교육학석사 학위논문

Stability of silica-based nanoparticles

실리카계 나노 입자의 안정성에 대한 연구

2017 년 8 월

서울대학교 대학원

과학교육과 화학전공

양 선 아

Stability of silica-based nanoparticles

지도교수 Yu, Junhua

이 논문을 교육학석사 학위논문으로 제출함
2017 년 8 월

서울대학교 대학원
과학교육과 화학전공
양 선 아

양선아의 석사 학위논문을 인준함
2017 년 8 월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

Abstract

Stability of silica-based nanoparticles

Yang, Seon-ah
Department of Science Education
(Major in Chemistry)
The Graduate School
Seoul National University

Silica has received huge attention and is broadly applied to biomedical application due to advantageous properties such as low toxicity, biocompatibility and tunable synthesis in particle size, porosity and shape render. Though stable in water and phosphate buffered saline, silica nanoparticles are eroded by biological media, leading to the exposure of AgNDs from AgND@SiO_2 nanoparticles and the quenching of nanodot luminescence. It have presented that a synergistic effect of organic components in cellular media, particularly the amino groups, accelerates the erosion. The results indicate that silica nanostructures are vulnerable to cellular medium and the tendency may depend on porosity and presence of foreign substance in silica layer. This study may be possible to tune the release of drug molecules from silica-based drug delivery vehicles through controlled erosion.

Keywords : silica nanoparticles, erosion, cell culture media
Student Number : 2015-21623

Contents

List of Figures

List of Tables

Ⅰ. Introduction

1.1. Strategy for stable nanoparticles

1.1.1. Aggregation of Nanoparticles in aqueous solution

Nanomaterials have demonstrated unique and remarkable characteristics that are not present in bulk. It attracted much attention to their potential to be applied to healthcare, cosmetics, electronics and information technology. So, well-engineered nanostructures have been required for controlling the properties in almost all areas of nanomaterial researches.[1-4]

However, many studies have found that it is difficult to keep the size of nanomaterials in colloidal system.[5] The dispersion of nanoparticles may not be favorable thermodynamically, because interfacial tension requires a high free energy.[6] For thermodynamically stable system, as the nanoparticles agglomerate to reduce the ratio of surface area to volume, total of free energy of the system may be reduced. From different angle, when nanoparticles are generated in colloidal system and the ratio of surface area to volume is reduced, nanoparticles can be more adjacent to each other. The thermodynamic interaction in short-range facilitates attachment between the

particles.[7] Therefore, uniform-sized nanoparticles especially in colloidal systems has been disturbed by the tendency of aggregation.[8] Such propensity to growth of clusters leads to change of physicochemical properties such as reactivity and stability in colloidal system. Furthermore, biological interactions such as biocompatibility are also influenced by the growth of clusters.

For further applications of nanoparticles in colloidal system, in order to protect the aggregation of nanoparticles, it is important to understand fundamental aggregation kinetics and instinct colloidal behavior.

1.1.2. Considerations for stable nanoparticles in colloidal system

Derjaguin-Landau-Verwey-Overbeek (DLVO) theory has been widely applied in an effort to understand the aggregation of nanoparticles in colloid science. Classic DLVO theory simply explains the surface interactions thermodynamically by both van der Waals and electrostatic force. [9] and it helps to predict the aggregation tendency of nanoparticles. And then as Lewis acid-based interaction and non-DLVO force are introduced to existing DLVO theory, extended- DLVO (EDLVO) theory has been developed and permits to complex interfacial forces such

as hydration and osmotic effect.[10]

Some factors have been reported to affect dispersion in colloidal system, for example morphology, chemical composition and solution chemistries including pH, ionic strength. Shape of nanoparticles as well as the size representatively influences the aggregation kinetics. In DLVO theory, it is supposed that the shape of particles is spherical. However, the nanoparticles have a multitude of shapes such as nanotube, nanowire and nanoplates. The changes of shape influence to Van der Waals and electric double layer (EDL) forces. [11] The EDL forces explain the interactive orientation of different shapes from sphere. The change in EDL forces may be caused by different crystallographic orientations by atomic arrangements.[7] Solution pH and dissolved ionic solute also affect to stability in aqueous solution by changes of the surface charge. At low pH, excess of H^+ ion results in a positively charged particle surface, whereas high pH generally renders a negative surface charge.[7] On that account, the pH control results in the change of surface charge reversely and stabilization in dispersion. When the surface charge become neutralized or electric double layers are screened, the aggregation is more favorable. In addition, chemical composition influences to Hamaker constant, surface potentials and hydrophobic/hydrophilic properties[12]. Consequently, the changed factor affects the aggregation tendency.

1.1.3. Strategies for stability in colloidal system

In order that the nanoparticles can maintain the nano-size and dispersed state in aqueous solution, surface coating is typically introduced to prevent aggregation by the enhancement of the electrostatic, steric, or electrostatic repulsive forces between them [13-15] and provide additional functionality. Surface coating is introduced to the nanoparticles by various type of coating materials such as polymers, surfactants and polyelectrolytes and the dispersibility after the coating depends on the type of coating and repulsive force obtained from surface coating.

Adsorption or covalent bond of surfactants can impart the surface charge to nanoparticles and reduce the interfacial energy in solvent.[16] Therefore, it can prevent the nanoparticles from aggregation. The influences are dependent on characteristics of surfactants including molecular weight or type of head groups and solvents.[7] In other hands, large molecular weight polymers usually provide steric repulsive forces due to stereotactic hinder.[16] Consequently, the surface coating can prevent it from approaching of adjacent molecules.

Especially in the case of inorganic nanoparticles for biological application, a core-shell nanostructure is usually applied to various applications. As the structure involves the core part surrounded by a layer of another material by ionic or covalent

bonds, chemical stability, dispersibility, luminescence properties and controlled release of the core are improved. [17-18]

1.2. Why Silica is attracted for biological application?

Silica has received huge attention as coating materials especially in biomedical fields. Advantageous properties such as low toxicity, biocompatibility and tunable synthesis in particle size, porosity and shape render silica broadly applicable.

1.2.1. Physicochemical properties

Silica showed reasonable stability especially in aqueous media. The stability of silica in colloidal chemistry could be explained in terms of steric and electrostatic forces that protect them from aggregation.[19] Dispersive partsof the Hamaker constant of silica in water showed low value than those of other nanoparticles at same size, which indicates the attraction energy– mainly van der Waals interactions between two silica particles is much lower.[20]In addition, cations and positively charged molecules strongly can be attached to negative charged polymeric silicate layer under basic conditions.[21]Furthermore,

these studies facilitates the control of their solubility in various solvents. [22]

Besides, silica nanoparticles usually have good optical transparency and low conductivity depending on specific surface area, density and concentration of silanol group.[23-24] in optics, the transparency means incident light can pass through the materials without or with less scattering. The exceptional properties have been widely studied in the approach of surface defect. The transparency is known to occur by relatively large energy gap between valences to conduction band, approximately 9eV. The large energy gap leads to short range distance in the crystal structure and then can explain that the crystalline of SiO_2 is mainly α-quartz by sp^3 hybridizationm.[25] However, in the presence of the defects, the characteristics of the silica nanoparticles can be degraded because the energy gap between the electronic states localized in defects is less than the energy gap in pure silica.[25] So, silica nanoparticles are extensively studied for their potential as photonic crystals, chemical sensors, biosensors, markers for bio imaging and catalysts, etc.

