Synthesis of gold nanoshells based on the deposition-precipitation process

James C. Y. Kah^a,
Nopphawan Phonthammachai^b,
Rachel C. Y. Wan^b, Jing Song^b,
Timothy White^b, Subodh Mhaisalkar^b,
Iman Ahmad^b, Colin Sheppard^a,
Malini Olivo^{c*}

^a Division of Bioengineering, National University of Singapore, Singapore

^b School of Materials Science and Engineering, Nanyang Technological University, Singapore

^c Division of Medical Sciences, National Cancer Centre Singapore, Singapore

^c Bio-Optical Imaging, Singapore Bioimaging Consortium, Biomedical Sciences Institutes, Singapore

* Corresponding author. Mailing address: Division of Medical Sciences, National Cancer Centre Singapore, 11 Hospital Drive, Singapore 169610.

Telephone: +65 64368317 Facsimile: +65 63720161

Email: dmsmcd@nccs.com.sg

Abstract

The synthesis of gold nanoshells involves the preparation of precursor seed particles consisting of nanoparticulate gold loaded on a dielectric core as scaffolds on which the layer of gold shell can be grown. The common route in preparing these seed particles involves a two-step technique of synthesizing colloidal gold particles followed by attaching them to amine functionalized dielectric core. In this paper, we present the use of a single-step deposition-precipitation (DP) process, commonly used in the catalytic field, as a feasible alternative route to seeding gold hydroxide nanoparticles onto a silica core and growing a layer of gold shell around the core without the need for prior synthesis of colloidal gold. The various factors such as pH, temperature and time of reaction, as well as the effect of amine functionalization of silica on the deposition of gold were investigated.

Compared to bare silica nanoparticles whose low isoelectric point render the DP process of seeding nanoparticulate gold unfavorable, amine functionalization of the silica surface is able to alter its isoelectric point to facilitate the deposition of gold hydroxide nanoparticles and increase the uniformity and density of seeding. We have also established that by varying the pH and time of reaction, it is possible to control the size of the gold hydroxide nanoparticles and density of seeding. The highest seeding density was achieved at pH 8 where the surface charge on the amine terminated silica surface favored the attraction of complex gold anions and the hydrolysis of these complex anions at elevated temperature produced the insoluble gold hydroxide precipitate which was readily deposited on the silica. As the time of reaction was increased from 3 min to 60 min, these gold hydroxide nanoparticles also increased in size from 2 nm to 7 nm. We have also shown the progressive growth of the gold hydroxide seeds to eventually form gold nanoshells with a complete layer of gold shell with tunable optical response.

Keywords

Gold nanoshells, silica, surface functionalization, deposition-precipitation, gold hydroxide.

Introduction

Amongst the wide range of clinical imaging modalities available to clinicians today, advanced optical imaging systems such as optical coherence tomography (OCT), confocal reflectance endomicroscopy and diffuse optical tomography etc. offer promising clinical application particularly in the area of cancer imaging due to the high resolution they afford [1-3]. However, these novel optical systems rely on scattering processes in tissue which can be rather indiscriminate in highlighting diseased states [4]. The application of metallic nanoparticles such as gold nanoparticles as contrast agent in these reflectance-based imaging techniques is well appreciated [5], but limited to a narrow optical excitation range that are appropriate for use with only certain optical systems with matching optical wavelength. The narrow optical excitation range is due to its rather invariable optical plasmon resonance, typically at 520 nm. Although its plasmon resonance is known to vary with size, the plasmon resonance of gold nanoparticles hardly changes by more than 60 nm before their sizes become impractical for biological applications [6]. Hence, for most optical imaging systems that operate in the near infrared (NIR) wavelengths, the optical response afforded by these gold nanoparticles are not matched, resulting in sub-optimal

optical performance.

Gold nanoshells have been an emerging subject of recent research, mainly fueled by their unique tunable optical response to match the excitation wavelength of many optical imaging systems. Compared to gold nanoparticles, gold nanoshells are versatile for use with promising clinical imaging modalities that operate in the NIR such as the OCT [7, 8]. Gold nanoshells composed of a dielectric core, surrounded by a thin gold shell, whose relative thickness to the core size dictates the position of their peak optical resonance response, which can be tuned from visible range right up to the mid-infrared wavelengths [9]. Apart from their optical properties, these nanostructures also present inert biological activities

which make them appropriate for biological applications [10, 11] and they can be readily surface functionalized with targeting moieties for targeting to specific diseased sites [7]. The potential of gold nanoshells in biological applications have recently been demonstrated in several studies, including their use in molecular imaging, as contrast agents for OCT and also in photothermal therapeutic applications [12-15].

The common synthesis route of gold nanoshells involves the synthesis of the dielectric core material, typically silica or polystyrene and their surface functionalization with terminal amine groups to facilitate attachment of small colloidal gold, usually formed from a separate process, on the amine terminated core to form a precursor seed particle for

Figure 1

The approach for synthesis of gold nanoshells using the (a) common two-step process of seeding gold nanoparticulate on silica and (b) our proposed single step DP process of gold seeding on silica prior to subsequent shell growth

Table 1

Reaction volumes of various reactants used to synthesise the silica nanoparticles core of different diameter sizes

Size (nm)	Ethanol (ml)	H ₂ O (ml)	NH ₃ (ml)	NH ₄ OH (ml)	TEOS (ml)
78.9 ± 10.5	138.0	10.8	-	2.4	6.6
120.6 ± 8.4	92.0	3.1	-	4.45	6.0
210.9 ± 8.0	138.0	4.65	0.675	6.0	9.0
248.8 ± 16.7	138.0	4.65	3.75	6.0	9.0
298.9 ± 11.2	138.0	4.65	6.0	6.0	9.0
377.0 ± 14.7	138.0	10.8	15.0	6.0	9.0
439.7 ± 14.7	138.0	10.8	30.0	6.0	9.0

subsequent growth of the polycrystalline gold shell in a controlled manner (Figure 1a) [16-19]. As there is weak interaction between silica and gold, the grafting of an amine terminated silane coupling agent to the silica surface grants the silica a nucleophilic surface for coordinate bonding with the colloidal gold particles. In this common synthesis route, the colloidal gold particles function as nucleation sites for the additional gold to be added on to eventually form a shell. These small colloidal gold are typically formed from the reduction of gold salt by strong reducing agents [20, 21] and may require several days of aging [16], up to 2 weeks for their size to stabilize before they are seeded onto the surface functionalized silica core. Whilst this common two-step process of seeding i.e. formation of small colloidal gold followed by attachment to the core is effective, it is also laborious and costly in term of time and consumables.

