaths are induced in tumour tissue. Though the destruction process requires the combination of photosensitizing, light, and oxygen, there is enough advantage for photodynamic therapy in selective distraction of tumour tissues. However, one of the major problems of photodynamic therapy is in the poor water solubility of photosensitizing drugs, thus making their stable formulation for systemic administration are highly required. One of

the overcoming these problems are using solubilising agent such as nanocarrier. However, these nanocarriers often have the problem of drug leakage from carriers, resulting in fewer efficacies. Furthermore, rejection reactions caused by nanocarriers are one of the anxiety problems. Therefore, the ideal formulation for safe and for good efficacy photodynamic therapy requires the solubilising agent free method. Thus, in this time, as new method for the delivery of water insoluble drugs, which is free from using additional solubilising agents was reported. This new drug formulation was fabricated by the reprecipitation method. The resulting nanocrystals were monodispersed and taking stably dispersion, with diameter about 100 nm.

 In this research, there was an interesting finding that the fluorescence and photodynamic activity of the drug nanocrystals were initially quenched in aqueous media because of the peculiarity of crystal structure; however both recovered under *in vitro* and *in vivo* conditions with the treatment of serum, resulting in creating molecular form. The recovery of fluorescence and photodynamic activities were verified by confocal fluorescent microscopy observation of cells and cellular phototoxicity assay. Efficacy of the nanocrystal formulation *in vitro* as well as *in vivo* was found to be comparable with that of the same drug formulated in the conventional surfactant delivery vehicle. This study was first example of the use of organic nanocrystals prepared by the reprecipitation method.

3.2 Organic nanocrystals used for bioimaging

Fluorescence microscopy observation is one of the most broadly utilized imaging techniques in biomedical researches, which allows the noninvasive imaging of cells and tissues with molecular specificity. These imaging requires fluorescent dyes to enter cells and tissues before visualization. Currently used dyes include classes of the coumarin, rhodamine, fluorescein, and carbocyanine, as well as their derivatives. 3,3′-Dioctadecyloxacarbocyanine perchlorate [DiO: DiOC18(3)] is the long-chain dialkylcarbocyanine dye, and commonly used for visualizing the anterograde and retrograde neuronal tracers in living cells. This lipophilic carbocyanine is also employed for many other applications, including the tracking of cell migration and lipid diffusion in membranes through fluorescence recovery after photobleaching (Gordon et al., 1995), the labeling of lipoproteins (Lohne et al., 1995), and cytotoxicity assays (Johann et al., 1995). Nevertheless, the hydrophobic nature of these dyes require, in many cases, organic solvents [e.g. dimethylsulfoxide (DMSO) and dimethylformamide] and surfactants for successful cell imaging with increased water solubility of dyes. However, unfortunately, organic solvents and surfactants themselves tend to increase cytotoxicity *in vitro* and *in vivo*. On the other hand, the direct applications of micronized crystals have also been investigated, although such crystals are probably not small enough to allow the diffusion of dyes into cells, thus imaging efficency is poor.

Furthremore, not only DiO dyes, but also many of other fluorescent dyes are hydrophobic in nature, including perylene, which is widely studied hydrophobic material as functioning with high quantum yield in organic electroluminescence devices. Although using perylene is potentially useful for bioimaging, the preparation of aqueous dispersions of perylene dyes requires special formulation techniques, similar to those of DiO. One of the very useful technique for dispersing hydrophobic compounds in water is the "reprecipitation method", which has been used for the nanocrystallization of organic optoelectronic materials exhibiting size-dependent optical properties. Recently, by using this reprecpitation mehod, we demonstrated an organic solvent free bioimaging method employing nanocrystals of

hydrophobic fluorescent dye, and their applications to *in vitro* were evaluated by confocal fluorescent laser microscopy observation (Baba et al., 2009). The fluorescent dyes that we used were DiO and perylene.

The aqueous dispersions of DiO and perylene prepared using the reprecipitation method have been dialyzed for 6 h to remove acetone, thereby resulting in organic solvent–free dispersions were obtained (Fig. 4). The resulting particle sizes and morphologies were less than 150 nm for the DiO nanocrystals and 155 nm for the perylene nanocrystals, which was revealed by scanning electron microscopy observation, and these values correspond reasonably well with those determined through dynamic light scattering measurements (Fig. 5). The average ζ-potentials of the DiO and perylene nanocrystals were ca. +36 and –10 mV, respectively. The relatively high positive surface charge of the DiO nanocrystals, presumably derived from the positively charged molecular structure, imparted this water dispersion with excellent stability. In the case of the perylene nanocrystals, the factors causing their negative surface potential remains unclear, although negative charging of organic nanocrystals in water is well known empirically to inhibit particle aggregation. The aqueous dispersion of perylene nanocrystals was stable for more than 3 months. The crystallinity of their prepared DiO and perylene nanocrystals were confirmed by powder X-ray diffraction analysis.