1.2.2. Relatively Safety *in vivo*

Generally, silica is considered as relatively safe materials to

human body by approval of U.S Food and Drug Administration (FDA).[26] Biocompatibility of materials was defined as the ability of a material to perform with an appropriate host response in a specific application by a consensus conference of the European Society for Biomaterials.[27] And it indicates that biomaterials have met certain criteria of safety and efficacy in specific situations and chemically inert and potentially bioactive. Recent *in vitro* and *in vivo* studies of the biocompatibility of silica-based nanomaterials have been carried out, especially cellular uptake[28-29], haemolytic activity[30], distribution and elimination from the body, repeated dose toxicity, genotoxicity, and carcinogenicity. Numerous parameters- the size, porosity, and morphology, surface chemistry of the silica particles, and the location and manner of their physiological introduction have been found for factor of the biocompatibility. The studies indicate that appropriate biocompatibility and non-toxicity of silica nanoparticles can be accomplished by optimization of these parameters and thus can avoid side effects such as asbestosis and silicosis. Especially in application of drug delivery, toxicity *in vivo* is as much as most critical issue as capacity to deliver the drugs. It has been reported that mesoporous silica nanoparticles (MSNs) do not induce any cytotoxicity in a multitude of cell lines from several studies [31-32], while some growth was inhibited over 200 μg mL^{-1} of the concentration. Although many questions are still remained for

application of drug delivery system, bio-inspired silica synthesis routes can be used advantageously to reduce human cytotoxicity. And it provides improvement of biocompatibility both through implementation of being synthetic conditions and the resulting enhanced control over composite silica structure.[33]

1.2.3. Tunable synthesis and Surface modification

1.2.3.1. Control of particle size, porosity and shape

Stöber method firstly introduced hydrolysis by catalysis of ammonia and condensation in methanol, ethanol, or isopropanol as solvents and successfully produced uniform sphere shaped silica nanoparticles in 1968. [34] Further studies have reported that the particles size and the distribution can be precisely controlled by concentration of ammonia, solvent, reaction temperature and ratio of silicate additives to solvent. [35]

Much synthetic technologies of silica nanoparticles have been derived from Stöber method. For instance, modified Stöber methods allow to synthesize various shapes of silica nanostructure, such as porous or hollow sphere[36], tubes [37], and wires[38]. The mesoporous silica nanoparticles (MSNs) can be synthesized by modified Stöber method by introduction of surfactants as structure-directing agents.[39] Increased surface

area of mesoporous silica nanoparticles allows loads of drugs more efficiently. The characteristics of pore-size, structure and particle crystallinity can be designed by control of materials and the concentration.[40] The pore size distribution showed in narrow [41] and the pore diameter can be tuned in allowable range of effortless endocytosis in living animal and plant cell. [42] In the other examples, modified Sol-gel method using removable templates [43] and layer-by-layer (LbL) adsorption[36] have been used for synthesis of hollow particles. As outstanding properties has been reported to low bulk density, high specific area and high drug loading capacity, hollow structured silica nanoparticles have been recognized to higher applicability to biological application. Furthermore, desirable materials such as silver, gold, or various polymers, can introduce to core part of the templated particles.[44]

1.2.3.2. Facile surface chemistry

Surface functionalization and modification provide new abilities or improve the properties of the nanoparticles. For instance, introduction of some active groups such as polyethylene glycol (PEG) can increase the colloidal stability of nanoparticles and offer the opportunities to graft polymers or conjugate biomolecules. High density of silnaol group on the surface

especially makes it more easily to conjugate other functional groups to their surfaces. In addition, high specific surface area in three-dimensional structure modulated can provide wide space to interconnect to other reagents.

There are general two approaches for the conjugation of silica nanoparticles: non-covalent electrostatic adsorption and covalent coupling reaction. [45] The electrostatic adsorption is usually used for fixation of biomolecules including antisense oligonucleotides and peptides on the surface. For instance, the positively charged peptides containing protonated N-terminate interact with negatively charged silica surface. Hydrogen bonds between polar groups with silanol and siloxide groups on the silica surface also affect to the physical adsorption.[46] On the other hands, different strategies of covalent bonding are applied to biomolecules such as peptides, monoclonal antibodies and DNA by the kinds of functional groups on the surface. The surface modification with aminos, mercaptos and carboxyl group is allowed efficiently to bind to biomolecules.[47-49] Moreover, these surface functionalization is used for various application, such as modulate drug or chemical loading, nanoparticles dispersion, blood circulation, cellular uptake and site specific targeting.[50]

1.3. Silica nanoparticles in biological application

1.3.1. Application in bio imaging and bio sensing

Silica-based networks in three-dimensions can provide suitable environment for especially bio imaging application. One of the unique properties, optical transparency facilitates efficient optical detection through layers of silica. It has been employed for building biosensors. Silica encapsulated structures are mostly expected for improvement of chemical stability and dispersibility to colloids,[49, 51] enhancing luminescence properties, controlled release of the core, and so on. It is also possible to design various arrays of materials as core and shell. So tiny fluorescent molecules or metal particles with unique optical, magnetic, or Raman-active properties, such as quantum dots, noble metal or metal oxide particles and magnetic particles have been usually applied in form of core-shell structures. Recent researches in silica-based NPs have focused on biological applications including bio imaging, bio-labeling, bioassays, immunoassay, cell targeting, and biosensors.

Noble metal nanoparticles, representatively gold and silver, are one of attractive materials in bio-imaging application, due to their distinct physicochemical properties and localized Surface Plasmon Resonance (LSPR).[52] As silica coating provide

opportunities to easily control the characteristics of surface, improve stability and biocompatibility, noble metal nanoparticles @ SiO_2 can be applied in bio imaging as well as catalysis and self-assembled nanostructure.

1.3.2. Application as Carriers for drug delivery

Drug delivery system (DDS) has been developed to improve the kinetics of pharmaceuticals and bio-distribution of related drugs, or function as drug storage *in vivo.*[53] Nano-sized particulate carriers such as compositions of lipids, micelles [54], dendrimers, biopolymers[55] and inorganic nanomaterials[56-57] is included in the system and delivery of different cargo including drugs as well as imaging agents have contributed to development of nano-medical fields.[58-59] Successful biocompatible carrier has been required to have good loading capacity of drug molecules without any premature release of the cargo before reaching the destination.[42] Above all, silica has shown potentials to store and gradually release therapeutically relevant drugs for control of drug association and release timing.[42] And silica as the carrier contributes improved biocompatibility to existing drug delivery systems.

Mesoporous silica nanoparticles are one of most attractive carrier in medical application. As porosity of the mesoporous

nanoparticles provides large surface areas and pore volumes and the pore size as well as surface chemistry also can be precisely controlled, MSNs can enhance efficient release with high concentration and favorable interactions with the surrounding environment for controlled released applications [47] and facilitate additional application in bio imaging and photodynamic therapy with fluorescent labels. Furthermore, MSNs showed stronger resistance to heat, pH, hydrolysis induced degradations and mechanical stress than polymer based structure and can provide more stable and rigid frame work [42].

1.4. Stability of Silica based nanoparticles for biomedical application

As the organic-inorganic hybrid nanoparticles have been applied to biomedical application, the degradation of silica based nanomaterials received also attentions. It is necessary to understand stability of nanoparticles in biological media such as phosphate buffered saline (PBS) or cell culture media for the applications of biomedicine and the studies reported that the phenomena is related to the size, shapes, porosity and physiological components.[60-63] For instance, sphere-shaped MSNs showed faster degradation rate in fresh Dulbecco's Modified Eagle's Medium (DMEM) in comparison with the rod-

shapes, which were probably caused by larger surface area.[63]

The strategies to enhance the dispersibility have been widely known to control in removal process of surfactant and introduce polyethylene glycol (PEG) to surface of silica nanoparticles. In the case of the mesoporous, PEGylated MSNs have showed improved stability in biological media [64-65] as well as reduction of nonspecific reticuloendothelial system uptake [66].

Previous observations have tended to focus on phenomena of the degradation of silica nanoparticles in cell culture media rather than how cell culture media influences to stability of the silica nanoparticles. So, we did investigate influence of components in cell culture media to the degradation obtained from luminescent silver nanodots and improve the stability of silica in the media.

