Nanoparticle Synthesis in Vesicle Microreactors

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1. Introduction

Numerous microorganisms such as *E-coli* and yeast are capable of synthesizing inorganic micro- or nano-structures including gold, silver, CdS, ZnS and calcium/silicon-based materials in their intra- or extra-cellular matrix (Sanchez et al, 2005; Mandal et al, 2006; Bhattacharya et al, 2005). Even though the widespread speculations propose that a few enzymes or peptides present in the organic matrix (mainly in cell walls and periplasmic space) act as reducing and nucleation sites (Mukherjee et al, 2001; Ahmad et al, 2002; Dameron et al, 1989; Umetsu et al, 2005; Kröger et al, 2006; Naik et al, 2002), the molecular basis for the biosynthesis of these materials is not well established. Currently, there are two main directions of research in this field. The first one, as widely developed by Naik's group, for review see e.g. (Dickerson et al, 2008), is to use the technique of phage display library to screen potential peptide sequences which could selectively recognize inorganic and metal ions for material synthesis. Alternatively, the second direction is to directly perform chemical and physical structural analysis on biomolecules from biological organisms (Evans, 2008; Killian et al, 2008; George et al, 2008) and eventually mimic them by synthetic molecules (Kato et al, 2010; Meldrum and Cölfen et al, 2008; Sommerdijk et al, 2008). A progressive step along this second direction was recently reported by Nagasawa, Kato et al about calcium carbonate synthesis mediated by the Pif protein found in the pearl oyster *Pinctada Fucata* (Suzuki et al, 2009).

Although the above-described *in vitro* studies have been extensively reported, *in vivo* tests of the material mediation abilities of the biological and synthetic molecules may offer a potential for biologically inspired material synthesis (Naik et al, 2008). For this purpose, another key focus besides functional templating molecules is how to encapsulate (envelop) these molecules in biological organisms for performing targeted mediation functions. Nature already gives us a good answer to this question, that is, the cell membrane provides a good encapsulation function of enveloping and microcompartmentalization of reactive and functional molecules (Sweeney et al, 2004; Kloepper et al, 2005; Mukherjee et al, 2001). Thus, a desirable future research direction aims at encapsulating functional or reactive molecules in cell membranes and/or analogous model systems for evaluating their biomimetic mediation ability for material fabrication *in vitro* and *in vivo*. For instance, Iverson et al reported an interesting finding that semiconductor CdS nanocrystals, around 2 - 5 nm in diameter could be formed intracellularly by *E-coli* (Sweeney et al, 2004). However, direct use of the cell imposes obstacles, because of the following factors: First, cells, even the simplest

form, have complex supramolecular structure, which usually shields the desired reaction from direct observation. Second, their fragile body also makes it difficult to apply harsh characterization techniques on them, which otherwise are widely employed in artificial materials science. Therefore, a good approach to overcome these difficulties is to use simplified cell analogs as an alternative to perform such studies.

Vesicles could be considered as an unique kind of "a simplified cell", i.e. an artificial container enclosed by a self-assembled envelop (Dimova et al, 2006 and 2007; Smith et al, 2007; Tang et al, 2006; Christensen et al, 2007; Hales et al, 2006; Venturolia et al, 2006; Jelinek et al, 2007; Luisi, 2007; Morigaki et al, 2007; Walde et al, 2010). This kind of self-assembled envelope mimics the basic skeleton of the cell membrane, while excluding many other complex functional blocks embedded in it. Accordingly, this kind of structure is very interesting and promising for biologically inspired research and future applications. It has been demonstrated that vesicles are able to provide a very flexible model for cell-based functional researches, such as photosynthesis, enzyme function profiling, gene synthesis and expression etc. (Tung et al, 2003; Walde et al, 2001; Yukito et al, 1996; Luisi 2007). Whereas direct research on material synthesis in cells is difficult and complex, performing similar reactions in vesicles could be expected to be much simpler and straightforward. Moreover, the construction of functional artificial cells becomes possible by performing bottom-up synthetic reactions in vesicles. In this chapter, we introduce one of the first attempts to construct such a model system for step-by-step investigation toward cell-based nanoparticle synthesis.

The molecules to form such bilayer self-assembled vesicular structures are various including diblock copolymers, lipids, polypeptides and surfactants (Discher et al, 1999, Pochan et al, 2004; Lorenceau et al, 2005; Angelova et al, 1986; Tangirala et al, 2007; González-Pérez et al, 2007; Holowka et al, 2007; Bellomo et al, 2004). Here, we focus on lipid vesicles. Lipid bilayer vesicles can be multilamellar or unilamellar, with various sizes ranging from nanometers to micrometers. The difference between multilamellar and unilamellar vesicles, as the names suggest, is that the former consists of a cavity enclosed by a multi-layer structure, while the latter represents a container formed by a single bilayer. Multilayer vesicles could provide a good accommodation space for ions and other reactive solutes binding to their multilayer walls, while high quality unilamellar vesicles with a single bilayer structure provide a closer analogue to cells than multilamellar vesicles. Unilamellar vesicles could be further categorized according to their sizes into three types: small unilamellar vesicles (SUV), large unilamellar vesicles (LUV) and giant unilamellar vesicles (GUV). The former two categories have sizes up to hundreds of nanometers while the latter one has dimensions in the range up to hundred micrometers. Various methods such as hydration, extrusion and electroformation have been developed for vesicle preparation. The hydration method usually produces a mixture of multilamellar and unilamellar vesicles. Extrusion can be used to obtain SUV and LUV suspensions with a narrow size distribution and diameters in the hundred-nanometer range. Electroformation provides a convenient way to produce high quality and large amount of GUVs with diameters up to hundred micrometers (Angelova et al, 1986; Dimova et al, 2006; Walde et al, 2010).

Material synthesis in lipid vesicles is inherently related to the properties of the lipid membrane. From the viewpoint of a cellular analogue, the lipid membrane in unilamellar vesicles has maximum structural proximity to the plasma membrane, thereby providing the most suitable type of simplified artificial cellular system for bio-inspired material synthesis research. However, some significant differences exist between pure lipid vesicles and the

cell membrane. For example, the cell membrane is a complex assembly including many glycoproteins, peptides, and enzymes. These biomolecules can be involved in particular biological functions such as stimuli-responsive ion transport and gating through ion channels with on/off control across the cell membrane (Swartz, 2008). In contrast, the pure lipid membrane in unilamellar vesicles is a simple permeation barrier. Because of the absence of regulating proteins, in its natural state and without extra physical or (bio) chemical stimuli, this kind of lipid membrane is impermeable to ions and macromolecules, while water can freely permeate through the membrane to ensure osmotic balance. This lack of permeability to ions and other solutes allows lipid vesicles to function as a new type of confined micro- or nano-biological reactors for micro- or nano-material synthesis.