The obtained UV–Vis absorption and fluorescence emission spectra of these nanocrystals are significantly different from that of their solution form, namely we observed suppression and broadening in the absorption spectra and decreases in emission intensities. The decreases in fluorescence intensities are well-known aggregation effects for fluorescent molecules. In our case, this phenomenon was due to the low solubility of the hydrophobic dyes, with corresponding aggregation effects in aqueous system. A decreased fluorescent yield will induce a decreased imaging efficiency.
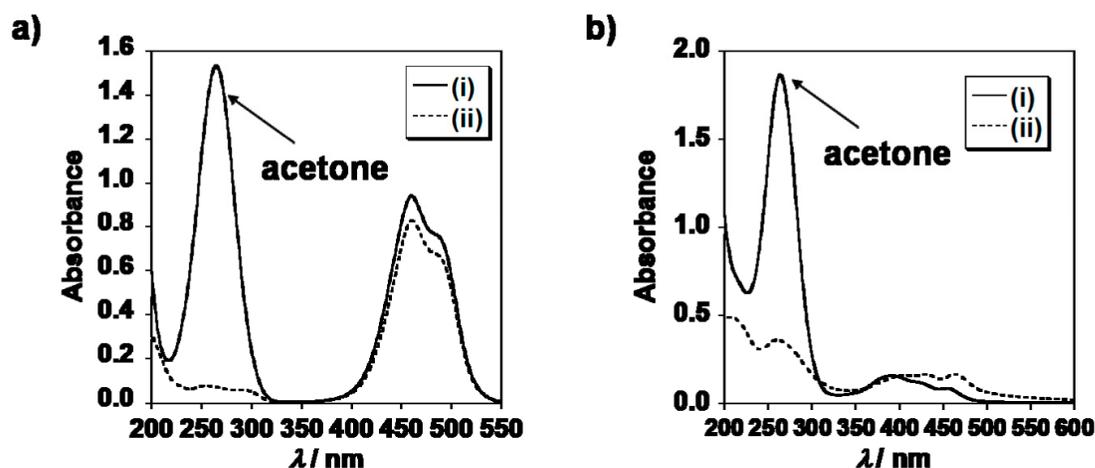

Fig. 4. Absorption spectra of water dispersions of (a) DiO nanocrystals and (b) perylene nanocrystals. (i) before and (ii) after dialysis. The disappearences of the absorbance of acetone (at λ_{max} = 265 nm) were confirmed.
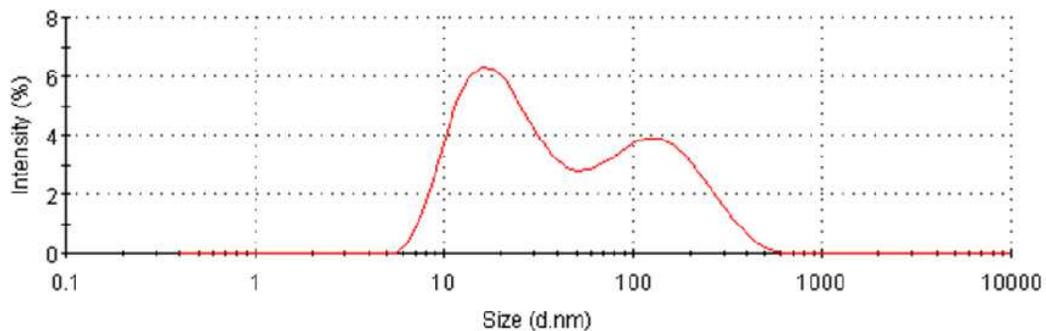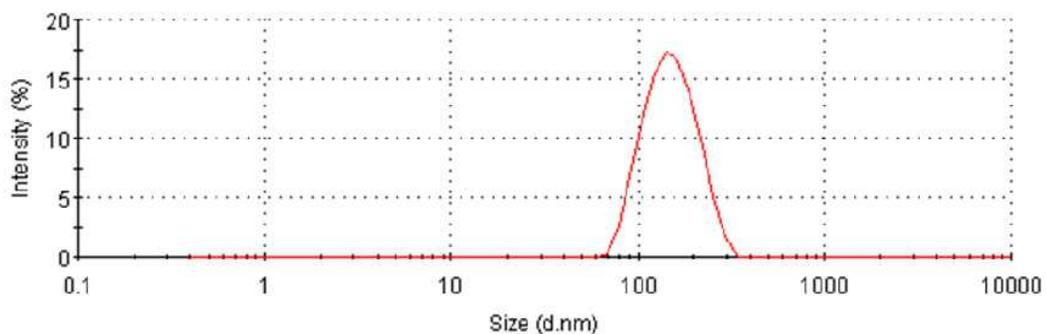
Fig. 5. Size distributions of (a) DiO and (b) perylene nanocrystals dispersed in water. The peak sizes of the DiO particles were 22 nm (intensity: 58%) and 146 nm (intensity: 42%); the peak size of the perylene particles was 155 nm (intensity: 100%; i.e., monodispersity).

However, there was interesting finding that the fluorescence of the DiO and perylene nanocrystals were recovered by elapsed time under *in vitro* conditions in the presence of 10% fetal bovine serum. It seemed that this recovery in fluorescence in the presence of fetal bovine serum induced the DiO and perylene nanocrystals to reasonably good soluble. Although the exact mechanism for the improved solubility is not cleared yet, but we consider that it may rely on the affinity interactions between the hydrophobic moieties of the proteins and the hydrophobic surfaces of the nanocrystals, which cause the dissolution of nanocrystals, resulting in converting nanocrystals into the molecular forms of the dyes, thereby, leading to fluorescence recovery.