Ⅱ. Experimental Section

2.1. General

2.1.1. Chemicals

Phosphate buffer saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM, D6434), fetal bovine serum, silver nitrate (99.9999%), sodium borohydride, tetraethyl orthosilicate (TEOS), N-[3-(Trimethoxysilyl)propyl]ethylenediamine (NED), mPEG5K-Silane (average Mn 5,000), mesoporous silica nanoparticles (200 nm in diameter), propylcarboxylic acid functionalized mesoporous silica nanoparticles (200 nm in diameter), propylamine functionalized mesoporous silica nanoparticles (200 nm in diameter), glycine, sarcosine, glycine methyl ester, diethylenetriamine, riboflavin, tyrosine, threonine, folic Acid, glucose, potassium phosphate dibasic trihydrate, and $Au@SiO_2$ nanoparticles (20 nm gold core) were purchased from Sigma-Aldrich and used as received. Sheep blood was purchased from Thermo Scientific. Single strand DNAs (ssDNAs) were synthesized by Integrated DNA Technologies.

Deionized water (DI) at Milli-Q grade water were used in all the preparation.

2.1.2. Instruments

TEM images were obtained on a JEM 2100 transmission electron microscope (JEOL Ltd.) at 200kV. The size distribution of nanoparticles was obtained from multiple TEM images. Energy Dispersive Spectroscopy (EDS) results were acquired on a X-maxT (Oxford instruments) connected to JEM 2100F transmission electron microscope. Emission spectra were obtained on QM-40 (Photon Technology International, Inc.), respectively. Microscopic emission images were obtained on an Olympus XI-81 microscope with a ×60 objective (NA 1.35) and an Andor LucaEM S 658M camera. An Eppendorf cooling centrifuge 5415 R was used to collect silica nanoparticles. Samples at 37℃ were stored in a CO_2 incubator at 37℃ (Thermo Fihser Scientific). Infrared spectra were obtained on a FT-IR Fourier Transform Infrared Spectrometer (TENSOR27, Bruker). DLS was analyzed with a Dynamic Light Scattering Spectrophotometer (DLS-7000).

2.2. Preparation and measurement of samples

Preparation of Silica encapsulated gold nanoparticles (Au@SiO_2 NPs) Au@SiO_2 nanoparticles were concentrated by centrifugation and re-suspended in 1 mL of fresh deionized water, PBS, DMEM, DMEM supplemented with 10% FBS, and sheep blood, respectively. After 5 min, 30 min, 1 h, 3 h, 6 h and 9 h incubation in a CO_2 incubator (5% CO_2, 37℃), samples were centrifuged at 16,000 rpm and collected highly concentrated. For protection of damage of silica shell during centrifugation, the Au@SiO_2 was fixed for 9 h before TEM examination.

Preparation of Mesoporous Silica nanoparticles (MSNs) Mesoporous silica nanoparticles (amino-modified, carboxylic acid-modified, and no modification; respectively 0.1 mg) were re-suspended in 1 mL of DMEM. After 5 min, 30 min, 1 h, 3 h, 6 h, and 9 h incubation in a CO_2 incubator (5% CO_2, 37℃), samples were centrifuged at 16,000 rpm to collect the mesoporous silica nanoparticles.

Measurement of depth of dents on MSNs The depth of dents on the silica nanoparticles was obtained in TEM image on the

assumption that its original shape was sphere. The depth was measured difference between the thinnest part and hypothetical circle of spherical shape. The depth of the dent was counted as the degree of erosion.

Preparation of Silica encapsulated silver nanoparticles (Ag@SiO_2 NPs) Single stranded DNA stabilized silver nanodots emitters were prepared according to published data.[67] Single stranded DNA (ss DNA) and silver nitrate were mixed at a DNA base/Ag^+ ratio of 2:1, followed by the reduction with aqueous sodium borohydride. After chemical reduction for a day, the AgNDs were used as probes. SsDNA for the 615-emitter was used as a sequence of CGCGC12CGCG, GGGGC8CCCC.[68]The hydrodynamic size, as determined by dynamic light scattering (DLS), is examined in only deionized water (DI).

Ⅲ. Results and Discussion

3.1. Degradation of Silica layers in bio-medium

3.1.1. Degradation of Silica layers in cell culture medium

We investigated interaction between silica coating and the cell culture medium DMEM, which is widely used for *in vitro* studies. Thickness of the silica layer indicates how much silica coating is influenced from surrounding environment. The thickness was obtained by measurement of the thinnest part on silica shell surrounding $Au@SiO_2$ in TEM image. The commercially available $Au@SiO_2$ have approximately 20 nm of average diameter and the thickness of silica shell is approximately 11.3 ± 1.6 nm, as control group (Figure 1a).

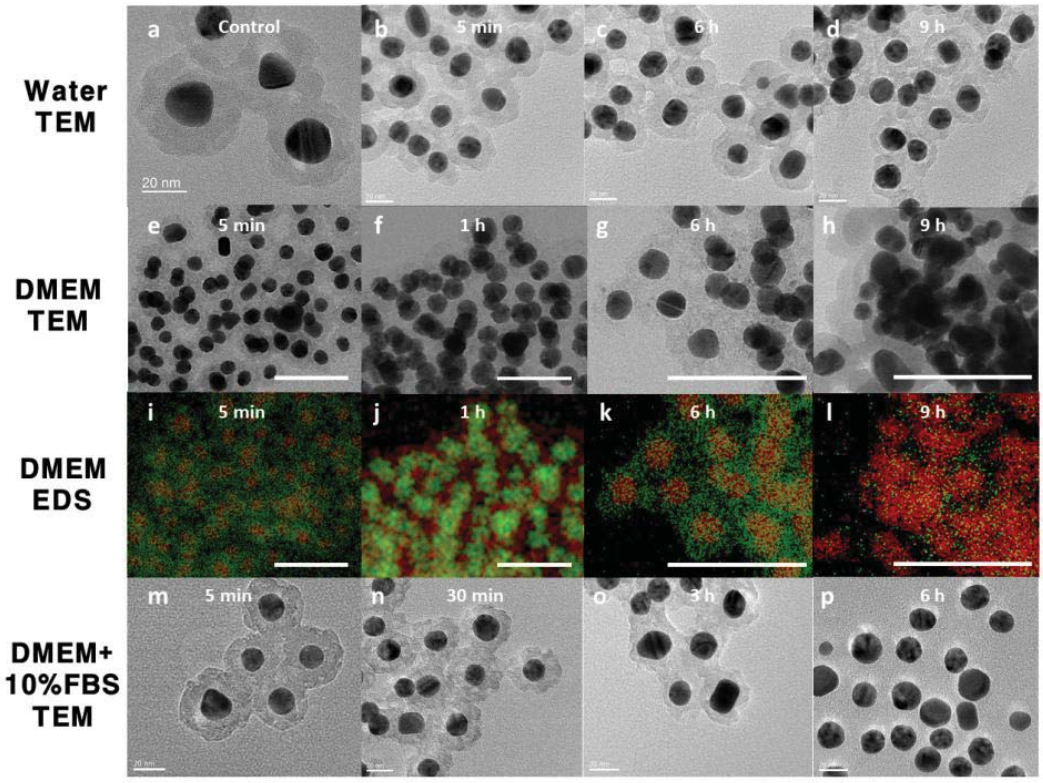

Figure 1. *in vitro* stability of silica shells in different medium. TEM images of control group (a), in deionized water at 5 min, 6 h and 9 h, respectively, Scale bars: 20 nm (b–d), and in DMEM at 5 min, 1 h, 6 h and 9 h, respectively, Scale bars, 100 nm (e–h). EDS images of Au@SiO_2 in DMEM at 5 min, 1 h, 6 h and 9 h, respectively (i–l), signal of gold (red dots) and silicon (green dots), Scale bars: 100 nm. TEM images in DMEM + 10% FBS at 5 min, 30 min, 3 h and 6 h, Scale bars: 20 nm (m–p).