The synthesis in confined micro/nano-containers (Shchukin et al, 2004) can be divided into two main types: one involves exact templating where the product has the same shape and size as the container, and the other – not. Exact templating is usually achieved in SUVs and LUVs, and until now, nearly most of the reported syntheses of particles in the nanometer range have been realized in this way (Korgel et al, 1996 and 2000; Rafaeloff et al, 1985; Khramov et al, 1993). However, in order to obtain a clear picture about bio-fabrication processes in cells with much bigger sizes e.g. in the micrometer scale, such synthesis in nano-reactors does not provide a suitable model. Certainly, an important factor affecting the reaction and the final product is the dimensions of the physical confinement in which this reaction is carried out. Thus, some significant features of the underlying mechanism of bio-fabrication in cells may be lost when using LUV or SUV nanoreactors as a reaction model. On the contrary, performing inorganic synthesis reactions in GUVs should be expected to shed light on cell-based nanoparticle synthesis and the corresponding mechanism, since GUVs have dimensions in the cell-size scale (micrometer).

Until now, the investigations on various biological activities using GUVs as prototypes of cells have been widely performed covering many aspects: preparation (Angelova et al, 1986; Larsen et al, 2003; Takakura et al, 2003; Mohanty et al, 2003; Pautot et al, 2003), membrane related processes like fusion, fission, budding (Walde et al, 2010; Wang et al, 2010; Hanczyc et al, 2004), cellular processes and mechanisms like adhesion, communication, endocytosis, exocytosis (Marrink et al, 2003; Haque et al, 2001; Chen et al, 2005; Rustom et al, 2004; Menger et al, 1992, 1997 and 2002; Hanczyc et al, 2003; Espinoza et al, 1999; Ichikawa et al, 2004; Davidson et al, 2003), structure and shape transformation (Suezaki, 2002; Sasaki et al, 2004; Boon et al, 2002; Lee et al, 2005; Hamada et al, 2005; Tomšić et al, 2005; Brückner et al, 2001), drug release (Menger et al, 1998; Barragan et al, 2001; Park et al, 2000; Sun et al, 2003; Vandenburg et al, 2002), micromanipulation (Karlsson et al, 2001; Marmottant et al, 2003), compartmentation (Jesorka et al, 2005; Bucher et al, 1998; Nomura et al, 2001) and microreactors (Vriezema et al, 2005; Tung et al, 2003; Walde et al, 2001; Morgan et al, 1997; Esch et al, 1986; Kang et al, 2003; Krafft et al, 2001; Moffitt et al, 1995; Kommareddi et al, 1996; Rassy et al, 2005; Regev et al, 2004; Faure et al, 2003; Nishikawa et al, 2004; Kim et al, 2000; Wu et al, 2005; Monnard et al, 2003; Fischer et al, 2002; Tsumoto et al, 2001; Nomura et al, 2003; Noireaux et al, 2004; Yu et al, 2001). The last application, namely GUVs as microreactors, has been developed in some realms such as enzyme-catalyzed reactions (Walde et al, 2001; Yukito et al, 1996), photosynthesis reaction (Tung et al, 2003), biochemical reaction (Luisi, 2007), polymerization (Morgan et al, 1997; Esch et al, 1986; Kang et al, 2003; Krafft et al, 2001), inorganic particle synthesis (Moffitt et al, 1995; Kommareddi et al, 1996; Rassy et al, 2005; Regev et al, 2004; Faure et al, 2003; Nishikawa et al, 2004; Kim et al, 2000; Wu et al, 2005), gene (Monnard, 2003; Fischer et al, 2002; Tsumoto

et al, 2001) and protein synthesis (Nomura et al, 2003; Noireaux et al, 2004; Yu et al, 2001). The new research aspect of nanoparticle synthesis in GUVs described in this chapter pertains also to this category. The reaction volume for such bio/chemical reactions in GUVs is typically on the order of femto- or picoliters, depending on the sizes of the GUVs used. Obviously, the features of the tiny nanometer products of such reactions would depend on the overall shape of the container, and the reaction initiation and pathway. Syntheses in GUVs also have the unique exclusive advantage that the vesicle containers and the corresponding product inside them may be monitored in real time under a light microscope. This on-line monitoring enables us to capture *in situ* some important information about material growth and reaction kinetics relevant among others to cell-based synthesis and representing frontier research topics in current chemical and material sciences.

The research based on the use of GUVs as microreactors for synthesis of inorganic nanoparticles inside GUVs was firstly initiated in 2006, and reported officially in 2009 (Yang et al, 2009). To the best of our knowledge, the studies introducing similar approaches are scarce. Namely, a proposal concerning the possibility of using electroporation of GUVs in order to synthesize inorganic nanoparticles in the vicinity of the lipid membrane was made by Schelly (Schelly 2007). Thus, the breakthrough reported in 2009 is the first report on the use of GUVs as confined containers for performing the synthesis of inorganic semiconductor nanocrystals. Briefly, we succeeded in inducing, controlling and directly observing the formation of CdS quantum dots and nanoparticles in GUV, an artificial cell system whereby the membrane container remains intact. Our study, for the first time, extended confined vesicular reactions to micrometer-scale cell-size reactors for the synthesis of nanomaterials. Differently from the drastic experimental conditions used previously for the synthesis of nanoparticles in vesicles, the processes we employed in this report are quite simple and mild, and effectively mimic intracellular mixing and membrane fusion, which naturally occur in cells.

One of the implementation pathways is to induce the reaction between two reactants loaded in two different GUVs. One GUV contains a solution of CdCl_2 and the other contains Na_2S . The synthesis of CdS nanoparticles is then simply initiated by conducting electrofusion between these two kinds of vesicles. This electrofusion-based synthesis is a good model for cellular fusion-based reaction systems. Another pathway involves an attempt to mimic the cell culturing-based systems, often used in bacteria-based inorganic nanoparticle synthesis. GUVs are in-situ grown in electroformation chambers, where they remain attached to the substrate via lipid nanotubes. The initial solution contained in the GUVs is Na_2S , and the synthesis reaction of CdS nanoparticles is initiated by gradually replacing the external solution with CdCl_2 solution, which enters the GUVs via the nanotubes. This is a good mimic to bacteria culturing, however, with accelerated speed. Below, we describe these two protocols in details. We emphasize that they can be applied to arbitrary reactants (not necessarily CdCl_2 and Na_2S) provided suitable concentration conditions are selected.