Different surface seeding strategies were also reported by other groups to prepare the precursor seed particles. Examples of these strategies include the use of electrostatic attraction to attach preformed anionic colloidal particles onto a polycationic polyethyleneimine functionalized polystyrene microspheres, as demonstrated by Ji et. al [22]. Besides using preformed colloidal particles, the electroless plating approach is another strategy where the metal ions or complex cations were absorbed onto the silica surface by the negatively charged Si-OH groups on the surface through electrostatic attraction and were subsequently reduced *in situ* to form the seed particles [23, 24]. Another possible approach employed the use of the solvent-assisted route where the polystyrene core was immersed in a mixed solvent of ethanol and acetone containing the gold salt, causing the surface layer of the polystyrene colloid to swell by the mixed solvent and permeated by the gold ions. The gold ions were subsequently reduced such that the swollen layer of the polystyrene colloids was uniformly loaded with gold nanoparticles [23]. Apart from seeding using colloidal gold or silver, Lim et. al. seeded tin (Sn) particles on the surface of silica, which in this case, the Sn particles formed on the silica surface acted as a linker site between the silica core and the gold shell as well as an efficient catalytic surface for the reduction of gold ions on the silica surface [25].

The deposition-precipitation (DP) process of loading small gold particles on a metal oxide support base developed by

Haruta's group has been widely investigated in the catalytic field to form supported nanoparticulate gold catalysts for low temperature oxidation of carbon monoxide [26-30]. The DP method, in essence, entails placing the support in contact with an aqueous solution of gold (III) chloride, the pH of which has been raised by the addition of a base, so that upon heating, oxidic precursor particles are formed on the support. The size and density of the nanoparticulate gold on the substrate is easily controlled by the concentration of gold (III) chloride, type of support base, reaction temperature and time and the pH of reaction. This DP method is the easiest to handle and is used now for producing commercial gold catalysts in a highly controlled manner. In this study, we adapted the DP method as an alternative route to seeding nanoparticulate gold onto the silica core as nucleation sites in a single-step process to form the precursor seed particle without the need for prior synthesis of colloidal gold (Scheme 1b). This precursor seed particle was then used to form the gold nanoshells. The various factors such as the pH, temperature and time of reaction, as well as the presence of surface functionalization on the deposition of gold were investigated and discussed. We also demonstrated the gradual growth of the gold shell on our precursor seed particle prepared from this DP method. The effects of other reactants on the growth of the shell will also be briefly discussed.

Materials and methods

Materials

The reagents used for the synthesis of silica nanoparticles include tetraethyl orthosilicate (TEOS, 99%, Merck), ammonia (NH₃, 27%, Merck), ammonia hydroxide (NH₄OH, 14.2 M, ISPL), absolute ethanol (C₂H₅OH, 99.9%, Merck) and milliQ water. 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich) dissolved in 25 parts water and 75 parts absolute ethanol to form a 12 mM APTES stock solution was used as an amine terminated silanizing agent to functionalize the surface of the silica nanoparticles with an amine terminal group. Hydrogen tetrachloroaurate (III), also known as gold (III) chloride (HAuCl₄·3H₂O, 99.99%, Alfa Aesar) was prepared in 6.35 mM and 25 mM aqueous solution for the DP process of seeding gold hydroxide (Au(OH)₃) nanoparticles and for growing of

the gold shell respectively. A solution of 0.1M sodium hydroxide (NaOH, Merck) was used to adjust the pH during the DP process of seeding. Potassium carbonate (K_2CO_3 , 99%, Sigma-Aldrich) was used to raise the pH of $HAuCl_4$ for its hydrolysis to form gold hydroxide solution for growing the gold shell. Both the sodium borohydride ($NaBH_4$, 98%, Sigma-Aldrich) and sodium citrate dihydrate ($HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$, 99%, Aldrich) were used in the reduction process of the gold hydroxide solution to grow the gold shell.

Synthesis of silica core and surface functionalization

The dielectric monodisperse silica nanoparticles were synthesized using the Stöber procedure [31], which involved the hydrolysis and condensation of TEOS in a sol-gel process. The volume of individual reagents in the reaction mixture used for producing the different sizes of silica nanoparticles were summarized in Table 1. The mixtures were allowed to stir vigorously overnight for the reaction to complete. The white turbid suspension was subsequently centrifuged and washed in ethanol and distilled water for at least 5 times at 10,000 rpm for 30 mins each before resuspending them in 80% by mass of absolute ethanol prior to surface functionalization. Using this protocol, spherical and monodispersed silica nanoparticles from a size range of 50 nm to 440 nm in diameter were synthesized. The silica nanoparticles were then surface functionalized by grafting them with 12 mM APTES in volume ratio of 3:7 under constant heating and vigorous stirring at 80°C for 1 h to give a terminal amine group on their surface. Under this condition, the APTES used was in molar excess to achieve a complete surface functionalization. The amine grafted silica particles were then cooled to room temperature and washed with at least 7 cycles of centrifugation and redispersion in absolute ethanol and distilled water at 8,000 rpm for 30 mins each to remove residual reactants before resuspending them in 1 ml of water for every 0.3 g of silica used for surface functionalization with amine.