The degradation of the silica coating depending on each medium are differently generated as shown in TEM and EDS imaged (Figure 1). The thickness of silica coating layer in DI water decreased gradually and reached to 8.1 ± 1.8 nm in 9 h at 37℃ (Figure 1b–d). The results are consistent with earlier studies indicated that the silica coating is slowly dissolved in

deionized water.[69] On the contrary, the degradation in the presence of DMEM was comparatively accelerated at same temperature (Figure 1e–h). The dents in silica coating started to increase after 1 hour. The energy dispersive spectroscopy (EDS) results visually and clearly provided the distribution of silica around gold nanoparticles as concentration of dots in some difficult TEM images to distinguish from surrounding. After 9 h, the silica shell almost disappeared around the gold nanoparticles in EDS image (Figure 1i–l). Furthermore, the silica shell in DMEM + 10% FBS was almost degraded and only bare AuNPs was observed in TEM image (Figure 1m–p).

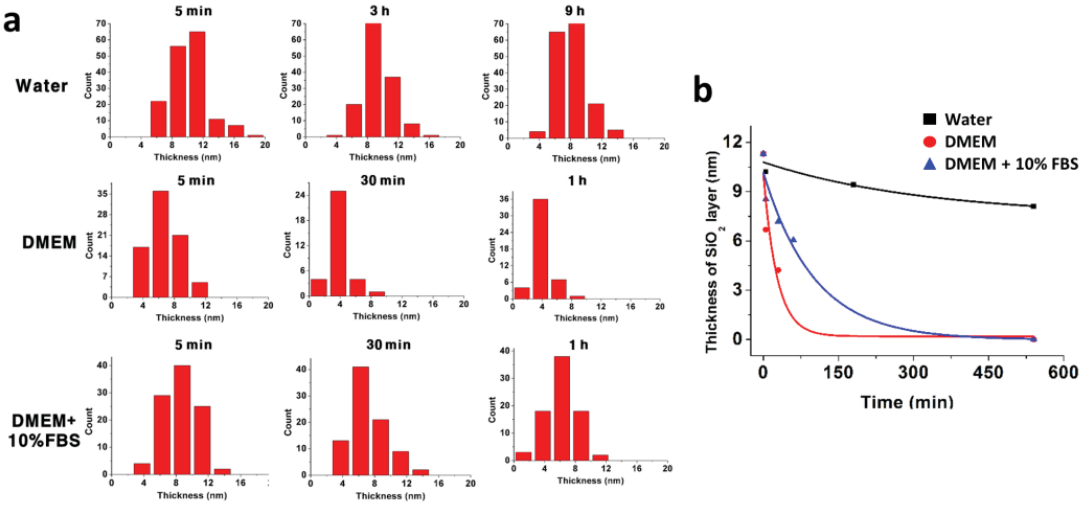

Figure 2. Size distribution of the thickness of SiO_2 layer in Au@SiO_2. Au@SiO_2 nanoparticles were incubated in water at 5 min, 6 h and 9 h and in DMEM at 5 min, 30 min, and 1 h measured in TEM images (a). Plots of the thickness of silica shells versus time in water (black), DMEM (red) and DMEM + 10% FBS (blue), measured in TEM images (b).

The distribution tables consist of the silica layer thickness in different medium, which are obtained by measurement in TEM image. The distribution tables present differently degradation degree of the silica shell (Figure 2). The average of thickness in DMEM decreased from 6.69 ± 2.01 nm to 4.22 ± 1.41 nm for 25 min, although the average in DIW was slightly changed from 10.2 ± 2.42 nm to 8.11 ± 1.76 nm for about 9 h. The DMEM showed rapidly deteriorated than in DIW and the distribution in graph of 10% FBS contained DMEM shifted slowly to thinner than DMEM. Although Ryabchikova E.I. et al. didn't show difference of degradation in cell culture media with or without serum due to long-termed scales,[70] we clearly suggested the influence of the addition of serum. In the presence of 10% FBS, we assumed that slightly decreased decay rate is caused by absorbed macro molecules from FBS, which showed opposite tendency to observations of mesoporous nanoparticles. [63]

3.1.2. Degradation of Silica layers in blood

In attempt to examine the degradation closely to physiological conditions, we tested the stability of silica *ex vivo*. Au@SiO_2 nanoparticles were prepared in incubation with sheep blood at

37℃ and checked by TEM and EDS after rinse. After 5 min, the silica coating was severely degraded in blood and only few was remained around the gold nanoparticles as shown in TEM image (Figure 3a). The silicon signals are distributed away from the gold core in EDS (Figure 3b-d). These results may suggest that cracks had formed before the harvest as sample preparation for electron microscope examination. The ratio of silicon signals by the time indicates erosion in blood was faster even than the silica in only DMEM (Figure 3e). After the incubation in blood for 9 h, the nanoparticles were situated around cells and silica shell almost damaged by components of blood (no coating left) as shown in TEM image (Figure 3f-h).

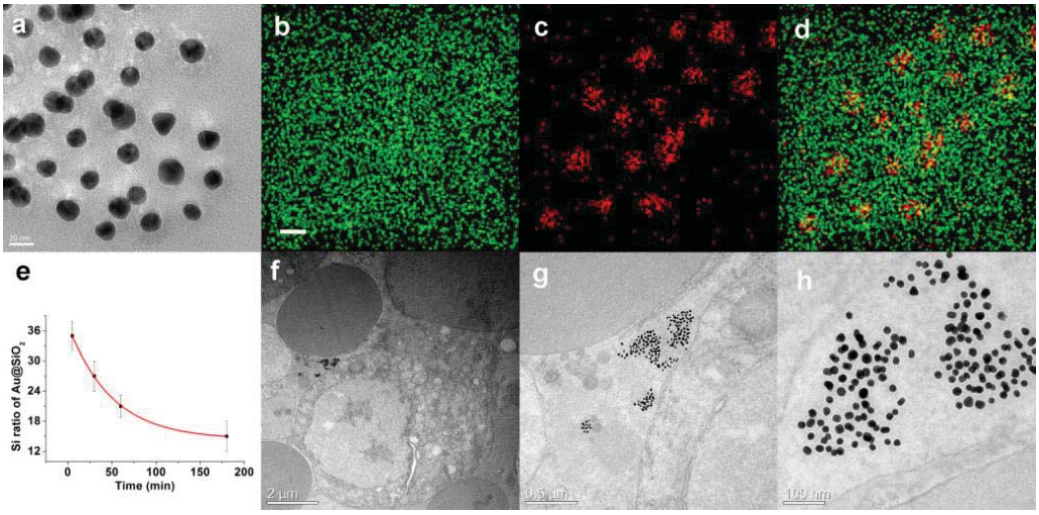

Figure 3. *ex vivo* stability of silica in sheep blood. Images of Au@SiO_2 incubated in blood for 5 min by TEM (a) and EDS (b-d), signal of silicon (green) and gold (red), Scale bars 20 nm. Plots of the EDS intensity of silicon by the time, measured from EDS images (e). Images of Au@SiO_2 in sheep blood at various magnification by TEM, Scale bars, 2 μm, 0.5 μm and 100 nm, respectively (f-h).

3.2. Influence of surface modification

3.2.1. Influence on surface functionalized mesoporous silica nanoparticles (MSNs)

In an effort to investigate cause of silica degradation, several type of functionalized mesoporous silica nanoparticles (MSNs) such as control, amino group modified and carboxylic group modified MSNs were prepared in the presence of DMEM at 37℃. The MSNs did not have homogeneous size distribution with commercially available MSNs in approximately 0.2 μm in diameter (Figure 4a). It may make it difficult to compare the decrease of diameter in the silica nanoparticles. However, different tendency of damage on the surface were shown obviously depending on their surface modification in the presence of DMEM (Figure 4a-b). As some parts in nanoparticles is forced to etching from the surrounding medium, the size of the nanoparticles can decrease and the depth of dents reversely increase. Several sites seriously damaged on the surface of MSNs were found in TEM images. Increasing dent is counted as severely damaged degradation in silica nanomaterials.