2. Experimental section

Growing GUVs via Electroformation. Giant vesicles made of L- α -phosphatidylcholine from egg yolk (Egg-PC, Sigma, St. Louis, MO) were grown using the electroformation method (Angelova et al, 1986, Dimova 2006). In principle, the choice for the lipid is not limited to this lipid and any phosphatidylcholine (or a mixture of phosphatidylcholines), which is in the fluid phase at room temperature may be employed. Egg-PC was our choice because it is

a natural and abundant lipid. The procedure for electroformation used here is described in detail elsewhere (Riske et al, 2005). Briefly, Egg-PC was dissolved in chloroform to form 2 mg/ml lipid solution. For observation of the vesicles with fluorescence microscopy the following dyes were used: 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC₁₈) from Molecular Probes (Leiden, The Netherlands; excitation wavelength at 551 nm and emission wavelength at 569 nm) was added to the lipid solution at concentration 0.1 mol %, and perylene from Sigma-Aldrich (Steinheim, Germany; with excitation wavelength at 440 nm and emission wavelength at 450 nm) was added at concentration 0.4 mol %. Typically, for the CdCl₂-loaded vesicles we used DiIC₁₈ as fluorescence marker and for the Na₂S-loaded vesicles we used perylene. A small drop (~ 50 µl) of lipid solution was placed onto a glass slide coated with indium tin oxide (ITO) and spread evenly on the surface. Two such coated ITO glasses were placed in a vacuum desiccator at room temperature for at least 2 h to evaporate the organic solvent. For the vesicles grown in the presence of CdCl₂, we found out that drying at higher temperature (60°C) yields more and bigger vesicles. A closed chamber was assembled from the two ITO glasses (the sides with conductive coating were facing each other) and a 1 mm thick rectangular Teflon spacer with two holes as solution inlet and outlet. The CdCl₂- and Na₂S-loaded vesicles for the electrofusion protocol were typically grown in parallel. The growing solutions of CdCl₂ or Na₂S in 100 mOsm sucrose were introduced through the inlet to fill the respective chamber. The chambers were connected in parallel to an alternating current (AC) field function generator (Agilent 33220A 20 MHz function/arbitrary waveform generator) and an alternating voltage with amplitude of 0.7 V (peak-to-peak) and frequency of 10 Hz was applied for 4 h. The magnitude of the AC voltage was a key parameter for the formation of CdCl₂-loaded vesicles. In 0.03 mM CdCl₂ solution, GUVs were formed at low voltage (< 1.7 V). At higher voltages, the vesicles would start rupturing. However, for electroformation of Na₂S-loaded vesicles, even in 3 mM Na₂S solution, this effect was not observed. Presumably, CdCl₂ affects the membrane properties making the bilayers either more fragile or inducing additional tension, as the binding of Cd²⁺ effectively impeded the separation of lipid film, which is an essential condition for electroformation of GUVs.

Electrofusion Protocol for Synthesis of CdS Quantum Dots. After the completion of the electroformation process, the vesicles were detached from the ITO glass substrate by lowering the field frequency to 5 Hz and setting the voltage to 0.5 V for 20 min. Then, the vesicles were removed from the electroformation chamber, diluted up to 10-40 times with isotonic glucose solution, and transferred into an electrofusion chamber (Eppendorf, Germany). The effective dilution is important for lowering the concentration of CdCl₂ or Na₂S outside the vesicles and to ensure no interference of neighboring vesicles in the image when confocal imaging is performing. The glucose-sucrose asymmetry creates a refraction index difference between the interior and the exterior of the vesicles. The glucose-sucrose asymmetry also creates a density difference stabilizing the vesicles at the bottom of the chamber. The latter enhances the contrast of the phase-contrast microscopy images (vesicle images appear dark on a bright background). The electrofusion chamber consists of a Teflon frame with two cylindrical electrodes (500 µm in diameter) with a gap distance of 200 µm. The chamber was connected to a Multiporator (Eppendorf, Germany) to align the vesicles (AC field of 3 V, 2 MHz for 90 s applied a few times) and electrofuse them (direct current pulses of field strength 0.5-2 kV/cm and 150-300 µs duration). The observation of the vesicles was performed with an Axiovert135 microscope (Zeiss, Jena, Germany) equipped with 20x and 40x phase-contrast objectives. The fusion process was also monitored by a

laser scanning confocal microscope (Leica, TCS SP5, Germany) with excitation at 458 nm (Ar laser) for perylene and 561 nm (DPSS laser) for DiIC_{18} .

Batch Electrofusion for Synthesis of CdS Quantum Dots in Large Scale. Before doing batch electrofusion, the vesicles swelled in CdCl_2 solution were incubated with an ion-exchange resin (Amberlite IR-120, H^+ form, Sigma-Aldrich, Germany) to remove the Cd^{2+} ions in the vesicle exterior media. The resin was carefully washed and activated according to the instructions of the manufacturer. Before use, the exchanging ability of the resin was checked by UV-Vis spectra to guarantee the highest performance of the resin. After removing the cadmium ions outside the GUVs, the vesicles solution with CdCl_2 entrapped in the vesicles was mixed with the solution of vesicles loaded with Na_2S . The mixture was then exposed several times to electric pulses in a helix fusion chamber (Eppendorf, Germany). Immediately after that, the solution was analyzed with a series of techniques including fluorescence spectra analysis (luminescence spectrometer LS50B with excitation at 400 nm, Perkin Elmer, Beaconsfield, England) and transmission electron microscopy (TEM) (Omega 912 TEM, Carl Zeiss, Oberkochen, Germany, with 100 kV accelerating voltage).

Slow Content Exchange Protocol for Synthesis of CdS Nanoparticles. A programmable syringe pump was used to slowly exchange the solution in the exterior of the vesicles in the electroformation chamber at a controlled rate. To ensure complete solution replacement, at least four times the volume of the chamber was flowed through it. We typically electroformed the vesicles in Na_2S aqueous solutions and then replaced these solutions with CdCl_2 solutions. The osmotic balance across the vesicle membranes, and thus the preservation of the vesicle volumes was ensured by the presence of e.g. 100 mM glucose in the salt solutions. Because the salt concentrations used were very low (on the order of 1 mM), the osmolarity of the initial Na_2S solution and the exchanging solution are nearly the same as confirmed by osmolarity measurements (Osmomat030, Gonotec, Berlin). During and after the exchange, the solution flown out of the chamber and the one inside the chamber were carefully collected and examined with fluorescent spectroscopy and TEM.