DP process of seeding gold hydroxide nanoparticles

The amine grafted silica nanoparticles were then seeded with $Au(OH)_3$ nanoparticles on its surface using the DP process. Briefly, 4.5 ml of 0.1M NaOH was added to a 6.35 mM solution of $HAuCl_4$ and allowed to stir for about 15 mins before the solution became mild yellowish. The addition of NaOH raised the pH of the $HAuCl_4$ and allowed hydrolysis of $HAuCl_4$ to take place to form gold hydroxide solution. 1 ml of the amine grafted silica particles was then added to this gold hydroxide solution and the solution was heated at 70°C for 30 min under vigorous stirring where the color of the mixture turned from milky white to orange-brown as an indication of successful loading of $Au(OH)_3$ nanoparticles on the amine grafted silica. The product was then centrifuged and washed with distilled water for at least 5 times at 800 rpm for 1 h each and the final brown pellet redispersed in distilled water to give a final volume of 40 ml of precursor seed solution. Based on mass calculation, the concentration of the 300 nm

diameter precursor seed particles in the seed solution was of the order of 2.3×10^{11} particles per ml. In this study, the pH, temperature and heating time of reaction were varied to investigate the effects of these factors on the size and seeding density of the $Au(OH)_3$ nanoparticle seeds. The pH was varied by changing the volume of 0.1M NaOH added to the $HAuCl_4$. The $Au(OH)_3$ nanoparticles were deposited on a range of different sizes of silica cores as described in Table 1. The direct seeding of $Au(OH)_3$ nanoparticles on silica surface without the terminal amine group was also investigated. In this case, the synthesized silica nanoparticles were added directly to the gold hydroxide solution and the mixture heated to proceed with the DP process.

Growth of gold shell

The $Au(OH)_3$ nanoparticles deposited on the surface of the silica acted as nucleation sites and were allowed to grow in size by further addition of more gold reduced from $HAuCl_4$ until they eventually coalesce to form a continuous complete layer of gold shell around the silica core. In growing the shell, the pH of $HAuCl_4$ was adjusted to around 10.1 by addition of 60 mg of K_2CO_3 to 1.5 ml of 25 mM $HAuCl_4$ diluted in 100 ml of water and allowing the solution to stir in the dark overnight at room temperature for the $HAuCl_4$ to hydrolyze and age to give a colorless gold hydroxide solution. This solution is subsequently referred to as K-gold. The 40 ml of precursor seed particles were ultrasonically redispersed and 0.5 ml of the seed solution was added to different volumes of K-gold before a freshly prepared 6.6 mM $NaBH_4$ was added at 1 ml for every 10 ml of K-gold to reduce the complex gold hydroxide anions in the K-gold onto the $Au(OH)_3$ seeds. This reduction resulted in an almost immediate color change: red, purple, blue or green depending on the shell thickness and degree of completeness. The ratio of seed solution to K-gold solution controls the amount of gold available for deposition on the silica surface, hence the growth of the shell depended on the amount of K-gold used. Different volume ratios of K-gold to precursor seed particles from 5:1 to 300:1 were varied to demonstrate the progressive growth of the shell. In this reduction step, 1 ml of 10 mM sodium citrate dihydrate per 20 ml of K-gold was also added to slow the reaction and stabilize the gold nanoshells by acting as a capping agent. Using this approach of synthesis, the core size and shell thickness of the gold nanoshells could be varied by changing the size of the silica core nanoparticles and the amount of $HAuCl_4$ for reduction respectively.

Characterization of gold nanoshells

The synthesized silica, precursor seed particles and gold nanoshells at different stages of shell growth was imaged under the transmission electron microscopy (TEM) (Jeol JEM-2100F) operating at 200 kV. Samples were prepared by placing a drop of solution on a carbon coated copper grid and allowing the grid to dry on filter paper. The average size and size distribution of the silica nanoparticles were determined from the TEM images using the ImageJ software

from National Institutes of Health, USA. The optical extinction spectra of gold nanoshells at different stages of shell growth were measured in water using a UV-Vis spectrophotometer (Shimadzu UV-2401 PC) in the wavelength range from 400 nm to 900 nm. Solution spectra were obtained by measuring the absorption of a dilute solution in a cell with a pathlength of 10 mm. To compare these experimentally measured spectra with theoretical prediction of the spectra, a program for the calculation of optical spectra of core-shell structures based on Mie theory [32] was written in MATLAB. The calculations require the following as input: the refractive indices of the silica core (1.43, assumed to be constant) and the medium ($n = 1.33$), the frequency-dependent dielectric

constant of gold [33], the size of the core, and the thickness of the shell. Extinction spectra were initially calculated with the data for the silica core radii obtained by TEM measurements and the thickness of the gold shells was calculated by subtracting the silica core radii from the total particle radii also obtained by TEM. These spectra were then adjusted to the measured extinction spectra by a fitting procedure.