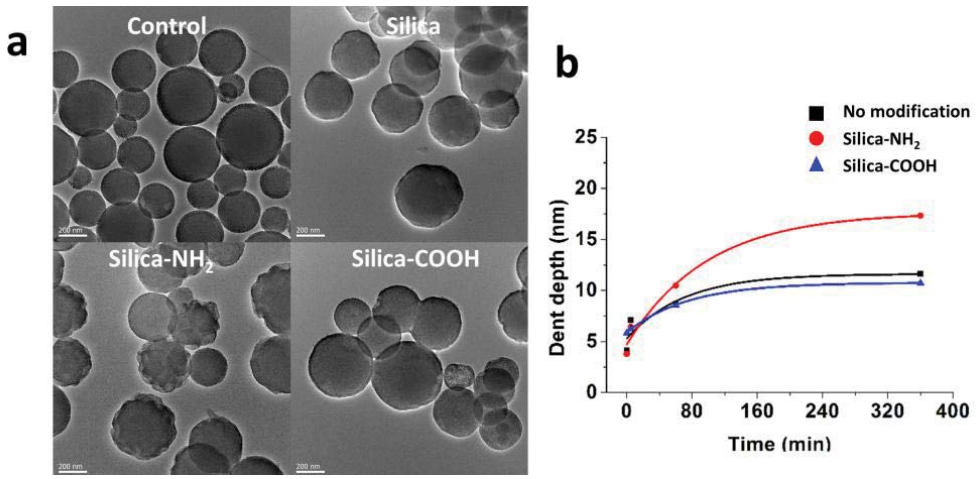

Figure 4. Degradation of mesoporous silica nanoparticles in DMEM. Representative TEM image of mesoporous silica nanoparticles. TEM images of control group, amino group-modified and carboxylic group-modified MSNs in DMEM at 6 h, Scale bars, 200 nm (a). Plots of the dent depth of MSNs versus time in DMEM (b).

As the incubation time passed, degradation on surface of the silica nanoparticles started to appear (Figure 4a-b). Surface of Propylcarboxylic acid functionalized MSNs was degraded at a similar rate with that of the non-modified, whereas depth of dents in propylamine functionalized MSNs increased (Figure 4b). Although size was distributed broadly in standard deviation, the dents depth of propylamine functionalized increased from 3.62 nm to 10.0 nm for 6 h.

These results suggested that surface modification of MSNs influences the rate of degradation. Propylamine-modified MSNs showed deeper depth of dents than propylcarboxylic acid modified MSNs. The severe etching by amino groups may indicate that amino-rich compounds make monomeric units of

silica stabilized and the speed of silicic acid poly-condensation reduced. The electrostatic attraction between the negatively charged silica nanoparticles and the protonated amino groups may facilitate strong bonding between nitrogen and surface of silica and then enhance the dissolution of silica nanoparticles.[71] The amino-rich groups in DMEM synergistically etched the silica surface, resulting in a significant increase in the degradation rate of silica.

3.2.2. Influence of specific functional group of DMEM components

To monitor the influence on the silica coating from each gradient in cell culture medium, luminescent silver nanodots was used as probe. The AgNDs has proven potentials of efficient and economical sensor in bio imaging.[72-73] As silica coating is eroded, the exposed luminescent AgNDs may be quenched (Figure 5a). We prepared single stranded DNA stabilized silver nanodots encapsulated in silica nanoparticles (AgND @ SiO_2) with appropriately 7 nm of diameter and the AgNDs emitted at 615 nm @ 560 nm excitation (Figure 5b).

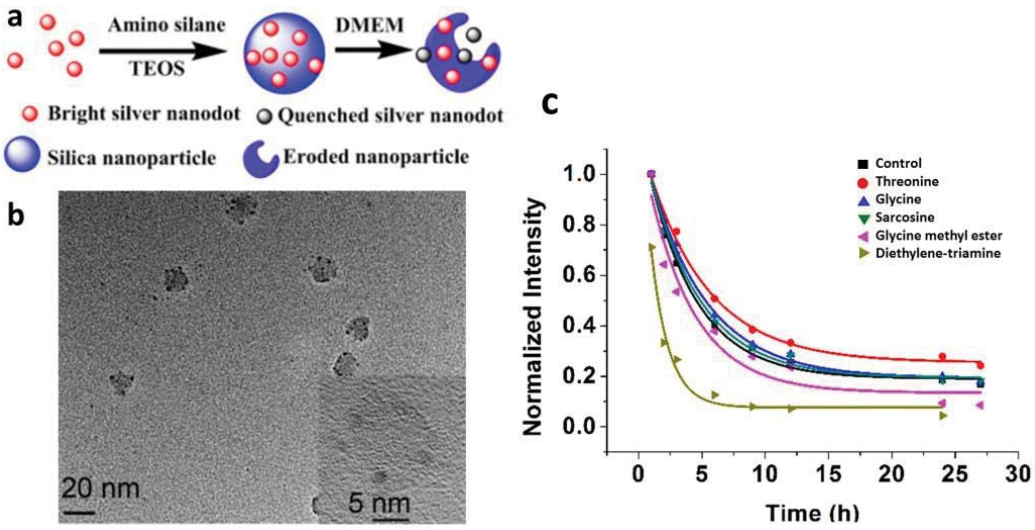

Figure 5. Detection of silica degradation with luminescent silver nanodots. Schematic presenting optical detection of silver nanodots in silica nanoparticles by the degradation (a), TEM image of silver nanodot-encapsulating silica nanoparticles (AgND@SiO_2). Scale bar, 20 nm, Inset image of a high resolution image of the above. Scale bar, 5 nm (b), Normalized luminescence intensity of the above AgND@SiO_2 in the presence of control (black), threonine (red), glycine (blue), sarcosine (dark cyan), glycine methyl ester (pink) and diethylene-triamine (dark yellow) in DI-water (c).

The AgND@SiO_2 nanoparticles were kept in one DMEM component or additional conditions added to DI-water and the intensity of luminescence was obtained as time goes by. The slope of emission intensity can be related to degradation rates of silica layers influenced from each component (Figure 5c). Lifetime of luminescence also can present the tendency of quenching and furthermore decay of the silica by functional group (Table 1). The results showed that specific functional

group influenced to degradation rate of silica nanoparticles.

Intensity of the nanodots in presence of amino group rich compounds such as diethylenetriamine drastically decreased than other plots. The steep slope in the presence of the *amino group* rich in diethylenetriamine, supported previous findings about the effect of amino group from MSNs, However, the amino group showed different tendency by attached location and influence from related functional group. In the case of primary amino acid attached to β−carbon, while tyrosine with para−methyl phenyl group was little difference from glycine, threonine, which is connected with hydroxyl group, showed relatively shorter lifetime in 4.2 h^{-1}. The secondary amino group in the amino acid barely accelerated the degradation rate of silica, because they may act as zwitter ions in water. The secondary amine group−protected such as sarcosine (N−methyl glycine) barely accelerated the decay rate. On the contrary, the lifetime in glycine methyl ester measured in 2.0 h^{-1} was even shorter than 4.5 h^{-1} of glycine. The carboxylic acid group in amino acid may provide stable environment and have less of effect on the silica than substituted compounds by methyl. This result is consistent with the fact that carboxylate ions interact weakly with silica and did not influence on the degradation of silica.[74] In addition, hydroxyl−rich compounds including riboflavin and glucose also showed gradual slope in the plots (Figure 5c). Hydroxyl group may not affect the decay.

Table 1. Photoluminescence decay of silver nanodots in $AgND@SiO_2$ in the presence of small organic molecules. Lifetime of luminescence was obtained from exponential fitting of the Figure 5c.

Compounds	Control	Alanine	Tyrosine	Threonine	Glycine	Sarcosine	Glycine methyl ester	Glucose	Diethylene-triamine
Luminescence Lifetime (h^{-1})	3.1	2.5	3.3	4.2	4.5	3.6	2.0	3.0	1.5
Standard deviation	0.46	0.36	0.55	0.40	0.67	0.63	0.14	0.72	0.46

3.3. Improvement of stability of silica coating in bio-medium

3.3.1. Surface modification by Polyethylene glycol (PEG)

Surface modification was introduced in order to improve the function of the silica coating for protection of the quenching of luminescent silver nanodots in DMEM. As the thickness of the silica layer increased, the degradation time of silica layers was extended and luminescent stability of the silver nanodots in DMEM was improved. Silver nanodots encapsulated in the large silica nanoparticles presented a survival half-life of 2.6 ± 0.3 h in DMEM, significantly longer than that of unprotected silver nanodots in DMEM (0.07 ± 0.02 h) as shown in the Figure 6.