3. Results and discussion

3.1 Electrofusion protocol

For the general interest, before reporting results from the electrofusion protocol, we will introduce a short discussion on the background about this protocol.

For the simplest "A+B" type reaction (that is, the reaction is initiated when reactant A encounters reactant B), three models could be summarized to describe possible protocols when utilizing vesicles as microreactors (Figure 1). In Model 1, all reactants are incorporated into the vesicle interior or the membrane bilayer and the reaction occurs at the membrane or inside the vesicle. In Model 2, reactant A (such as an enzyme) is incorporated into the vesicle membrane or interior and reactant B (such as a substrate) is dispersed in the media surrounding the vesicles. The reaction is then initiated when reactant B diffuses across the membrane. Actually, nearly all of the work reported in the literature is based on these two approaches (Korgel et al, 1996 and 2000; Ralaeloff et al, 1985; Khramov et al, 1993). Up to now, there are only few reports on reactions realized via the fusion of two vesicles encapsulating reactant A and B, respectively (Model 3). The corresponding feature of this model is that the triggering mechanism for the reaction between A and B to occur is vesicle fusion.

Fig. 1. Three models for reactions taking place in vesicles.

In nature, fusion is a spontaneous and important event, by which a myriad of physico-chemical processes occur (Leabu *et al.*, 2006; Chernomordik *et al.*, 2006; Jahn *et al.*, 2006; Wang *et al.*, 2006; Tsaadon *et al.*, 2006; Ogura *et al.*, 1995; Wilmut *et al.*, 1997) such as fertilization from egg-sperm fusion, formation of multinucleated muscle cells from fusion of myoblasts during embryonic development, signaling cascades. Fusion has been also widely observed in artificial and natural colloidal systems from crystal growth via droplet condensation to lipid vesicle (Strömberg *et al.*, 2000; Riske *et al.*, 2006) or polymersome (Zhou *et al.*, 2004 and 2005; Yan *et al.*, 2004; Vriezema *et al.*, 2003) recombination.

For fusion-initiated nanoparticle synthesis, the starting complementary reactants are separately loaded into different colloids, and then the reaction is triggered by the fusion of these reactive colloids to make the reactants meet each other. An instant benefit for this strategy is that the precise temporal and spatial control on the synthesis process could be easily achieved: these colloidal cartridges could be stored or transported to any place and then the reaction can be initiated at a desired time point or location. Successful examples of this approach have been reported for fabrication of semiconductor nanoparticles in microfluidic channels through the fusion of droplets containing the starting reactants (Shestopalov *et al.*, 2004; Hung *et al.*, 2006). Moreover, in a recent report, Beaune *et al.* described potential bioimaging ability for tumor treatment when vesicles are entrapped with fluorescent magnetic nanoparticles and quantum dots (Beaune *et al.*, 2007).

An important condition for successful fusion-initiated reactions is to stimulate the vesicles in order to obtain an effective fusion event since vesicle fusion is inefficient in the absence of external stimuli. Fusion can be induced artificially by means of membrane stress (Cohen *et al.*, 1982; Shillcock *et al.*, 2005), ions or organic reagents (Estes *et al.*, 2006; Neil *et al.*, 1993; Lentz, 2007; Haluska *et al.*, 2006; Kunishima *et al.*, 2006), DNA (Stengel *et al.*, 2007), proteins (Jahn *et al.*, 2006; Peters *et al.*, 1999 and summary by Walde *et al.*, 2001), laser beams (Steubing *et al.*, 1991; Weber *et al.*, 1992; Kulin *et al.*, 2003) or electric fields (Strömberg *et al.*, 2000). The last one is termed electrofusion. Among these fusion methods, electrofusion becomes increasingly important due to its reliable, fast and easy handling procedure (Riske *et al.*, 2006). This method utilizes discrete intense electric pulses (kV/cm) of short duration (μ s).

The electrical breakdown of the membranes of cells or vesicles which are in contact can lead to fusion. The process is completed within milliseconds. Due to its general applicability and mild conditions, electrofusion has been extensively used and the procedures further developed for many years (Strömberg et al, 2000) in a wide variety of biological experiments with cells and vesicles, from the creation of hybridomas and new cell lines to *in vitro* fertilization and the production of cloned offspring, like the sheep Dolly and her equals (Zimmermann et al, 1986). Genetic and biochemical reactions, such as DNA transcription, translation and gene expression, are often achieved by fusion. According to Zimmermann (Zimmermann et al, 1986), the electrofusion process could even have been an important step in evolution after the first simple cells had come into being. Therefore, it is expected that the research on electrofusion-based reactions and chemistry *in vivo* or *in vitro*, could have a great development potential in many fields such as origin of life and birth, biomineralization, bioengineering, cell science, tissue engineering, bio-inspired and new materials synthesis.

Electrofusion-based reactions in GUVs strongly rely on the physical behavior (deformation, poration and fusion) of these vesicles subjected to either alternating current field or strong direct current (DC) pulses. In recent years, our group has systematically investigated this topic by experimental and theoretical modeling (Riske et al, 2005; Riske et al, 2006; Haluska et al, 2006; Dimova et al, 2007; Aranda et al, 2008; Riske et al, 2009; Vlahovska et al, 2009; Dimova et al, 2009; Yamamoto et al, 2010). Based on the findings from our and other research groups, successful electrofusion is based on four important features of the membrane and the vesicles: 1) The lipid membrane is impermeable to reactants (ions and macromolecules), while water can freely permeate through the membrane to assure osmotic balance; 2) Fast and effective fusion could be initiated with a short electric pulse with characteristics above the poration threshold of the membrane; 3) Leakage of reactants during the fusion process is negligible (Riske et al, 2005); 4) Vesicles with complementary reactants can be brought together to form a reactive couple for electrofusion. Features 1) - 3) stem from the intrinsic properties of lipid membranes, while the last one, feature 4), is inherent to giant vesicles and can be achieved either via micromanipulation or exposure of the GUV suspension to AC fields causing vesicle alignment due to dielectric screening.