(a)

(i)   **(ii)**

(iii)   **(iv)**

(b)

(i)   **(ii)**

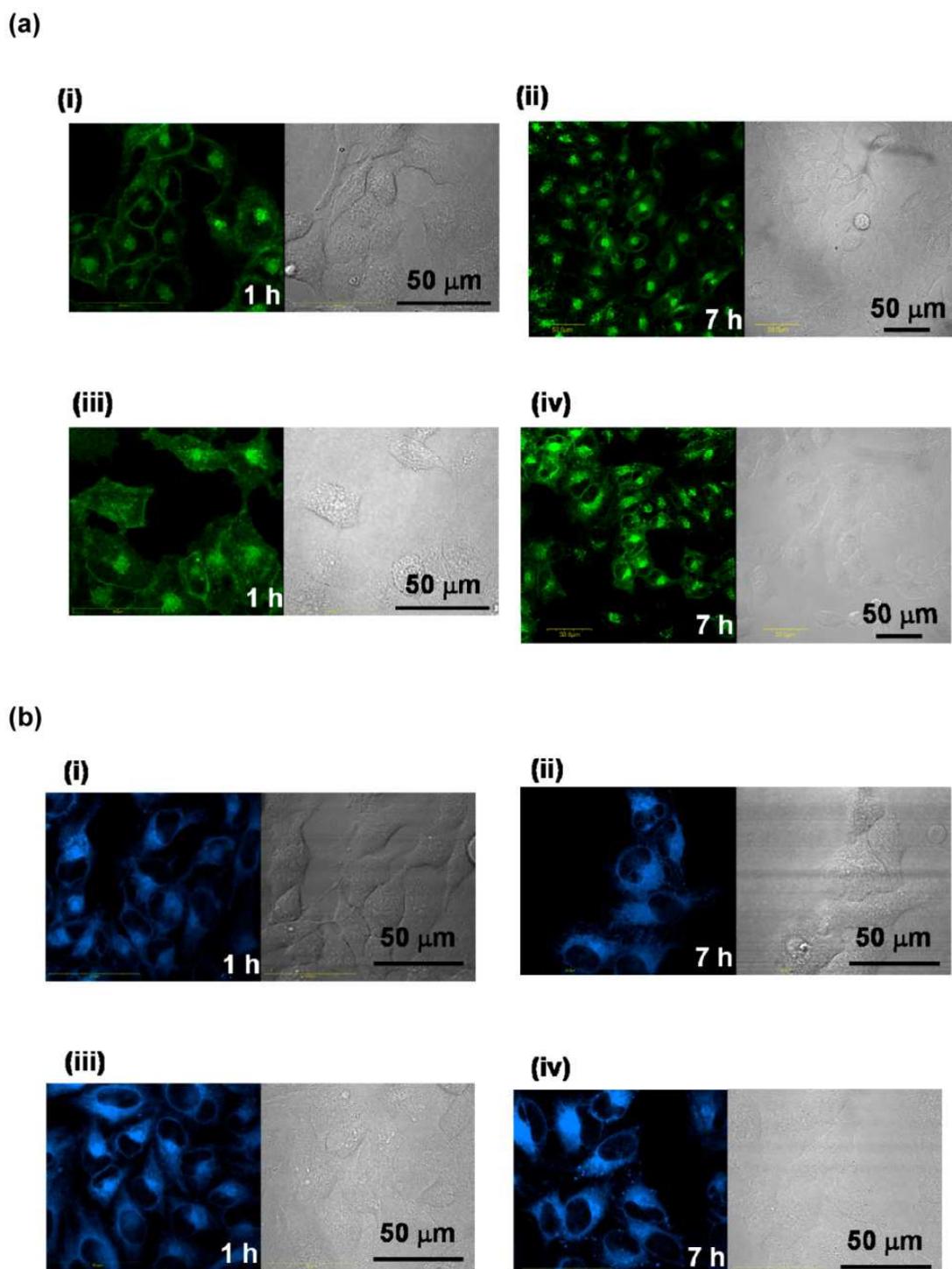**(iii)**   **(iv)**

Fig. 6. *In vitro* fluorescent confocal images of cells recorded 1 h and 7 hrs after incubation with (i), (ii) nanocrystals of (a) DiO and (b) perylene, and (iii), (iv) dyes solution in DMSO of (a) DiO and (b) perylene.

To evalute if the organic nanocrystals were useful for the bioimaging of living cells *in vitro*, we performed confocal fluorescence imaging of tumor cells using the DiO and perylene

nanocrystal formulations (Fig. 6). Although the fluorescence signals in the nanocrystals were initially weak, it increased after cellular uptake (1 h) as similar to the behavior of nanocrystals in the fetal bovine serum-containing medium in bothe case of perylene nanocrystals and DiO nanocrytals. Compared with the using of solution form of dyes disolved in DMSO, where exhibiting higher fluorescence signals from at the initial stage of dye dosing in culture medium, nanocrystals revealed comparable levels of cellular uptake by elapsed time. Therefore, the long-term cellular uptake of the DiO and perylene dyes in their nanocrystal formulations were similar to that of the dyes in their solution formulations. The DiO dyes specifically stained the cell membrane; the perylene dyes stained the cytoplasm.