Results

Effect of amine terminated surface functionalization

The influence of surface functionalization of the silica cores with a terminal amine group on the deposition of $\text{Au}(\text{OH})_3$

Figure 2

The DP process of seeding gold hydroxide nanoparticles on (a) ungrafted naked silica cores with (b) a higher magnification image of a single precursor seed particle. This seeding result is compared to (c) silica cores grafted with terminal amine groups and (d) its corresponding higher magnification image of a single precursor seed particle showing the details of the gold hydroxide nanoparticles

nanoparticles during the DP process of seeding was investigated. Both amine grafted and ungrafted silica cores were subjected to the same seeding conditions in DP and the results are shown in Figure 2 for a 30 min heating with 6.35 mM HAuCl_4 adjusted to pH 8 with 4.5 ml of 0.1 M NaOH. The presence of the terminal amine group on the surface of amine functionalized silica results in $\text{Au}(\text{OH})_3$ nanoparticles that were smaller and uniform in size and shape, although large gold clusters were occasionally observed (Figure 2c). The formation of these large clusters is discussed in more detail as we examine the effect of heating duration. The $\text{Au}(\text{OH})_3$ seeds formed were homogeneously distributed on the silica surface with high seeding density i.e. small inter-particle distances and

high coverage on the silica core. The seed particles were typically about 4 nm in size. In contrast, the $\text{Au}(\text{OH})_3$ seeds on the bare surface of the ungrafted silica cores were generally larger and less uniform in size and shape. The seed particles in this case range from 4 nm to as large as 15 nm. The distribution of these seed particles was also less homogenous with regions of different seeding density observable, although in general, the seeding density and hence surface coverage is lower on the ungrafted silica compared to the amine grafted silica cores. This result clearly shows the influence of the amine terminated surface functionalization on the DP process of seeding $\text{Au}(\text{OH})_3$ nanoparticles in terms of the seed size, uniformity and surface coverage.

Figure 3

The DP process of seeding gold hydroxide nanoparticles at (a) pH of 4, (b) pH of 6, (c) pH of 8 and (d) pH of 11 on the surface of amine functionalized silica cores to illustrate the influence of pH on the seeding efficiency using the DP method

Effect of pH

The pH in which the DP process occurs was varied by adding different amounts of 0.1 M NaOH to the 6.35 mM HAuCl₄ to investigate how the pH and hence the degree of hydrolysis of HAuCl₄ affects the loading of Au(OH)₃ nanoparticles on the amine grafted silica. Four different amounts of NaOH was used: 1.6 ml, 3.3 ml, 4.5 ml and 6 ml, and these results in approximate pH of 4, 6, 8 and 11 respectively after 15 min of stirring the hydrolyzed HAuCl₄ solution prior to adding the amine grafted silica. The DP process of loading Au(OH)₃ nanoparticles under these four pHs with 30 min heating is shown in Figure 3. At low pH of around 4, the solution was pale yellow after the DP reaction and the TEM image revealed that the density of the seed was low and was not very homogeneously distributed with regions showing higher density of Au(OH)₃ nanoparticles compared to others (Figure 3a). The seed particles were generally small, typically < 5 nm, although the variation in size distribution is large with seed particles as big as 10 nm. As the pH increases to 6, the solution turned orange. One dominant observation under the TEM was the presence of two populations of seed particles with different sizes formed on the amine grafted silica core (Figure 3b). The smaller seed particles with sizes of < 2 nm were seeded with high density on the surface. These small particles were as fine as 1 nm and they achieved

an almost complete coverage on the surface. The large seed particles with mean size of about 7 nm were scattered amongst the smaller seeds in a non-homogeneous manner and with much lower seeding density. These two populations were not observed when the pH was raised further to pH 8 where similar observations were made as that shown in Figure 2d. At this pH, the solution after the DP reaction was dark orange and the density of Au(OH)₃ nanoparticle seeds on the silica core remains high and homogeneously distributed with a narrow size distribution centered at around 4 nm (Figure 3c). Further increase in the pH to 11 reduced the density of the Au(OH)₃ nanoparticle seeds although the particles remained rather homogeneously distributed on the silica core (Figure 3d). The lower density of seeds results in a pale yellow solution similar to that observed at pH 4, but in this case, the density of the seeding is slightly higher than that compared to pH 4. The Au(OH)₃ seed particles had a large variation in size distribution, ranging from 4 nm to as big as 20 nm. From the results of seeding under different pH, we conclude a general pH dependence of the size and seeding density of the Au(OH)₃ seeds on the silica core and that to achieve a high density of Au(OH)₃ loading for subsequent effective shell growth, a rather narrow optimal working range of pH at around 8 needs to be effected.

Figure 4

Comparison of DP process of seeding small gold nanoparticles on amine functionalized silica cores for different heating duration of 3, 30 and 60 min at temperature > 67 °C. Images on the top row were taken with the same magnification of 150,000X while images on the bottom row were taken with the same magnification of 20,000X

Effect of temperature and duration of reaction

The DP reaction for the formation and deposition of $\text{Au}(\text{OH})_3$ nanoparticles on the amine functionalized silica cores was observed to occur only at temperature over 65°C . The mixture containing the hydrolyzed HAuCl_4 solution at pH 8 and amine grafted silica remained milky white at $< 65^\circ\text{C}$, but started to turn pale yellow at around 65°C before eventually turning dark orange as heating continued. It was found that the temperature of reaction had little influence on the size and seeding density of the seeds. The DP reaction heated at 68°C and 86°C for the same duration of less than 20 min showed no observable differences in the size of the $\text{Au}(\text{OH})_3$ seeds or their density of seeding (results not shown). However,

the duration of heating at temperature $> 67^\circ\text{C}$ affected the size of the deposited $\text{Au}(\text{OH})_3$ nanoparticle seeds. This influence of heating duration was investigated by heating the mixture (with pH 8) at 70°C for 3, 30, and 60 min and the results were shown in Figure 4. Generally, the mean $\text{Au}(\text{OH})_3$ nanoparticles seed size increased with the duration of heating from 2 nm for a 3 min reaction time to 4 nm for a 30 min reaction time and around 7 nm for a 60 min reaction time. Beyond an hour of heating, the size of the $\text{Au}(\text{OH})_3$ seeds was observed to remain unchanged at 7 nm. However, the more prominent observation with prolonged heating was the higher tendency for formation of large gold clusters, typically larger than 20 nm as the heating proceeded beyond 30 min

Figure 5

Growth of the gold hydroxide nanoparticle seeds by reducing the aged gold hydroxide (K-gold) solution on the (a) precursor seed particles to progressively form a complete layer of gold shell i.e. $\text{Au}(0)$ with increasing K-gold-to-seed ratios of (b) 5:1, (c) 20:1, (d) 40:1, (e) 70:1, (f) 100:1, (g) 160:1, (h) 200:1 and (i) 300:1 for a silica core size of 300 nm in diameter. All images in figures taken with the same magnification with the scale bar shown in (a)

(Figure 4c). The formation of these large gold clusters would subsequently affect the growth of gold shell on the precursor seed particles as these gold clusters could also serve as catalytic sites that compete with the $\text{Au}(\text{OH})_3$ nanoparticles seeds for the reduction of additional gold during the shell growth process.