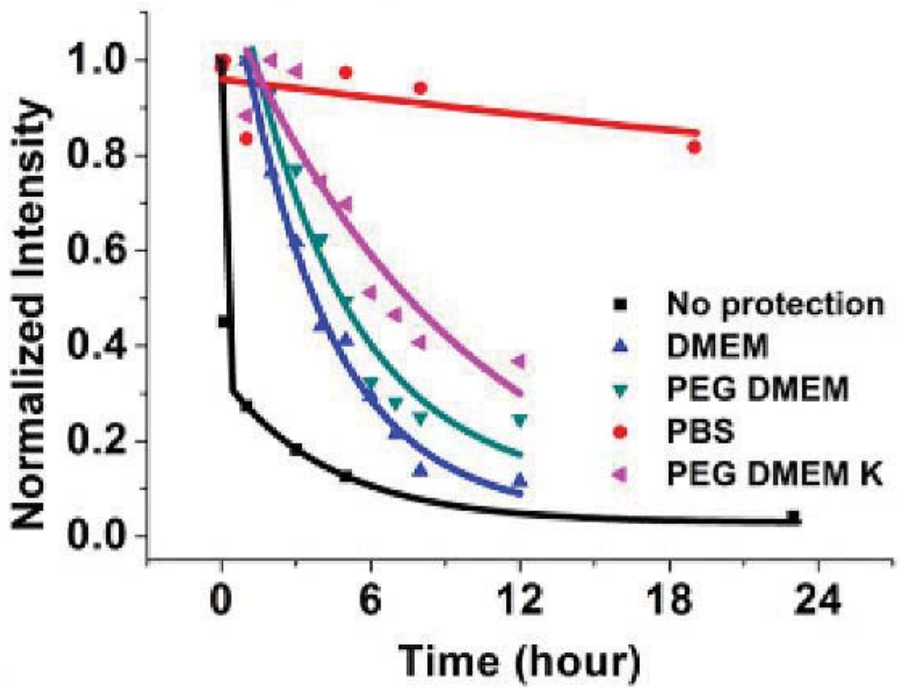

Figure 6. Comparison of silica stability under various conditions. Plot of luminescence intensity decay of silver nanodots @ 37℃ under various conditions, no protection of silica in DMEM (black) and in PBS (red), encapsulated silica nanoparticles (blue), PEG modified (pink), and PEG modified in addition of high concentration potassium ion (green) in DMEM.

PEG is one of general strategies for improvement of disperse and protection from protein adsorption. mPEG (mPEG5K-Silane, average Mn 5,000) was introduced to increase the thickness of silica coating and then improve the stability in DMEM. The PEG-modified AgND@SiO_2 exhibited strong infrared signals indicative of PEG (Figure 7a) and the hydrodynamic radius significantly increased hydrodynamic radius from 110 ± 15 nm to 1150 ± 220 nm after

modification (Figure 7b). The surface coating of only enhances increase of its half-life to 3.0 ± 0.2 h (Figure 6).

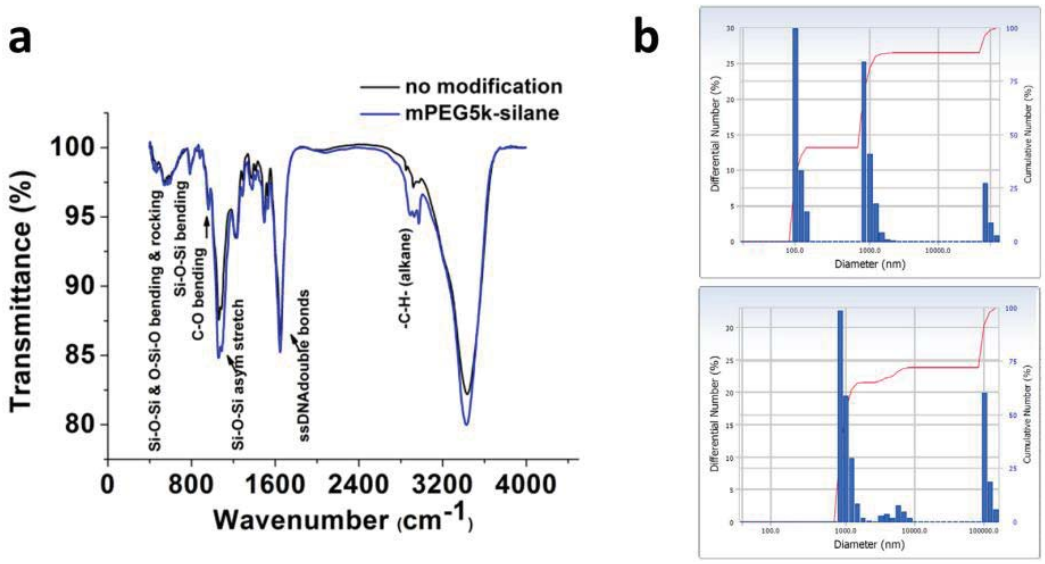

Figure 7. PEG modified silica nanoparticles. IR spectra of Silver nanodot-encapsulating silica nanoparticles (no modification, black) were modified with PEG (mPEG5k-silane, blue). Both nanoparticles were frozen dried in preparation for check of infrared spectrometer (a). Dynamic light scattering spectra obtained from DLS; no modification (upper), mPEG5k-silane (lower) respectively (b).

However, coating of polymer on the surface presented limitedly improvement of the silica stability in DMEM. Surface coatings of macro compounds may be hard to fully cover the surface, while small molecules can access the silica surface.

3.3.2. Addition of potassium ion to DMEM

As ahalf-life of the luminescent silver nanodots increased significantly to 57 ± 10 h in PBS even longer than that in water (Figure 6), the slowly decreased slope indicated that specific components of PBS, which is not contained in DMEM, may contribute stability of the silica surface in aqueous solution. EDS images of the stabilized silica nanoparticles presented that, the signal of potassium was highly overlapped with that of silicon element contrary to that of phosphate, chloride and sodium which is main components in PBS (Figure 8).

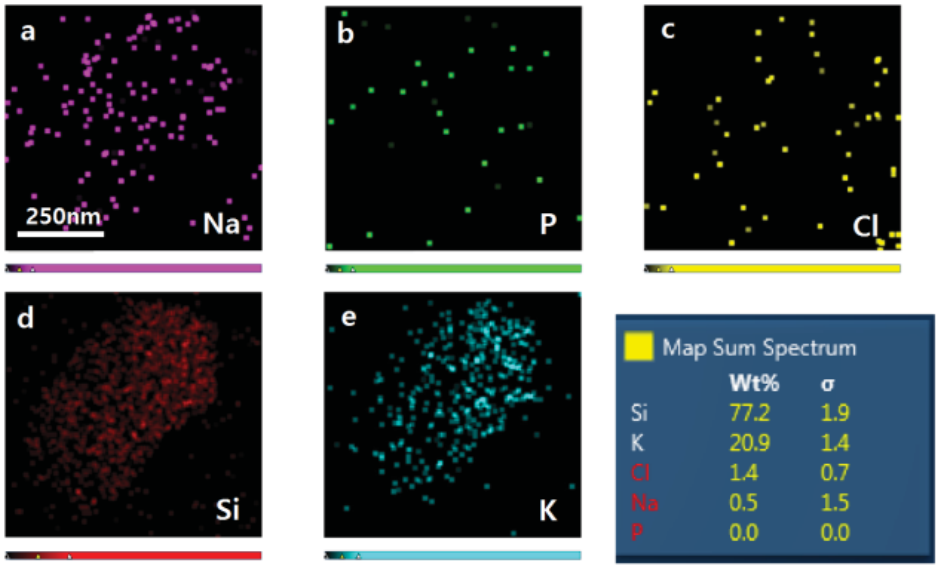

Figure 8. EDS images of Silica nanoparticles in presence of PBS. Silica nanoparticles were treated in PBS and prepared by centrifugal ultrafiltration with washes with DIW water three times. Signal of sodium (a), potassium (b), chlorine (c), silicon (d), and chlorine (e). Scale bars, 250 nm.