On the other hand, we investigated whether nanocrystal formation was really necessary for efficient cell imaging, especially remarking on the crystal size. Aqueous dispersions of microcrystals, with the average sizes were in the micrometer range, were prepared through sonication of the bulk crystals in water. The concentrations of these dispersions were adjusted to be the same as those of the nanocrystals. Figure 7 displays scanning electron microscopy images of the DiO and perylene microcrystals used for cell imaging. In both cases, their sizes were on the order of several to a few tens of micrometers. In microcrystals, no significant recovery in fluorescence occurred in the cell culture medium containing 10% fetal bovin serum during 6 hrs after the addition of the DiO microcrystals, and whereas a slight recovery of fluorescence in the case of the perylene microcrystals was observed. Presumably, one of the main reasons for the significantly lower fluorescence recoveries of the microcrystals was in their size. When the size of a crystal increases, its surface area per unit volume decreases, as a result, its interactions with the serum components become weak, and resulting in decreased solubility. Figure 8a presents the confocal microscopy images we obtained when applying the DiO microcrystals. Interestingly, only the surrounding areas where the microcrystals were attached to the cells
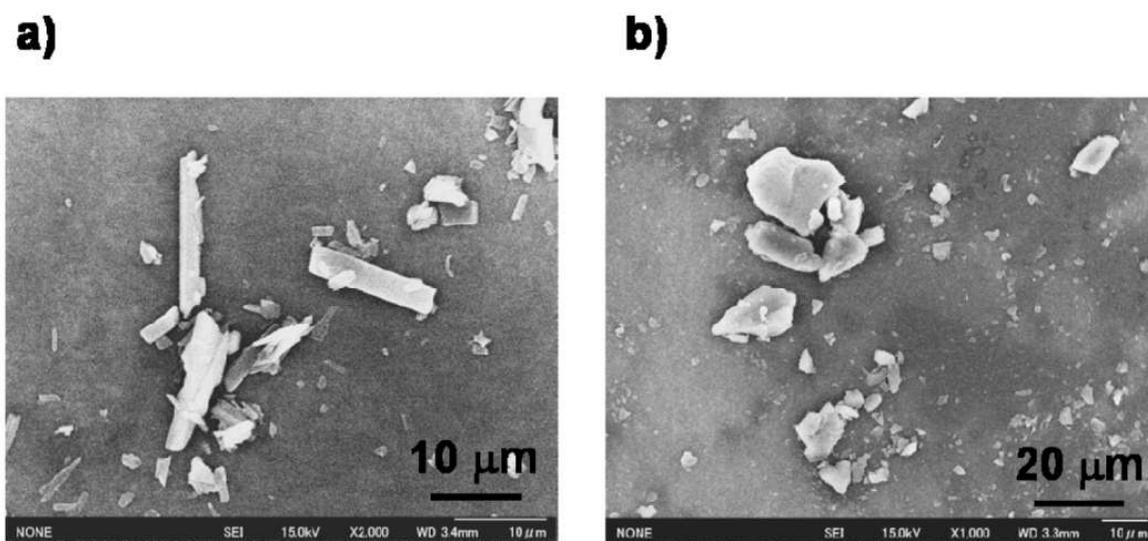

Fig. 7. Scanning electron microscopy images of (a) DiO and (b) perylene microcrystals.

displayed fluorescence (e.g., yellow circle in Figure 8a). The direct application of DiO microcrystals to fluorescence imaging of cells is a commonly used technique. Nevertheless, we observed no fluorescence from those cells not presenting any attached DiO microcrystals,