Growth of gold shell

The precursor $\text{Au}(\text{OH})_3$ seeded silica formed from seeding at pH 8 under a seeding temperature of 70°C for 30 min (Figure 5a) were then used for the further growth of a layer of gold shell around the silica by reducing more hydrolyzed HAuCl_4 i.e. gold hydroxide solution from the aged K-gold on them. Different K-gold-to-seed ratios were used to progressively grow the seed particles until they coalesce together with their increase in size and eventually form a complete layer of shell. We illustrate the typical growth of the seed particles on a silica core size of 300 nm diameter as an example. The results for increasing K-gold-to-seed ratios of 5:1, 20:1, 40:1, 70:1, 100:1, 160:1, 200:1 and 300:1 for growing a shell around the 300 nm core are shown in Figure 5b to 5i respectively. From our results, we observed the progressive growth in the seed size at lower ratios up to around 70:1, beyond which they started to coalesce together at 100:1. At a ratio of 200:1, the amount of gold ions was barely sufficient to form a complete layer of gold shell around the silica core with a thickness of about 10 nm. This amount is consistent

Figure 6

Measured UV-Vis extinction spectrum of the gold nanoshells at different stages of seed growth on the (a) precursor seed particles arising from a range of different K-gold-to-seed ratio of (b) 5:1, (c) 20:1, (d) 40:1, (e) 70:1, (f) 100:1, (g) 160:1, (h) 200:1 and (i) 300:1 for a silica core size of 300 nm. The theoretically predicted spectrum (j), shown in dashed line based on the Mie theory for a 300 nm diameter silica core with a 28 nm gold shell is also shown together for comparison

with that obtained from mass calculation for the same gold nanoshells dimension. Beyond this, at higher ratios, the shell thickened to reach a thickness of about 30 nm at a ratio of 300:1. We noted that the same technique could be applied to grow the shell on other silica core sizes. As the $\text{Au}(\text{OH})_3$ nanoparticle seeds were in the order of 4 nm and they need to grow and coalesce together before they form a complete shell covering, we observed that this technique of growing the shell around the silica core imposed a minimum shell thickness of around 10 nm (Figure 5(g)). Thicker gold shell can be grown at even higher K-gold-to-seed ratios using this same technique. The UV-Vis extinction spectra of the gold nanoshells at different stages of seed growth for the 300 nm diameter core corresponding to the same K-gold-to-seed ratios as in Figure 5 were measured and shown in Figure 6. The theoretically predicted extinction spectrum based on Mie theory for a single gold nanoshell with a silica core diameter of 300 nm and shell thickness of 28 nm is also shown for comparison. The extinction spectrum of the initial precursor seed particles loaded with $\text{Au}(\text{OH})_3$ nanoparticles showed a typical optical response of a particle scatterer having a decreasing optical extinction with increasing wavelength and an absence of any optical resonance. As the $\text{Au}(\text{OH})_3$ seeds were reduced to gold, i.e. Au^0 seeds and began to grow in size, an optical response at peak of 560 nm, typical of the surface plasmon resonance of large gold nanoparticles, was observed. The peak of this optical response gradually shifted towards higher wavelength and increased in their intensity as the seeds grew further in size until a complete shell was obtained (Figure 6(g)). In this case, the peak plasmon resonance for this configuration was determined to be around 800 nm. Further thickening of the gold shell caused a blue shift back in the wavelength of the peak optical response as observed in Figure 6(i). When the measured extinction spectrum of the gold nanoshells having a 300 nm diameter core and 28 nm shell was compared to theoretically predicted spectrum based on Mie theory for the same dimensions, we noted that the measured spectrum generally agreed well with the theoretical spectrum with small differences that can be attributed to the heterogeneity within the population of gold nanoshells formed. Such heterogeneity is inevitable due to the size variations in the silica core as well as the small differences in the thickness of the shell formed.

Discussion

In this paper, we have demonstrated the use of DP process as an alternative route to loading nanoparticulate gold onto silica that serve as nucleation as well as catalytic sites for further growth of a layer of gold shell in the synthesis of gold nanoshells. Compared to the widely-used two-step technique of synthesizing small colloidal gold particles from reducing HAuCl_4 by alkaline tetrakis(hydroxymethyl) phosphonium chloride (THPC) first, followed by attaching them to the surface functionalized silica core [16-19], this method results