Fig. 2. Electrofusion of vesicles as a method for nanoparticle synthesis: vesicles containing reactant A or B are mixed (in A- and B-free environment) and subjected to an AC field to align them in the direction of the field and bring them close together. A DC pulse initiates the electrofusion of the two vesicles and the reaction between A and B proceeds to the formation of nanoparticles encapsulated in the fused vesicle. Reproduced from (Yang et al, 2009). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

The concept of utilizing electrofusion of two GUVs to initiate a reaction was first proposed by Chiu, Zare et al (Chiu et al, 1999), who used a microelectrode to trigger electrofusion. The study showed that ultrafast reaction kinetics could be conveniently investigated because

electrofusion takes place only within milliseconds and the mixing of reactants upon fusion could be very fast (μs). Kulin et al (Kulin et al, 2003) further pointed out that this method may be a useful tool for quantitative studies in combinatorial chemistry involving only femtoliters of reagents. In the latter report ultraviolet laser light was used to fuse two liposomes (10 μm in size) containing chemical reagents and the encapsulated materials could be accurately conserved without leakage to the external media.

Different from the above-mentioned approaches, we used the electrofusion process to conduct nanoparticle synthesis. As shown in Figure 2, two couples of hybrid GUVs, a CdCl_2 -loaded GUV in red color (A, Cd-GUV) and Na_2S -loaded GUV in green color (B, Na-GUV) could be effectively aligned by AC field and fused with a DS pulse. Similar to pearl-chain formation in cell suspensions subjected to an AC field (Zimmermann, 1986), the two types of vesicles are brought together and align along the direction of an exogenous AC field. This field-induced self-arrangement makes reactive vesicles match well for the CdS synthesis reaction: half of the aligned vesicle couples are A-B couples. After that, a strong DC pulse (typically pulses of 0.5 - 2 kV/cm field strength and 150 - 300 μs duration suffice) is applied to initiate electrofusion between A and B. Electrofusion proceeds smoothly to form one hybrid GUV where mixing of CdCl_2 and Na_2S occurs for the CdS nanoparticle synthesis. The product, in this case, quantum-dot-like CdS nanoparticles, is visualized under laser excitation as a fluorescent bright spot in the fusion zone (Figure 3). Subsequent inspection of vesicle sections above and below the

Fig. 3. a) – c) Electrofusion of vesicles as a method for nanoparticle synthesis: confocal scans of vesicles loaded with 0.3 mM Na_2S (green) and 0.3 mM CdCl_2 (red) undergoing fusion. d) – f) Intensity line profiles along the dash-dotted lines indicated by red arrows in a) – c), respectively. The direction of the field is indicated in a) with an arrow. Before fusion, a), d), the vesicle interior shows only background noise similar to the external solution as indicated by the shaded zone in d). After fusion, b), c), e), f), fluorescence from the product is detected in the interior of the fused vesicle. The time after applying the pulse is indicated on the micrographs. The fluorescence signal was acquired in the ranges 565 - 765 nm (red channel) and 462 - 558 nm (green channel). Reproduced from (Yang et al, 2009). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

scanning plane showed no presence of membrane inclusions (free vesicles or membrane fractions) in the fusion zone. Therefore, the source of fluorescence is provided by the formed nanoparticles. The intensity from this spot increases gradually from 0 s (the starting time point of electrofusion), and reaches maximum around 10 s. We thus infer that the reaction begins at the electrofusion point and quickly reaches a balance around 10 s. This signal then decays over time (Figure 3 and 4). The mechanism behind the decay of the signal from the CdS product is still not clear at present and under investigation.

During the investigation on this electrofusion approach, we also studied the effect of salt concentration on the process of vesicle electroformation and on the effectiveness of electrofusion. In the concentration range from 0.00003 mM to 3 mM, Na₂S solutions showed weak effect on the electroformation of GUVs. In contrast, CdCl₂ solutions showed obvious salt-related influence on the electroformation, particularly at concentrations above 0.03 mM. The total effect could be summarized as shrinking of the diameter of Cd-GUVs and decrease in the vesicle yield. The reason for this could be related to the binding of Cd²⁺ ions to the polar headgroups of the lipids in the membrane effectively impeding the separation (swelling) of the lipid film from the substrate, which is an essential condition for electroformation of GUVs. We further found that fusion of GUVs loaded with CdCl₂ solution at concentration above around 0.1 mM is not effective and was rather inhibited (Figure 5). On the other hand, GUVs loaded with Na₂S at similar concentrations showed

Fig. 4. Confocal scans of vesicles loaded with 0.3 mM Na₂S (green) and 0.3 mM CdCl₂ (red) undergoing fusion after being exposed to an electric pulse (excitation wavelengths: 458 nm and 561 nm). The fluorescence signal has been acquired in the ranges 565 - 765 nm (red channel) and 462 - 558 nm (green channel). The field direction is indicated by the arrow in the first snapshot. The acquisition time is indicated on every image, where $t = 0$ s corresponds to the last snapshot before applying the pulse. Several seconds after fusion, a fluorescence signal is detected from the product in the vesicle interior (note that only fluorescence from objects located in the focal plane is visible in the images; particles out of focus are not detected). The signal, both from the membrane and from the vesicle interior, decays with time, and the mechanism for the latter decay is under investigation.

Fig. 5. Confocal scans of the electrofusion between two GUVs loaded with 3 mM CdCl_2 solution. As shown in this figure, the electrofusion between two GUVs- CdCl_2 could not occur under high salt concentrations, e.g. 0.3 and 3 mM. However, the electrofusion of CdCl_2 loaded vesicles could proceed under lower salt concentration such as 0.03 mM.

Fig. 6. Confocal scans of the electrofusion between two GUVs loaded with 3 mM Na_2S solution. As shown in this figure, the electrofusion between two GUVs encapsulating Na_2S could proceed smoothly under high salt concentrations.

normal electrofusion behavior (Figure 6). A possible interpretation for the inability of Cd -GUVs to fuse is that Cd^{2+} ions could influence the mechanical properties of the membrane and in particular hindering the formation of a fusion pore (through its divalent binding to the pore surface), which is a necessary intermediate stage during electrofusion. However, attempts for electrofusion between two hybrid GUVs (a Cd -GUV and a Na -GUV) loaded with 0.3 mM salt solutions, demonstrated that electrofusion could proceed smoothly. This showed that the commonly accepted fusion pore-mediated mechanism may not be necessarily valid for electrofusion.

After successfully exploring single electrofusion events, we applied this method to batch electrofusion in order to achieve large-scale synthesis of nanomaterials. The latter is beneficial for practical applications. In addition, analytical methods like TEM, selected area electron diffraction (SAED) and fluorescence measurements are easier to apply to samples containing a large fraction of fused vesicles and thus a larger amount of the fusion product. In order to increase the yield from the electrofusion protocol we applied a special electrofusion chamber where a larger fraction of vesicles could be fused. This was achieved by increasing the volume of the concentrated vesicle solution located between the two

electrodes. For this purpose, we used a helix fusion chamber (Eppendorf, Germany) (Figure 7), which consists of two coiled helical electrodes with a gap distance of 250 μm and a total inter-electrode volume of 250 μl . This volume is more than 20 times larger than the one between the two straight cylindrical electrodes used for the microscopy experiments, thus allowing higher yields.