meaning that this imaging technique is poorly efficient. Figure 8b reveals that, in the case of the added perylene microcrystals, the fluorescence imaging was less efficient than that obtained using the corresponding nanocrystal formulation (Fig. 6b, ii) Clearly, the low solubility of the perylene microcrystals in the serum led to poor imaging. Additionally, we also imaged cells lacking added dyes as a control, and we observed no fluorescence signals under the corresponding measurement conditions (Fig. 8c). Thus, the fluorescence images we obtained using nanocrystals, dyes in DMSO treatments, and microcrystals were not the result of autofluorescence of the cells.
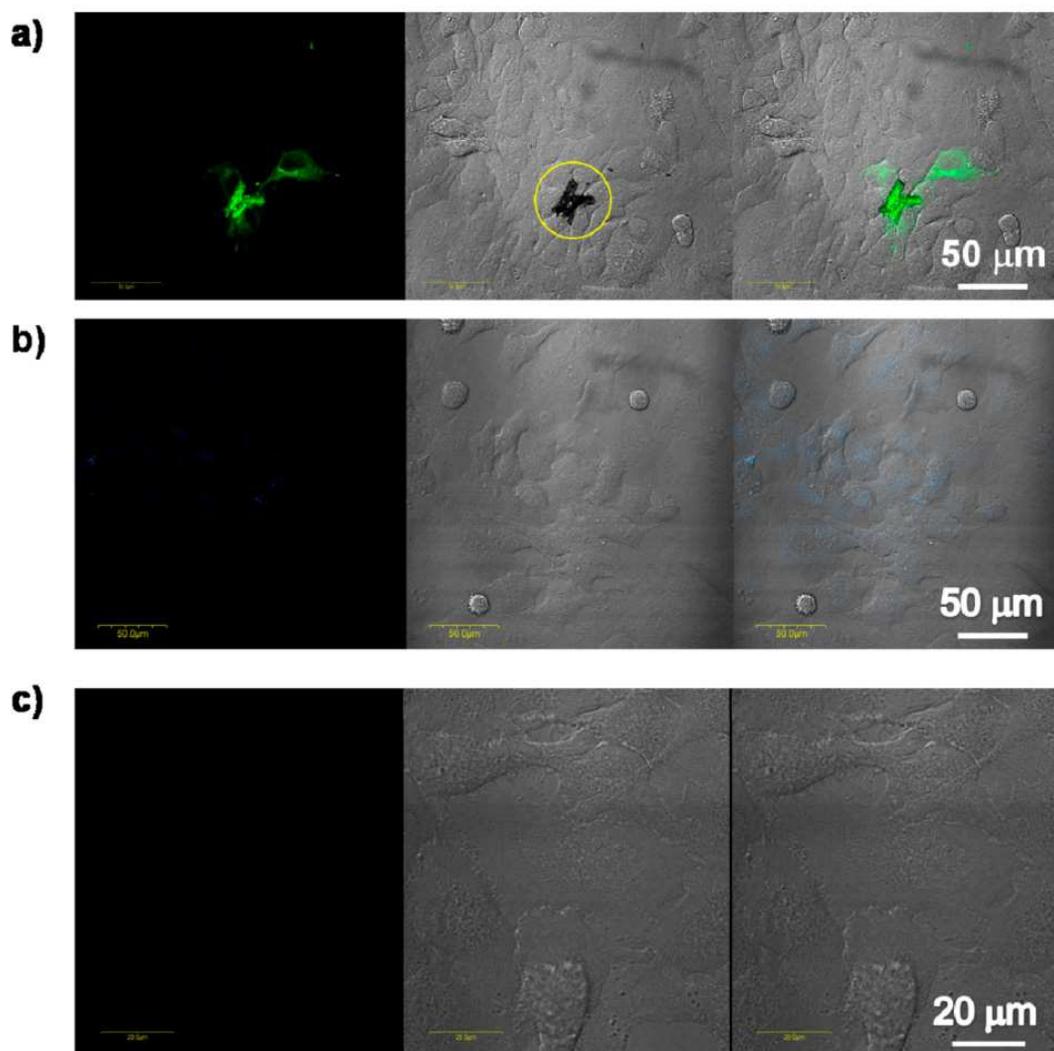

Fig. 8. Fluorescent confocal images of (a) DiO microcrystals, (b) perylene microcrystals, and (c) control: the pictures of fluorescent images (left), tranceperant images (middle), and overlaped images of the left and middle (right). The yellow circle in (a: middle) represents the microcrystals. These fluorescent images were obtained after 7 hrs incubation.
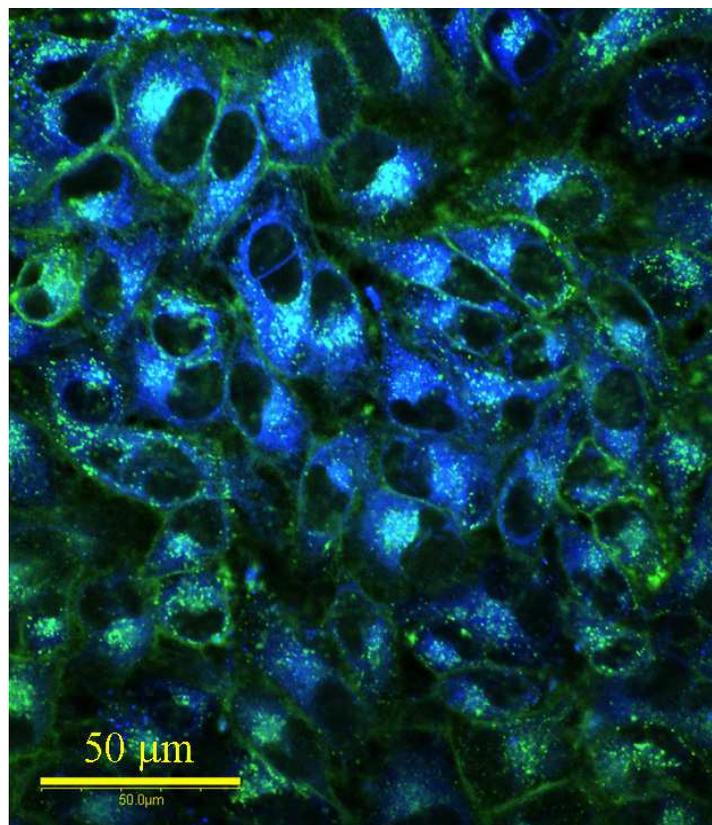
Fig. 9. Double staining images of cells using DiO and perylene nanocrystals. The green and blue fluorescent colours come from DiO and perylene, respectively.