in a single-step process to form the precursor seed particles. The process of deposition-precipitation of $\text{Au}(\text{OH})_3$ on oxide support starting from a gold precursor in the form of an aqueous solution of HAuCl_4 has been widely used in the catalytic field involving seeding such gold particles on metal oxide substrate to form nanoparticulate gold catalyst [26-30]. It is clear from recent work on the characterization of gold on alumina, that addition of base to HAuCl_4 hydrolyzes the chloroauric anion i.e. $[\text{AuCl}_4]^-$ in solution to form six major species of the general form $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ depending on the extent of hydrolysis [34]. The extent of hydrolysis in turn depends on the pH which gives an indication of the amount of OH^- available for hydrolysis. To promote the exchange of chloride in $[\text{AuCl}_4]^-$ with OH^- during hydrolysis, the aqueous solutions should be warmed to a temperature in the range of 60 – 80°C [28]. Out of these six species, only $\text{Au}(\text{OH})_3$ precipitates [34]. As a result, the gold is only partially deposited on the support as the remaining species remain dissolved in the solution. It is the control of hydrolysis to tune the speciation of $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ that subsequently influence the amount of nanoparticulate $\text{Au}(\text{OH})_3$ seeds formed on the substrate as well as their size and density on the silica. Therefore, this method has to be strictly controlled in terms of the pH value, the resulting dimension and seeding density of the $\text{Au}(\text{OH})_3$ nanoparticles being dependent on it. For typical HAuCl_4 concentration of the millimolar range used in most DP process, careful control of the pH in the range of 6 to 10, and temperature in the range of 60 to 80°C enable selective crystallization and deposition of $\text{Au}(\text{OH})_3$ nanoparticles only on the surfaces of metal oxide support without precipitation and crystallization in the liquid phase. It was found that the $\text{Au}(\text{OH})_3$ nanoparticles loaded onto the silica nanoparticles using this controlled hydrolysis and DP method were stable towards magnetic stirring and ultrasonication.

The nature of the support substrate surface also exerts a tandem effect with the extent of hydrolysis of HAuCl_4 on the deposition of the $\text{Au}(\text{OH})_3$, as demonstrated in our results. The unfortunate drawback of DP method is that it is not effective in the presence of metal oxides supports whose isoelectric point (IEP) is below 5 [28]. The deposition of $\text{Au}(\text{OH})_3$ on bare silica surface (IEP $\approx$ 2) without any surface functionalization has shown to be poor as demonstrated by our seeding results on bare silica nanoparticles shown in Figure 2(b). The reason for this is that above this pH i.e. pH > 2, its surface is negatively charged which hinders the attraction of $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ to its surface for subsequent deposition. In this work, we address the problem associated with the low IEP of silica nanoparticles by functionalizing their surface with a terminal amine group prior to seeding the $\text{Au}(\text{OH})_3$ nanoparticles. We have shown that by surface functionalizing the silica nanoparticles with APTES, we were able to deposit smaller $\text{Au}(\text{OH})_3$ nanoparticles and at a much higher density. Direct silanation of silica nanoparticles using a silane-coupling agent such as APTES is attractive for improving stability and control of surface properties. Use of APTES is a typical example

of obtaining cationic amine groups on nanosize particles [35]. The grafting of APTES on the silica nanoparticles caused a drastic change in the IEP of silica particles from 2 to 9. These amine groups on the silica surface were thus used to effectively modify the isoelectric point of silica nanoparticle. At this pH of 9, the surface of amine grafted silica becomes minimally charged, making the surface favorable for attraction of $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ and subsequent deposition of $\text{Au}(\text{OH})_3$. This favorable electrostatics coupled with dominant formation of $[\text{Au}(\text{OH})_3\text{Cl}]^-$ from the hydrolysis of HAuCl_4 at around pH 9 [36] (to be discussed in more detail below) results in high density of $\text{Au}(\text{OH})_3$ nanoparticles on the silica. Furthermore, it is also known that the introduction of a support with polar surface groups i.e. silica with surface-functionalized -NH₂ groups leads to the acceleration of the hydrolysis reaction near the functionalized surface [37]. The formed nanoparticles of hydrated metal oxides are immediately adsorbed onto the surface of the silica. Such practice of surface functionalization with a suitable terminal group to adjust the IEP of metal oxide bases could potentially hold important implications in reducing the size and improving the dispersion of the nanoparticulate catalytic sites, hence improving catalytic activity of catalysts derived from similar DP process in the catalytic field.

Besides the influence of surface functionalization, we have also shown how the various factors such as pH and time of reaction affect the seeding process. Perhaps one of the key factors controlling the seeding density of $\text{Au}(\text{OH})_3$ nanoparticles on the silica core is pH. In this case, two conditions in the DP process that are sensitive to the pH come into play: the hydrolysis of HAuCl_4 to give $[\text{Au}(\text{OH})_3\text{Cl}]^-$ as the dominant species that would precipitate [34] and a surface with appropriate IEP to facilitate the adsorption of $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$. These two conditions must both be satisfied to achieve good seeding on the silica. It has been found from previous studies that preparation at a mildly basic pH range of 7 to 9 is preferable for most oxide support [38] because at this pH, the value of x in $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ is close to 3 i.e. $[\text{Au}(\text{OH})_3\text{Cl}]^-$ and, as mentioned, precipitation is the most efficient with this species. This pH range is also compatible with the IEP of most metal oxide [28] for electrostatic attraction and deposition as discussed previously. In our study, the amine grafted silica substrate surface has an IEP $\approx$ 9. At pH below this IEP, the surface is positively charged and is capable of adsorbing more of the negatively charged gold species. However, at a much lower pH of 3 to 4, there is little hydrolysis of the Au – Cl bond to form $\text{Au}(\text{OH})_3$, and many studies have shown that the soluble $\text{AuCl}_3 \cdot \text{H}_2\text{O}$ is the major species with traces of other $[\text{AuCl}_x(\text{OH})_{4-x}]^-$ species at this pH [29, 36]. Therefore, there is a low precipitation of $\text{Au}(\text{OH})_3$ species and its density of seeding remains poor and inhomogeneous despite the favorable electrostatic adsorption from the substrate. As the pH increases to 6 while maintaining below the isoelectric point, the extent of hydrolysis increases due to increasing replacement of more Au – Cl with Au – OH in the complex $[\text{AuCl}_x(\text{OH})_{4-x}]^-$ anion. More of the precipitating