Another important prerequisite for the successful batch electrofusion is to remove the free Cd^{2+} ions from the exterior of the CdCl_2 -loaded vesicles before mixing them with the Na_2S -loaded ones. The reason is that the presence of reactive Cd^{2+} ions in the GUV exterior would result in an undesired reaction between Cd^{2+} and S^{2-} outside the vesicles when mixing the two vesicle populations. Apart from that, this undesired reaction would further influence the osmotic balance across the vesicle membrane potentially leading to vesicle rupture. Therefore, the CdCl_2 -loaded vesicles were placed in contact with an ion-exchange resin (Amberlite). The resin was thoroughly washed beforehand to remove small molecular impurities (3 times in deionized water), then activated according to the instructions of the manufacturer (acid wash with 2N HCl, at least 3 times), and rinsed at least 10 times with deionized water to $\text{pH} > 5$. The vesicles electroformed in CdCl_2 solution were then added to the resin at optimized condition (200 mg resin for 2 ml CdCl_2 -loaded GUV solution under very slow stirring for 1 hr). After the removal of the Cd^{2+} ions outside the vesicles, 1 ml solution of Cd-GUV was mixed with 1 ml solution of Na-GUV. Half of the mixture was used as a reference sample, while the rest was subjected to electrofusion in the helix fusion chamber in 4 portions of 250 μl each. The AC field (9 V, 2 MHz) was applied to each portion for about 15 min, followed by one pulse of 300 V and 150 μs duration. The 4 aliquots were then collected and subjected to further analysis using TEM and fluorescence spectroscopy.

Fig. 7. The schematic illustration of the helix electrofusion chamber. Two populations of GUVs loaded with CdCl_2 or Na_2S are mixed and filled into the space of beaker (1). After sealing, the chamber is connected to an electronic function generator providing AC or DC fields. After setting the desired AC and DC programs, the helix electrode (2) in the beaker is used to apply AC and DC fields for GUV alignment and electrofusion. After the field application, the solution in the beaker is gently taken out and subjected to further spectroscopy analysis.

The TEM images from the mixed samples before applying a DC pulse showed that they contained only some irregular formless sediment (Figure 8), which is commonly found in samples produced by the direct mixing of CdCl_2 and Na_2S solutions without any confinements. The SAED pattern showed two polydisperse diffraction rings, which are due to multiple overlapping from the following CdS crystalline lines: (100), (002), (101) for the inner diffraction ring and (110), (103), (112) for the outer ring (Li et al, 2003). This implies

that the product was polycrystalline with poor crystalline orientation. Polycrystalline CdS products before electrofusion originate from the TEM sample preparation process: during the preparation process, Cd-GUVs and Na-GUVs break upon the evaporation of the water, and their CdCl_2 and Na_2S content is released so they can react with each other along common mixing ways. In contrast, in the samples to which a DC pulse was applied, we found only dispersed tiny particles (Figure 9). Their diameters are lower than 10 nm and their SAED pattern showed the unique feature of monocrystallines (dotted pattern). The diameter of these CdS nanocrystals is lower than the Bohr radius of a CdS particle, and therefore in the quantum dot size regime. As also observed in the above-described confocal microscopy experiments, the emerging fluorescent emission in the interior of the fused vesicle formed upon the electrofusion of a Cd-GUV and a Na-GUV is attributed to the formation of these monocrystallines with quantum confinement effect for fluorescence emission. The fluorescent spectra discussed below further supported the photoluminescent property from these CdS quantum dots.

Fig. 8. A TEM image and a SAED pattern from a solution of mixed Na_2S - and CdCl_2 -loaded vesicles not subjected to electrofusion. The reagent concentration in the vesicles was 0.3 mM. Only irregular sediments with poor crystalline structure are formed due to vesicle rupture upon deposition on the TEM grid and subsequent content mixing.

Fluorescence spectra further demonstrated the formation of CdS nanocrystallines (Figure 10). Before applying a DC pulse, the fluorescence signal showed only a very small peak around 460 nm, which is from the Na-GUV solution itself. After applying the DC pulse to the mixed solution, enhanced fluorescence signal was observed at 460 nm, which can be attributed to the band gap emission and suggests high quality of the nanocrystals (Korgel et al, 2000; Khramov et al, 1993; Gratt et al, 2003). The enhanced fluorescence signal confirmed that a reaction has occurred after applying the pulse. In addition, the peak site around 460 nm demonstrated blue shift from the fluorescence peak of bulk CdS materials (600 nm), which further reflects quantum confinement effects by the 10 nm particles observed in TEM. It should be mentioned that this enhanced fluorescence had poor reproducibility when the vesicle solutions were not handled carefully. This could be attributed to the fragility of

Fig. 9. a) TEM image and b) SAED pattern from the product in the solutions after vesicle fusion. The salt concentration in the vesicles was 0.3 mM. Dispersed single-crystalline nanoparticles with diameter between 4 and 8 nm are detected. Reproduced from (Yang et al, 2009). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Fig. 10. Fluorescent spectra of the samples before (solid black curve) and after electrofusion (dashed red curve). As a control test, the fluorescence signal from Na_2S -loaded GUV solution is also plotted (dash-dotted green curve). The excitation wavelength is 400 nm. The resulting fluorescent emission at around 460 nm is strongly enhanced after electrofusion.

GUVs, which often results in undesirable breaking of the membranes and release of reactants. The absence of emission at higher wavelengths (up to 700 nm) associated with deep trapped states due to surface or core defects, indicates the high quality of the nanocrystals. It should be also noted that for the fluorescent spectra measurements, the excitation wavelength was 400 nm while the confocal scans in Fig. 3-4 were obtained by excitation with a laser at 458 nm. Therefore, the emission wavelength under confocal scanning exhibits a red-shift compared to that in the fluorescent spectra measurements.

Before we proceed with the second protocol for nanoparticle synthesis in GUVs, we will address some common features, advantages and application perspectives of the electrofusion approach.