We, therefore, checked influence of the addition of potassium ions ($K2HPO_4$, 30 mM) into DMEM and observed significantly improved stability of silica with a survival half-life of 6.7 ± 0.5 h in DMEM (DMEM K in Figure 6). The outstanding stability of silica nanoparticles in PBS was also supported in previous *in vitro* research. Nevertheless, the negatively-charged nature of silica nanoparticles allows cations, particularly potassium ions, to adsorb onto the nanoparticle surface. It is not evident why silica nanoparticles showed a preference for potassium ions over sodium ions. This may be due to a specific arrangement of charges and defects on the surface, just like crown ether, which matched the size of

potassium ions and significantly increased the affinity for potassium.[75] It has been reported that potassium ions increase the mechanical strength of silica glass,[76] which was also accompanied by decreased solubility of silica in a salt solution of PBS, resulting in improved silica stability.[77] In addition, the formation of a potassium layer may form a shell to shield the silica from hydrolysis.

Ⅳ. Conclusion

In summary, silica based nanoparticles were influenced by the components of cell culture media as well as *ex vivo*. In presence of 10% FBS in DMEM, silica layers encapsulated around AuNPs showed slower degradation rate than the rate in only DMEM. FBS may act as act as stereo-tactical hinder when the silica layer is formed densely in contrary to the observation using MSNs [63]. Therefore it suggested different influence of FBS depending on the porosity of silica nanoparticles. And the kinetic data of luminescent silver nanodots encapsulating silica nanoparticles presented accelerated decay of silica shell from particularly amino-rich compounds in cell culture medium. Silica as coating materials in core-shell nanoparticles was degraded more severely than the pure silica nanoparticles, because foreign substance such as gold and silver nanodots may weaken the connection of silica networks. The enhanced stability in addition of potassium showed some potential to improve the stability of silica nanoparticles for *in vitro* studies.

The results provide some preliminary insights into the degradation of silica nanoparticles in cellular medium from a synergistic effect of organic components. It may be possible to control the erosion and furthermore apply to design of drug

delivery system as the vehicles to tune the release of drug molecules as drug delivery vehicles.

V. References

1. S. Y. Chou, P. R. Krauss, P. J. Renstrom, *Science,* 1996, **272**, 85.
2. T. Suntola, *Materials Science Reports,* 1989, **4**, 261-312.
3. K. Ariga, A. Vinu, Y. Yamauchi, Q. Ji, J. P. Hill, *Bulletin of the Chemical Society of Japan,* 2011, **85**, 1-32.
4. D. Macwan, P. N. Dave, S. Chaturvedi, *Journal of Materials Science,* 2011, **46**, 3669-3686.
5. A. Albanese, W. C. Chan, *ACS nano,* 2011, **5**, 5478-5489.
6. B. Xing, C. D. Vecitis, N. Senesi, *Engineered Nanoparticles and the Environment: Biophysicochemical Processes and Toxicity*. John Wiley & Sons: 2016; Vol. 4.
7. W. Zhang, Nanoparticle aggregation: principles and modeling. In *Nanomaterial*, Springer: 2014; pp 19-43.
8. J. D. Wright, N. A. Sommerdijk, *Sol-gel materials: chemistry and applications*. CRC press: 2000; Vol. 4.
9. M. Hermansson, *Colloids and Surfaces B: Biointerfaces,* 1999, **14**, 105-119.
10. E. M. Hoek, G. K. Agarwal, *Journal of Colloid and Interface science,* 2006, **298**, 50-58.
11. M. Elimelech, J. Gregory, X. Jia, *Particle deposition and aggregation: measurement, modelling and simulation*. Butterworth-Heinemann: 2013.
12. C. J. Van Oss, *Interfacial forces in aqueous media*. CRC press: 2006.
13. X. Li, J. J. Lenhart, *Environmental science & technology,* 2012, **46**, 5378-5386.
14. D. D. Jurašin, M. Ćurlin, I. Capjak, T. Crnković, M. Lovrić, M. Babič, D. Horák, I. V. Vrček, S. Gajović, *Beilstein journal of nanotechnology,* 2016, **7**, 246-262.
15. J. S. Gebauer, M. Malissek, S. Simon, S. K. Knauer, M. Maskos, R. H. Stauber, W. Peukert, L. Treuel, *Langmuir,* 2012, **28**, 9673-9679.
16. C. Lutz, J. A. Steevens, *Nanomaterials: risks and benefits*. Springer Science & Business Media: 2008.
17. J. Zhang, R. Misra, *Acta Biomaterialia,* 2007, **3**, 838-850.
18. S. Kim, B. Fisher, H.-J. Eisler, M. Bawendi, *Journal of the American Chemical Society,* 2003, **125**, 11466-11467.
19. M. Kaur, H. Zhang, L. Martin, T. Todd, Y. Qiang, *Environmental science & technology,* 2013, **47**, 11942-11959.
20. H. E. Bergna, W. O. Roberts, *Colloidal silica: fundamentals and applications*. CRC Press: 2005; Vol. 131.
21. G. Vigil, Z. Xu, S. Steinberg, J. Israelachvili, *Journal of Colloid and interface science,* 1994, **165**, 367-385.
22. I. Pastoriza-Santos, J. Pérez-Juste, L. M. Liz-Marzán, *Chemistry of Materials,* 2006, **18**, 2465-2467.

23. I. Rahman, P. Vejayakumaran, C. Sipaut, J. Ismail, C. Chee, *Materials Chemistry and Physics,* 2009, **114**, 328-332.
24. Y. Shi, C. J. Seliskar, *Chemistry of materials,* 1997, **9**, 821-829.
25. H. S. Nalwa, *Silicon-based material and devices, two-volume set: materials and processing, Properties and Devices*. Academic Press: 2001; Vol. 1.
26. S. C. Sahu, D. A. Casciano, *Nanotoxicity: from in vivo and in vitro models to health risks*. John Wiley & Sons: 2009.
27. D. F. Williams, E. S. f. Biomaterials, *Definitions in Biomaterials: Proceedings of a Consensus Conference of the European Society for Biomaterials, Chester, England, March 3-5, 1986*. Elsevier: 1987.
28. X. Huang, X. Teng, D. Chen, F. Tang, J. He, *Biomaterials,* 2010, **31**, 438-448.
29. J. Lu, M. Liong, S. Sherman, T. Xia, M. Kovochich, A. E. Nel, J. I. Zink, F. Tamanoi, *Nanobiotechnology,* 2007, **3**, 89-95.
30. I. I. Slowing, C. W. Wu, J. L. Vivero-Escoto, V. S. Y. Lin, *Small,* 2009, **5**, 57-62.
31. J. Lu, M. Liong, J. I. Zink, F. Tamanoi, *small,* 2007, **3**, 1341-1346.
32. I. Slowing, B. G. Trewyn, V. S.-Y. Lin, *Journal of the American Chemical Society,* 2006, **128**, 14792-14793.
33. P. Ducheyne, K. Healy, D. E. Hutmacher, D. W. Grainger, C. J. Kirkpatrick, *Comprehensive biomaterials*. Newnes: 2015; Vol. 1.
34. W. Stöber, A. Fink, E. Bohn, *Journal of colloid and interface science,* 1968, **26**, 62-69.
35. G. Bogush, M. Tracy, C. Zukoski, *Journal of non-crystalline solids,* 1988, **104**, 95-106.
36. F. Caruso, R. A. Caruso, H. Möhwald, *Science,* 1998, **282**, 1111-1114.
37. H. Nakamura, Y. Matsui, *Journal of the American Chemical Society,* 1995, **117**, 2651-2652.
38. N. I. Kovtyukhova, T. E. Mallouk, T. S. Mayer, *Advanced Materials,* 2003, **15**, 780-785.
39. F. Tang, L. Li, D. Chen, *Advanced Materials,* 2012, **24**, 1504-1534.
40. K. Unger, D. Kumar, M. Grün, G. Büchel, S. Lüdtke, T. Adam, K. Schumacher, S. Renker, *Journal of Chromatography A,* 2000, **892**, 47-55.
41. J. Zhang, X. Li, J. M. Rosenholm, H.-c. Gu, *Journal of colloid and interface science,* 2011, **361**, 16-24.
42. I. I. Slowing, J. L. Vivero-Escoto, C.-W. Wu, V. S.-Y. Lin, *Advanced drug delivery reviews,* 2008, **60**, 1278-1288.
43. A. Khanal, Y. Inoue, M. Yada, K. Nakashima, *Journal of the American Chemical Society,* 2007, **129**, 1534-1535.
44. J. Yang, J. Lee, J. Kang, K. Lee, J.-S. Suh, H.-G. Yoon, Y.-M. Huh, S. Haam, *Langmuir,* 2008, **24**, 3417-3421.
45. V. Shirshahi, M. Soltani, *Contrast media & molecular imaging,* 2015, **10**, 1-17.