$[\text{Au}(\text{OH})_3\text{Cl}]^-$ species are formed and the density of the $\text{Au}(\text{OH})_3$ nanoparticles increases correspondingly until a high seeding density of the $\text{Au}(\text{OH})_3$ nanoparticles is achieved at pH 8. At this pH, the hydrolysis forms $[\text{Au}(\text{OH})_3\text{Cl}]^-$ as the dominant species and their electrostatic attraction to the positively charged amine grafted surface ($\text{IEP} \approx 9$) facilitated their deposition. Further increase in pH to 11 beyond the IEP of the amine grafted surface causes adsorption of the negatively charged $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ complex to reduce rapidly due to repulsive negative charge from the surface [26]. Furthermore, since $\text{Au}(\text{OH})_3$ is amphoteric, its solubility increases due to the formation of $[\text{Au}(\text{OH})_4]^-$ at higher pH, thus making the soluble $[\text{Au}(\text{OH})_4]^-$ the most dominant species at high pH [29] instead of the precipitating $[\text{Au}(\text{OH})_3\text{Cl}]^-$. This reduces the efficiency of precipitation and results in a lower density of the $\text{Au}(\text{OH})_3$ nanoparticles on the silica as the amount of $\text{Au}(\text{OH})_3$ species available for deposition is decreased at high pH. Therefore, there is a narrow optimal working range of pH where the $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ can be attracted to the surface and with sufficient hydrolysis to form the precipitate for deposition to occur.

The DP reaction for the formation of $\text{Au}(\text{OH})_3$ nanoparticles on the amine grafted silica cores seem to take place at temperature above 65°C . The role of temperature probably accounts for the rate of precipitation and deposition of the $\text{Au}(\text{OH})_3$ nanoparticles which can take place as rapid as 3 min (see Figure 4a). Apart from affecting the rate of reaction, the size and seeding density of the $\text{Au}(\text{OH})_3$ nanoparticles are not affected much if the duration of reaction is not a limiting factor. Hence, we observed that beyond 65°C , there were little or no observable differences in the seeding with temperature at longer heating time of 30 min. The more dominant factor is the duration of reaction at temperature $> 65^\circ\text{C}$ where the size of $\text{Au}(\text{OH})_3$ increases with the duration of heating and gold clusters larger than 20 nm were formed with prolonged heating time of beyond 30 min. We attempt to account for the growth of the $\text{Au}(\text{OH})_3$ nanoparticles and formation of large gold clusters by briefly considering the surface energetics of the nanoparticles. As the precipitation and deposition of $\text{Au}(\text{OH})_3$ nanoparticles on amine grafted silica progresses with heating duration, the terminal amine groups on the silica available for attachment to $\text{Au}(\text{OH})_3$ nanoparticles starts to saturate and crystal growth of the existing $\text{Au}(\text{OH})_3$ nanoparticles become a more dominant process compared to further deposition of new $\text{Au}(\text{OH})_3$ nanoparticles on APTES. This growth arises from the continuous precipitation of $\text{Au}(\text{OH})_3$ in solution and aggregation of these new precipitates with existing deposited $\text{Au}(\text{OH})_3$ particles. This yields increasingly larger $\text{Au}(\text{OH})_3$ nanoparticles until their surface charge, which progressively becomes more negative due to the presence of chloride [26] as more $\text{Au}(\text{OH})_3$ aggregates, render them energetically unfavorable for further growth. In this case, prolonged heating causes the $[\text{Au}(\text{OH})_3\text{Cl}]^-$ in the solution to precipitate and aggregate with each other instead to form larger gold clusters of $\text{Au}(\text{OH})_3$ in the solution. Such nanoparticles formed in

solution compete with the deposited $\text{Au}(\text{OH})_3$ as reduction sites for K-gold, thus reducing the efficiency of shell growth.

The $\text{Au}(\text{OH})_3$ nanoparticles are seeded on silica to function as nucleation sites where they will be reduced to Au^0 nanoparticles while the K-gold is also simultaneously reduced to deposit more Au^0 on them in the process of growing the gold shell. In the growth of the gold shell, the pH of K-gold solution is important to nanoshell formation on the precursor seed particles as it controls the speciation of $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ which in turn affect its reduction on the $\text{Au}(\text{OH})_3$ nanoparticles. In this case, the gold to be reduced on the seeds was prepared by adjusting the pH of HAuCl_4 solution through the addition of K_2CO_3 to around 10.1, where hydrolysis of most of the Au-Cl bond occurs after an overnight aging in the dark to form the soluble gold hydroxide complex $[\text{Au}(\text{OH})_4]^-$ as the dominant species [29]. Amongst the six species of $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ discussed earlier, $[\text{Au}(\text{OH})_4]^-$ seems to have the lower tendency to be reduced in solution to form colloidal gold even with a strong reducing agent as evident from its slow and gradual color change when reduced. This is in contrast to the reduction of other $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ species formed at lower pH where we observed that the addition of NaBH_4 causes an instantaneous color change to form colloidal gold. This observation may possibly be attributed to a weaker reduction potential of $[\text{Au}(\text{OH})_4]^-$ compared to other species although the electrochemistry of different species of $[\text{Au}(\text{OH})_x\text{Cl}_{4-x}]^-$ is not well characterized in literature. During the reduction of K-gold, the $\text{Au}(\text{OH})_3$ nanoparticles also serve as catalytic sites to facilitate preferential reduction of $[\text{Au}(\text{OH})_4]^-$ on the seed particles for shell growth. We found that adjustment to other pH < 10 by addition of smaller amount of K_2CO_3 results in the formation of other dominant species that have greater tendency to be reduced in solution to form colloidal gold thus diminishing their preferential reduction on the seed particles and effectiveness in growing the gold shell. In reducing the K-gold solution of $[\text{Au}(\text{OH})_4]^-$ onto the precursor seed particles, we used freshly prepared NaBH_4 as the reducing agent. We note that by increasing the amount of NaBH_4 used for reduction also resulted in a corresponding increase in the tendency of gold nanoshells to aggregate. Furthermore, we also observed the formation of a brown scattering solution being formed when a large amount of NaBH_4 was added. The formation of such brown scattering solution has also been reported by others [17] and on closer examination under the TEM, this observation is due to the presence of large bulk gold particles (data not shown). However, if the amount of NaBH_4 used is too little, the solution will contain unreacted K-gold in solution. The amount used in our protocol represents a good compromise based on the molar ratio of reagents used for the redox reaction. In addition to NaBH_4 , sodium citrate dihydrate was also added during the reduction for shell growth. The use of sodium citrate to slow the reaction and stabilize the gold nanoshells during the reduction process has been reported by others [23, 37].