Obviously, this protocol provides us with a visualizing analytical tool to follow the reaction kinetics with high temporal resolution (Figure 3-4). Previously, we have demonstrated that fast fusion processes can be visualized at microsecond timescales using high-speed digital imaging (Haluska et al, 2006). This novel and facile electrofusion method is suitable not only for inorganic nanoparticle synthesis, but also for the online monitoring of ultrafast physicochemical processes such as photosynthesis, enzyme catalysis and photopolymerization, which at present requires complex and abstracted spectroscopy techniques. Our results also show that even without the mediation of biomacromolecules, nanoparticles can still be synthesized in biological compartments. This outcome provides a new insight in the developing research on biomineralization mechanisms.

Theoretically, the electrofusion-initiated reaction could be extended to chemical reactions with any stoichiometric ratio, because a large amount of vesicles can be aligned in the electric field, so that chain or step-type reactions could be achieved by entrapping A_1 - A_n reactants into 1- n vesicles respectively. For this purpose, a key condition is the controlled vesicle arrangement to encode them at pre-designed pattern. As shown above, AC field-induced alignment of GUVs shows the potential to control this prearrangement. However, it is difficult to control the number and the type of the aligned vesicles in the case where more than two vesicles need to be fused. Other methods have been already developed in this direction. For example, preliminary experimental and theoretical models have been reported based on vesicle recognition by biotin-avidin and electrostatic interactions (Farbman-Yogev et al, 1998; Walker et al, 1997 and Chiruvolu et al, 1994). We propose an alternative protocol, which may be more easily accessible, namely, first patterning the vesicles onto a planar surface and then fusing these pre-organized vesicles with a second vesicle population to complete the reactions (Lee et al, 2008; Christensen et al, 2007; Kim et al, 2008). The first step has been achieved by some smart designs, e.g. direct microcontact printing of vesicles (Liu et al, 2007) and ink-jet printing (Hauschild et al, 2005).

A possibly problematic feature of the electrofusion-induced reaction which may hinder the wide application of this approach is that vesicles are difficult to prepare by the electroformation method if high reactant (salt) concentrations are required. The reason is that the ions in the solutions could bind to the lipid headgroups suppressing the inter-bilayer separation and vesicle swelling. This effect was also observed in our experiments, showing that high concentrations of Cd^{2+} hinder electroformation and electrofusion of GUV-Cd. To overcome the problem facing electroformation at high salt concentration, Estes and Mayer et al developed a two-step approach: first, electroforming vesicles in solutions of low ionic strength and second, exchanging the solution inside the vesicles with a high ionic strength solution (Estes et al, 2006). More recently, a modification of the electroformation method was reported where high frequency AC field (500 Hz) was used to achieve effective electroformation under physiological conditions with sodium salts. (Montes et al, 2007; Pott et al, 2008) Concerning the difficulty of achieving electrofusion when hindered due to ion binding to the membrane, other fusion protocols may be utilized such as those based on the use of fusogenic molecules and laser beams. Further possible improvement of the protocol and allowing a better control over the reaction involves the use of monodisperse vesicles rather than polydisperse GUV samples used at present. The procedures of micro-contact printing (Taylor et al, 2003; Liu et al, 2007) and extrusion (Tangirala et al, 2007) have shown

certain potential to prepare vesicles or droplet populations with low polydispersity. Other interesting and effective methods, which have emerged recently include ink-jet printing for small vesicles (50-200 nm) (Hauschild et al, 2005), double emulsion template made by glass-capillary microfluidics (Shum et al, 2008; Kuroiwa et al, 2009) and direct pulsed microfluidic jetting (Stachowiak et al, 2008).

Fusion-based synthesis is a highly multidisciplinary and open project, with the close interplay between chemistry (biological, polymer and inorganic) and physics (biomembrane and polymer physics). This field can be broadened by exploring systematically a series of reactions initiated by the fusion of vesicles containing separated reactants. Some unexplored reactions include, but are not limited to, the biosynthesis of genes (RNA and DNA), proteins and carbohydrates in vesicles; enzyme-catalyzed reactions; biomineralization processes; host-guest interactions. The above investigations may open new ways of biosynthesis and give fundamental and valuable information about cell bioactivity during life origin, growth, organ and tissue formation; mimic bioactivities of a living cell; provide fundamental information for the role of functional interfaces in biomineralization in cells and some important aspects such as the detailed role of proteins in the synthesis of inorganic products, and finally, evaluate the biomineralization process *in vivo*.

3.2 Solution exchanging protocol

Fig. 11. Slow content exchange method for performing inorganic nanomaterial synthesis in GUVs: Vesicles formed in the presence of A (Na_2S) are still connected via nanotubes to the glass substrate of the electroformation chamber (see also the Fig. 12). The thickness of the nanotubes (tens of nm) and the size of the vesicles (tens of μm) are not in scale. Reactant B (CdCl_2) is slowly injected in the chamber. After diffusing through the nanotubes into the vesicle interior, B reacts with A to produce nanoparticles. The approximate timescales of the events are indicated in the pictures. Reproduced from (Yang et al, 2009). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Another protocol we used is based on the solution exchange around Na_2S -loaded GUV with a solution of CdCl_2 (Figure 11). In this approach, a microreactor array was formed on an ITO surface where the GUVs in Na_2S solution were attached to the substrate via nanotubes. The freshly prepared CdCl_2 solution could be gradually introduced into these vesicles via the nanotubes connecting them to the ITO surface. These nanotubes or tethers provide a good bridge connecting the GUV interior and the external media. They are clearly visible from microscopy images (Figure 12). The visualization of the tethers is achieved by preparing the vesicles on cylindrical platinum electrodes where side view observation of the growing vesicles is possible with xy-scans (contrary to the case when the vesicles are grown on ITO plates where z-scans are necessary to observe the tethers; the latter was not possible due to the large thickness of the ITO substrates used). Typically, these tethers can be broken (releasing the vesicles) if during the vesicle electroformation the frequency of the applied

AC field is reduced to about 5 Hz for ten minutes. For the protocol used here, we did not apply this latter step. Instead, the electroformation chamber that contained the vesicles tether-bound to the substrate was connected to a polyvalent syringe pump (Lambda Vit-fit, the Czech Republic), which slowly injected CdCl_2 solution into the chamber containing the Na_2S -loaded GUVs without detaching the vesicles from the substrate. The injection speed influences the stability, shape and transformation of the attached GUVs (Estes et al, 2005 and 2006). The optimal injection speed was about 1 ml/min. At this speed, the vesicles not connected to the ITO substrate via nanotubes flow out of the chamber. At the same time, the injection speed cannot be very quick in order to prevent the rupture of the surface-attached vesicles. As a result, the total amount of vesicles decreases.