46. S. V. Patwardhan, F. S. Emami, R. J. Berry, S. E. Jones, R. R. Naik, O. Deschaume, H. Heinz, C. C. Perry, *Journal of the American Chemical Society,* 2012, **134**, 6244-6256.
47. K. Möller, J. Kobler, T. Bein, *Journal of Materials Chemistry,* 2007, **17**, 624-631.
48. X. Liu, Z. Ma, J. Xing, H. Liu, *Journal of Magnetism and Magnetic Materials,* 2004, **270**, 1-6.
49. L. M. Liz-Marzán, M. Giersig, P. Mulvaney, *Langmuir,* 1996, **12**, 4329-4335.
50. J. Vivero-Escoto, *Silica Nanoparticles: Preparation, Properties, and Uses*. Nova Science Publishers: 2012.
51. X. Sun, Y. Li, *Angewandte Chemie International Edition,* 2004, **43**, 597-601.
52. S. M. Janib, A. S. Moses, J. A. MacKay, *Advanced drug delivery reviews,* 2010, **62**, 1052-1063.
53. T. M. Allen, P. R. Cullis, *Science,* 2004, **303**, 1818-1822.
54. J. M. Kim, Y. Sakamoto, Y. K. Hwang, Y.-U. Kwon, O. Terasaki, S.-E. Park, G. D. Stucky, *The Journal of Physical Chemistry B,* 2002, **106**, 2552-2558.
55. T. Coradin, J. Livage, *Comptes Rendus Chimie,* 2003, **6**, 147-152.
56. J. Kim, J. E. Lee, J. Lee, J. H. Yu, B. C. Kim, K. An, Y. Hwang, C.-H. Shin, J.-G. Park, J. Kim, *Journal of the American Chemical Society,* 2006, **128**, 688-689.
57. S. Giri, B. G. Trewyn, M. P. Stellmaker, V. S. Y. Lin, *Angewandte Chemie International Edition,* 2005, **44**, 5038-5044.
58. M. Liu, H. Du, Z. Wenjia, G. Zhai, *Materials Science and Engineering: C,* 2016.
59. A. S. Gupta, *Nanomedicine: Nanotechnology, Biology and Medicine,* 2011, **7**, 763-779.
60. Y.-S. Lin, C. L. Haynes, *Journal of the American Chemical Society,* 2010, **132**, 4834-4842.
61. D. Napierska, L. C. Thomassen, V. Rabolli, D. Lison, L. Gonzalez, M. Kirsch-Volders, J. A. Martens, P. H. Hoet, *Small,* 2009, **5**, 846-853.
62. W. Lin, Y.-w. Huang, X.-D. Zhou, Y. Ma, *Toxicology and applied pharmacology,* 2006, **217**, 252-259.
63. N. Hao, H. Liu, L. Li, D. Chen, L. Li, F. Tang, *Journal of nanoscience and nanotechnology,* 2012, **12**, 6346-6354.
64. V. Cauda, A. Schlossbauer, T. Bein, *Microporous and Mesoporous Materials,* 2010, **132**, 60-71.
65. Y.-S. Lin, N. Abadeer, C. L. Haynes, *Chemical Communications,* 2011, **47**, 532-534.
66. C. Fang, N. Bhattarai, C. Sun, M. Zhang, *Small,* 2009, **5**, 1637-1641.
67. S. Choi. Fluorescent noble metal nanodots for biological applications. Georgia Institute of Technology, 2010.
68. S. Choi, J. Yu, S. A. Patel, Y.-L. Tzeng, R. M. Dickson,

Photochemical & Photobiological Sciences, 2011, **10**, 109-115.
69. G. B. Alexander, W. Heston, R. K. Iler, *The Journal of Physical Chemistry,* 1954, **58**, 453-455.
70. E. I. Ryabchikova, B. P. Chelobanov, R. G. Parkhomenko, K. V. Korchagina, T. V. Basova, *Microporous and Mesoporous Materials,* 2017, **248**, 46-53.
71. E. Neofotistou, K. D. Demadis, *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 2004, **242**, 213-216.
72. S. Choi, S. Park, K. Lee, J. Yu, *Chemical Communications,* 2013, **49**, 10908-10910.
73. S. Park, S. Choi, J. Yu, *Nanoscale research letters,* 2014, **9**, 1-7.
74. H. Zou, S. Wu, J. Shen, *Chem. Rev,* 2008, **108**, 3893-3957.
75. J. Lehn, J. Sauvage, *Journal of the American Chemical Society,* 1975, **97**, 6700-6707.
76. M. Patschger, C. Rüssel, *Glass Technology-European Journal of Glass Science and Technology Part A,* 2015, **56**, 163-170.
77. W. L. Marshall, J. M. Warakomski, *Geochimica et Cosmochimica Acta,* 1980, **44**, 915919-917924.

국문초록

실리카는 낮은 유독성, 상대적으로 높다고 알려진 생체 적합성, 입자의 크기나 모양 그리고 다공성을 쉽게 조절하여 합성할 수 있는 유용성을 인정받아 다양한 산업분야에서 널리 사용되고 있다. 이러한 이점은 특히 생물의학 연구 분야에서 코팅 물질로 적용되는데 중요한 역할을 하였다.

이 연구에서는 실리카로 코팅된 은 나노 입자 및 투과 전자 현미경 이미지를 이용하여 체외 실험에서 널리 사용되는 세포배양액 내 실리카의 안정성을 측정하였다. 실험에 사용된 실리카 코팅된 은 나노 입자는 증류수와 인산완충용액에서는 안정한 반면, 생물학적 배양액에 의해 실리카 나노 입자의 표면이 분해되어 은 나노입자의 노출되어지기 때문에 나노입자의 형광세기의 약화로 이어지는 원리를 이용하여 실리카의 분해정도를 정량적으로 나타내었다. 그 결과, 세포배양액의 유기 구성 물질들의 상승적인 효과를 보여주었는데, 특히 아미노기의 경우 그 분해 속도를 가속시키는데 크게 영향을 미쳤다. 이러한 결과는 실리카 나노입자가 세포배양액 내에서 분해될 수 있으며, 이러한 경향성은 실리카 나노구조의 다공성이나 외부 물질의 존재에 의존하게 될 수 있음을 시사하였다.

이는 세포 배양액 내 실리카 나노입자의 분해에 대한 기초적인 이해를 제공하며, 향후 이러한 실리카 나노 입자의 분해의 조절을 통해 실리카 기반 약물 전달 매체로 약물 분자의 방출 양을 조절하는데 적용될 수 있을 것이다.

주요어 : 실리카 나노입자, 분해, 세포배양액

학 번 : 2015-21623