When we investigated double staining imaging of both the DiO and perylene nanocrystals in cells in culture medium we initially detected each fluorescence signal individually by confocal fluorescent laser microscopy observation, and Figure 9 presents the over laped pictures of them. DiO resulted in significant membrane staining (Fig. 9: green color), whereas perylene stained in mostly cytoplasmic (Fig. 9: blue color). One of the reason for this behavior might be due to differences in ζ-potentials of nanocrystals/dyes, namely the DiO particles had a positive ζ-potential (+36 mV), which may have aided in their trapping on the slightly negatively charged cell membrane. We also confirmed that the fluorescence obtained were not the result of autofluorescence of the cells. Although the detail mechanisms of endocytosis of the nanocrystals/dyes remained unclear, we were successful in recording a double-staining cell images using the formulation of organic nanocrystals.

3.3 Application of the reprecipitation method toward dye doped polymeric nanoparticles

We will introuduce the other approach for fabricating bioimaging tools using the reprecipitation mehtod, where the fluorescent dye doped biodegradable polymer nanoparticles were demonstrated (Baba et al., 2005). The polymer used was poly (D,L-lactide-co-glycolide), and the dyes used were infrared emitting dyes and two photon excitable fluorescent dyes. Current technologies in fluorescent imaging have advanced significantly in developing for multiprobe applications in the study of biological events. Especially, for further advanced fluorescent imaging capability, organic fluorophores absorbing and emitting in near infrared region have been developed. Such near infrared

dyes have the advantage for providing the better signals avoiding the autofluorescence caused by ultra violet or visible light. Secondly, tissue penetration of near infrared lighg is great due to low absorption of excitation light in tissue. However, many of these dyes have less solubility and emissions in aqueous systems, making them undiserable for biological applications. The infrared emitting dyes used as demonstration shows fluorescence around 1.1 to 1.35 μm in organic solvents, but was significantly quenched in aqueous media. Whereas, two photon excitable fluorescent dye has a polar D-π-A structure (Lin et al., 2004), in which the π-system is end-capped by an electron donor (D) and an electron acceptor (A), having one of the most effective molecular models for both second- and third-order nonlinear optical materials, thus such dyes have great potential for two photon imaging. However, as with these dyes lacks the solubility properties for the application of biological systems. The nanoparticle technology had the ability to encapsulate such hydrophobic dyes into some matrix for producing the aqueous dispersion, enabling functionality in biological systems. For example, the fabrication of dye-doped polymeric nanoparticle water dispersions were quite effective method for bioimaging of such hydrophobic fluorescent dyes. The biodegradable poly (D,L-lactide-co-glycolide) were useful for preparing particles, which have been well investigated as a drug and gene delivery vehicle (Jain, 2000). The applying of the reprecipitation method, which is simple and surfactant-free fabrication method, for the incorporation of hydrophobic, near infrared emitting dyes and two photon excitable emitting dyes into poly (D,L-lactide-co-glycolide) nanoparticles resulted in creating an aqueous dispersion of nanoparticle, with their sizes of less than 100 nm. Then, the optical properties of dye solution, dye nanocrystals water dispersion, and dye doped polymeric nanoparticle water dispersion for these two dyes were investigated, respectively. For infrared emitting dyes, the fluorescence from dye solution was in the spectral range of ~1.1 to 1.35 μm. Whereas, in the case of dye nanocrystal water dispersion, even at the same concentration of dye solution, the emission was not noticeable. This was because of the fluorescence quenching, estimated to be including collisional quenching, static quenching, excited state reactions, electron transfer and energy transfer. The main reason for dye fluorescence quenching in water dispersion might comes from the crystallization of dye, as it is well known for several kinds of organic molecules. Similar changes in absorption were usually associated with the appearance of the molecular *H*-aggregates, which were nonradiative. However, when fabricating the dye doped poly (D,L-lactide-co-glycolide) nanoparticle water dispersion, it exhibitted fluorescence in the aqueous environment. A series of systematic studys of the relationship between fluorescence and micelle microemulsion in water environment has been reported. Our results also indicated that dye was successfully encapsulated in the polymer matrix. Thus, crystallization of dyes were prevented, and also dyes were protected from contact with the water environment, thus fluorescence quenching was avoided. In the case of two photon excitable emitting dyes, optical properties were investigated especially forcusing on dye nanocrystals and dye doped polymeric nanoparticles. The size of dye nanocrystals was 35 ± 9 nm, and the sizes of these polymeric nanoparticles were controlled as ca. 36 ± 8 nm, 50 ± 11 nm and 96 ± 19 nm, respectively, using the reprecipitation mehod. According to the procedure of the reprecipitation mehod, when the initial concentration of polymer solution increased, the size of polymeric particles also increased. There have been several reports on fabrication of poly (D,L-lactide-co-glycolide) particles with sizes from several hundreds nanometer to few tens micrometer**.** However, polymeric nanoparticles less than 100 nm in size were succesuffly