After the introduction of CdCl_2 into the chamber, the cadmium ions slowly diffuse into the vesicle interior via the nanotubes. In this way, the CdS product is expected to form in the confined space inside the GUVs. Of course, the reaction proceeds also outside the GUVs because of the present Na_2S in the vesicle exterior. Thus, the solution initially flown out of the chamber contains the CdS produced in the vesicle exterior. To ensure complete replacement of the external solution, the total injection volume was at least four times the volume of the chamber. Finally, an AC field with low frequency (5 Hz) was applied for 20 min to detach the GUVs from the ITO substrate and this sample was collected separately for inspecting the product formed within the vesicles.

Fig. 12. Confocal scan through a chamber with vesicles electroformed on cylindrical electrodes. At the end of the preparation procedure, the vesicles remain connected to the substrate (electrode) via nanotubes (indicated by arrows). Note that tethers, which are slightly behind or in front of this scanning plane, are not imaged.

The fluorescence spectra of the solutions collected from the chamber (Figure 13) clearly indicated that after exchange, the solution in the chamber with GUV grafted to the surface showed an obvious increase of the fluorescence signal (red dashed line) compared to that from the solution flown out of the chamber before detaching the GUVs (black solid line). The fluorescent peaks emerged at two different sites: 460 and 570 nm. Compared with the fluorescent peak from bulk CdS material (600 nm), obvious fluorescent blue shift showed that certain quantum confinement effect existed in the samples from the chamber. This indicated that CdS nanocrystallines are present in the sample after the solution exchange.

We performed a parallel (reference) experiment to reflect the key role of the grafted GUVs. The same injection protocol was performed in a chamber containing no GUVs (i.e. using blank ITO glass without lipid coating). The obtained result showed that the solution in the chamber without GUVs present yields fluorescence signal (green dotted line) similar to that flown first outside the chamber. Both of these signals are also similar to the signal intensity obtained from the direct mixing of the two salt solutions.

Fig. 13. Fluorescent spectra of the sample containing the vesicles and the one flown out of the chamber. The solution in the chamber with GUVs after the content exchange protocol (dashed red curve) has significant absorption in the range 460–600 nm due to the formed nanoparticles. This signal is not observed from the solution flown out of the chamber (solid black curve). For comparison, the fluorescence spectra from direct mixing of CdCl_2 and Na_2S solutions with the same concentration (0.3 mM) is also plotted (dash-dotted green curve).

TEM images (Figure 15) provided further evidence for the formation of CdS nanoparticles by this solution exchange method. The nanoparticles had diameters around 50 nm and were polycrystalline as demonstrated by the two diffraction rings in the corresponding SAED pattern (Gorer et al, 1998). In contrast, the solution flown out of the chamber, which consisted of a mixture of the introduced CdCl_2 and the reagent Na_2S from the vesicle exterior, contained only irregular sediment with poor polycrystalline structure (Figure 15). For the slow content exchange protocol, our setup did not allow us to perform confocal microscopy scans and image analysis as in Fig. 3-4 because of the large thickness of the substrate. Thus we were not able to identify whether the nanoparticles were formed in the vesicle volume or in the nanotubes connecting them with the substrate. However, the drastic difference between the morphology of CdS material formed inside and outside the vesicles strongly suggested that templating role was present in this exchange system.

Besides the chemical reaction discussed above, the solution exchange method also provides an alternative way to prepare GUVs with a desired salt entrapped at a high concentration. For example, one could first form GUV populations at a low salt concentration, and then slowly exchange the content of the tethered vesicles with a solution of high salt concentration. We further noticed that when high concentration of CdCl_2 solution, e.g. 3 mM, is used as an exchange solution, the vesicles tend to adhere to each other. This finding

supports our explanation for the observation that Cd-GUVs could not be effectively grown and self-electrofused at high Cd^{2+} concentration (e.g. 3 mM). Presumably, electrostatic interactions of cadmium ions with the zwitterionic headgroups of the lipids increased the inter-membrane interaction and stiffness. Similar behavior from other divalent ions such Ca^{2+} , Mg^{2+} have been also previously found to induce tension on the membranes and accordingly, a marked increase in the membrane-membrane interactions between adjacent GUVs (Akashi et al, 1996; Bockmann et al, 2004; Sinn et al, 2006; Pabst et al, 2007).

Fig. 14. TEM images (two left images) and a SAED pattern (right) from polycrystalline CdS nanoparticles in the chamber after the slow solution exchange. The salt concentration in the exchange solution and in the vesicles was 0.3 mM. The diameter of the nanoparticles is around 50 nm. Reproduced from (Yang et al, 2009). Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Fig. 15. TEM image (left) and SAED pattern (right) from sediments with poor polycrystalline structure in the solution flown out of the chamber. The reagent concentration in the exchange solution and in the vesicles was 0.3 mM.

4. Conclusion and remarks

To summarize, our results suggest that the possible mechanism of cell-based nanoparticle synthesis, whether intra- or extra-cellular, may not necessarily be only peptide- or protein-driven or regulated. Simple chemical mixing of subpicoliter volumes due to fusion of carrier vesicles with cell membranes or slow influx in the intracellular space may be the possible

pathway of these syntheses. With the two protocols discussed above, the sizes of synthesized CdS nanoproducs could be tuned from 4 to 50 nm in diameter using two different protocols. The application and further improvement of the protocols may be sought in the following directions: 1) enhancing the yield and product quality for the electrofusion protocol can be achieved by applying batch electrofusion in optimized chambers; 2) optimization of the chamber substrate used in the solute exchanging protocol may allow us to elucidate the location (nanotubes of GUVs) for nanoparticle formation; 3) bio-organic/inorganic hybrid nanocomposite materials may also be prepared by our methods; 4) constructing GUV microreactor arrays on substrates can be employed for the construction of functional devices containing inorganic nano-functional blocks; 5) unexplored reactions, besides nanoparticle synthesis in GUV, include but are not limited to: biosynthesis of genes (RNA and DNA), proteins and carbohydrates in vesicles; enzyme-catalyzed reactions; biomineralization processes; host-guest interactions.

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The interaction between cells, tissues and biomaterial surfaces are the highlights of the book "Biomimetic Based Applications". In this regard the effect of nanostructures and nanotopographies and their effect on the development of a new generation of biomaterials including advanced multifunctional scaffolds for tissue engineering are discussed. The 2 volumes contain articles that cover a wide spectrum of subject matter such as different aspects of the development of scaffolds and coatings with enhanced performance and bioactivity, including investigations of material surface-cell interactions.

How to reference

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