prepared. Even though, two photon excitable fluorescent dye nanocrystals showed fluorescence in aqueous media, the fluorescence intensity of dye-doped polymeric nanoparticles was higher than that of the dye nanocrystals. Furthermore, we observed the increasing fluorescence intensity of dye doped polymeric nanoparticles, with an increasing in their particle size. The role of the polymer for influencing the fluorescence intensity should be similar to the case of infrared emitting dyes. Namely, when two photon excitable fluorescent dyes were surrounded by the polymer matrix, the crystallization of dyes and contact of dyes with the water environment were prevented, resulting in increasing fluorescent intensity. The reason for increasing the fluorescence intensity with an increasing the polymeric particle size was that when the ratio of polymer to dye increased, correspondingly, number of dyes individually embedded in the polymer matrix increased without interaction between dyes. The uptake of two photon excitable fluorescent dye nanocrystals and their dyes-doped polymeric nanoparticles by cancer cells in culture media were investigated. Confocal laser microscopy of two-photon imaging revealed a difference in the fluorescence intensity and staining pattern of fluorescence in cells incubated with dye nanocrystals (34 ± 11 nm in size) and the dye-doped polymeric nanoparticle (32 ± 10 nm in size), respectively. The fluorescence intensity obtained from cells incubated with dye-doped polymeric nanoparticles were about ten times higher than that of dye nanocrystals. These results suggested that the coating of fluorescence dye by adequate polymer was an important methodology for efficient cell imaging. We can say that not only nanocrystals formation, but alos dye-doped polymeric nanoparticles prepared by the reprecipitation mehod are effective way for efficient bioimaging.

4. Future perspective of organic nanocrystals in nanomedicine and biophotonics

4.1 Organic nanocrystals for biophotonics

Herein, we will report the new type anti-photo breaching fluorescent organic nanocrystals used in bioimaging, which have the potential to overcome the problems of quantum dots and molecular probe. Currently, using quantum dot and molecular probe for bioimaging and nanomedicine are numerously reported. Quantum dots have been applied for detection and imaging in several areas in the life sciences, ranging from microarray technologies to fluorescence *in situ* hybridization to *in vitro* imaging (Medintz et al., 2005). Despite many superior optical properties, such as size-tuneable absorption and emission, extremely broad and intense absorption enabling a unique flexibility in excitation, high fluorescence quantum yields even in the NIR wavelengths and large two-photon action cross-section as compared to organic dyes, the solutions for using quantum dots have so far been individual ones. Mainly, quantum dots need surface modification by complicated procedure to increase the water solubility and to avoid the cytotoxic caused by leaking of heavy metal ions. Additionally, we need patient to find a solution to the challenges of their particular experimental system against the benefits of the advanced spectroscopic features of quantum dots. On the other hand, the optical property of the molecular probe depend on the electronic transitions involved and can be fine-tuned by elaborate design strategies if the structure-property relationship is known for the given class of dye (Mason, 1999). The emission of organic dyes typically originates either from an optical transition delocalized over the whole chromospheres or form intramolecular charge transfer transitions. The

majority of common fluorophores, such as fluorescein, rhodamines, and most cyanines, are resonant dyes that are characterized by slightly structured, comparatively narrow absorption and emission bands that often mirror each other, a small solvent polarity-insensitive Stokes shift, high molar absorption coefficients, and niderate-to-high fluorescence quantum yields. The major problems of these dyes are both less water soluble in nature, thus need organic synthesis making hydrophilic salts form or using solubilising agents, and the weak resistance against photo breaching. We recently developed the new type of fluorescent organic nanocrystals for bioimaging. These organic nanocrystals have advantage in high water solubility, cytotoxicities-free caused by heavy metal ions, and quite strong against photo breaching. The organic nanocrystals were class of organic pigments, quinacridone deliverities. The organic pigments are very rigid compound, which have strong light resistance and weather fastness, thus have been used for several applications such as colour filter and coating materials. Therefore, making water dispersion of organic pigments is quite difficult because of their poor water solubility. However, we found that if applying the reprecipitation method, fine dispersion of quinacridone nanocrystals were obtained (Ujiiye-Ishii et al., 2006). Under the confocal laser fluorescent microscopy observation after dosing quinacridone nanocrytals in cell culture medium, we have been successfully obtained fine and tough fluorescent imaging during nearly 15 min in laser irradiation; despite the output power of laser source was nearly 100%. One can see that almost no fluorescent breaching was observed in the imaging pictures at the beginning and the ending of the observation (Fig. 10). Despite the merit of strong light resistance originated from crystal structure, the fluorescent intensity of quanicridone nanocrystals were not strong like such as fluorescein because of the quenching effect caused by crystallization. Thus, we are now further investigating to find the new approach that satisfies both high fluorescent intensity and light resistance. We believe that these kinds of right resistance fluorescent organic nanocrystals are candidate for new type of bioimaging tools in near future.
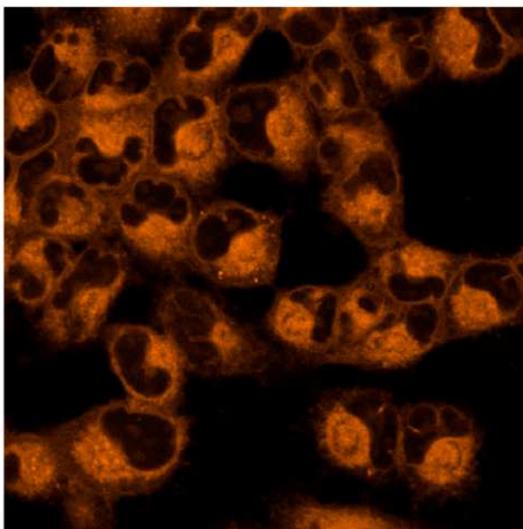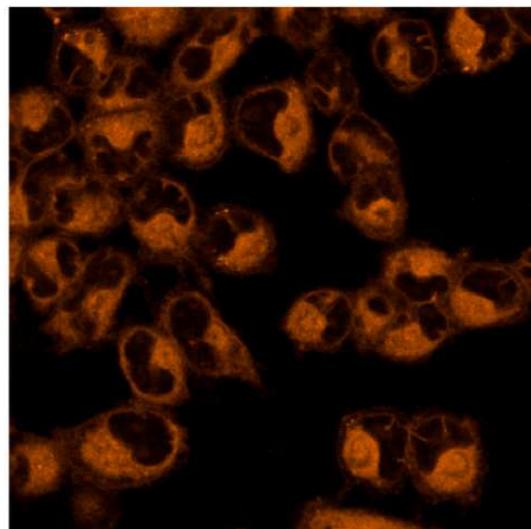

Fig. 10. Fluorescent confocal images of cells stained by quinacridone nanocrystals. The fluorescent images observed at the (a) beginning stage and (b) ending stage.

4.2 The size effect of nanocrystals for nanomedicine and biophotonics
The size is the important factor in biological events especially for cellular uptake, systemic circulation in the case of systemic administration, and solubility in living organism, which affect the efficacy of drugs and efficient fluorescent cell imaging. Actually, we found that hydrophobic nanocrystals that had poor solubility in water had good solubility in serum component, and which is peculiar to nano-sized crystals and not to more than micro-sized crystals. These findings give us the very important information for considering the strategy in nanomedicine and bioimaging for the selection of efficient and appropriate approaches. For the advanced applications, one of the strategies we can select for using organic nanocrystals in bioimaging and nanomedicine is taking the step of designing molecular structure at first, which have the hydrophobic characteristics and the sight specific targeting moiety. If the fluorescent dyes and drugs are hydrophobic, their stable organic nanocrystals aqueous dispersions are well prepared by the reprecipitation method. Upon, taking administration of these nanocrystals *in vivo* and *in vitro*, the nanocrystals will start dissolving in living organism, with gradually generating the drugs/dyes activity by taking molecular formulation. If the drugs and dyes that have site specific moiety, the drugs and dyes can bind to the desired part such as affected site and cells with minimally dosing with specifically. The dissolving kinetics, disposition, and body distribution of nanocrystals will be basically aligned by controlling the size of particles. These favourable peculiarities will be achieved by without applying any external solubilising agents such as organic solvents, surfactants, and nanocarriers. This nanocrystal technology in the fields of biology should be applied for not only limited scientific researches, but also for medical treatment in near future. This simplest nanocrystal approach, which we have demonstrated, will be mostly close way toward the clinical approach, which holds down the risk caused by carries and the cost caused by manufacturing. We can say that the nanocrystals aqueous dispersion or their dried powder will be useful for drugs/diagnostic fluorescent agents in such as administrations of oral, intravascular, inhalational, transdermal, nasotracheal, and ocular.

5. Conclusion

In this chapter we described three topics; first, we explained how to prepare the organic nanocrystals in aqueous dispersion system using the reprecipitation method. Second, we referred the recent our achievements of organic nanocrystals in nanomedicine and biophotonics. Third, we remarked the future direction of organic nanocrystals in nanomedicine and biophotonics. We believe that our organic nanocrystals technology, recent results, and ideas will be helpful especially for biochemist, biophysicist, nanoscientist, and medical scientist for tremendous advances in their specialized fields and in their multidisciplinary.

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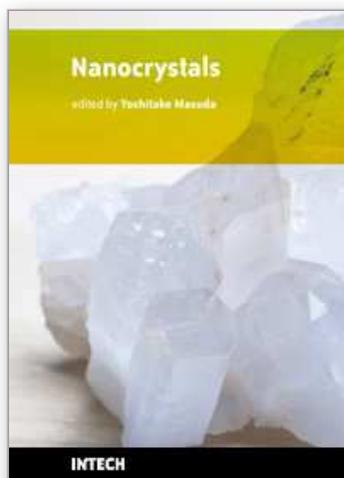
Nanocrystals

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This book contains a number of latest research developments on nanocrystals. It is a promising new research area that has received a lot of attention in recent years. Here you will find interesting reports on cutting-edge science and technology related to synthesis, morphology control, self-assembly and application of nanocrystals. I hope that the book will lead to systematization of nanocrystal science, creation of new nanocrystal research field and further promotion of nanocrystal technology for the bright future of our children.

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