Different K-gold: seed ratios from 5:1 to 300:1 were used to show the progressive growth of the $\text{Au}(\text{OH})_3$ nanoparticles

to eventually form a complete layer of gold shell. Based on calculation of the core size and concentration of the precursor seed particles as well as the dimension and density of the gold shell, we determined that a ratio of 190:1 was required to form a 10 nm thick uniform shell around the 300 nm diameter silica. This ratio concurs well with our experimental findings, where we observed a 10 nm thick shell formed at a ratio of 200:1. The changes in UV-Vis extinction spectra with different stages of shell growth were characterized. As the $\text{Au}(\text{OH})_3$ nanoparticles do not exhibit any surface plasmon resonance, the extinction spectrum of precursor seed particles loaded with $\text{Au}(\text{OH})_3$ nanoparticles shows the absence of any extinction peak. However, as these $\text{Au}(\text{OH})_3$ nanoparticles were initially reduced by NaBH_4 to Au^0 nanoparticles coupled with its slight growth in size at low K-gold ratios, the formation of an extinction peak at 560 nm was observed to indicate the formation and initial growth of the gold seeds. The UV-Vis spectra at higher K-gold: silica ratios (Figure 6) show a red shift in the plasmon resonance peak towards higher wavelength from 630 nm at 20:1 to about 900 nm at 160:1, indicating the growth of the seed and increasing plasmon interactions between the neighboring seed particles as they grow closer together. Apart from peak shifts, the extinction spectra also increase in intensity with the growth of the gold seed particles due to the increasing surface plasmon response and optical cross sections from the larger gold particles. The agreement of our experimentally measure spectrum with the theoretically calculated spectrum using Mie theory implies that the constituent scattering, absorption and backscattering spectrum as well as anisotropy of the gold nanoshells can also be predicted with good accuracy from Mie theory. These constituent spectra can subsequently be useful for examining the optical response of gold nanoshells for bioimaging or therapeutic applications based on the absorption or backscattering metric.

Conclusions

The use of a DP process of seeding nanoparticulate gold onto amine functionalized silica core particles to form the precursor seed particles has shown to be a feasible alternative route for the growth of a gold shell in the synthesis of gold nanoshells. Compared to the widely-used strategy to attach preformed gold nanoparticles from reduction of HAuCl_4 by THPC, our DP method has shown to be time and cost effective. While the common preparation technique for seeding the core material using THPC-Au nanoparticles requires a two-step process of synthesizing small colloidal gold particles first, followed by attaching them to the surface functionalized silica core, our proposed single-step DP process of seeding requires just 10 minutes of the heating reaction to form the precursor seed particles, without the need for prior synthesis of colloidal gold. Compared to bare silica nanoparticles whose isoelectric point of 2 renders the

DP process unfavorable, we have shown that the use of amine functionalization on the silica surface is able to alter the isoelectric point of the surface from 2 to 9 to facilitate the deposition of the $\text{Au}(\text{OH})_3$ nanoparticles and hence increasing the uniformity and density of seeding. We have also established that by varying the pH of the HAuCl_4 solution, as well as the time of reaction, it is possible to control seeding density and the size of the $\text{Au}(\text{OH})_3$ nanoparticle seeds. The highest seeding density is achieved at pH 8 where the surface charge on the amine grafted silica favors attraction of the complex gold anions and the hydrolysis of these complex anions produces the insoluble $\text{Au}(\text{OH})_3$ precipitate which is readily deposited on the silica to form the seed particles. As the time of reaction increases, these seed particles increase in size until a point where prolonged heating causes larger gold clusters to be formed in solution. We have used our proposed technique to seed $\text{Au}(\text{OH})_3$ nanoparticles on different sizes of silica cores and have also demonstrated the use of the precursor seed particles formed from this process to form the gold nanoshells. Just as the DP process of gold seeding proposed in this study was adapted from the DP process of loading gold particles on metal oxide substrate in catalytic field, the enhancement in seeding density with appropriate surface functionalization could likewise be adapted back to the catalytic field to improve the dispersion and total surface area of the nanoparticulate catalytic sites, thus improving the overall catalytic activity of the gold seeded metal oxide substrate.

About the authors

James Chen Yong Kah is currently pursuing a PhD in Bioengineering in the National University of Singapore (NUS). He received his B.Eng. (Electrical) (1st Class Honors) with minors in Bioengineering and Management of IT

from NUS. His current research is on the development of cancer targeting metallic nanostructures as optical probes for reflectance-based optical imaging, working jointly under the Colin Sheppard's bioimaging group in NUS and Malini Olivo's photodynamic therapy and optical imaging group in National Cancer Centre Singapore.

Noppawan Phonthammachai is currently a Research Fellow at the School of Materials Science and Engineering, Nanyang Technological University, Singapore. Her research interests are the designing of new potential materials

(apatite based-, functionalized-, compo-site, nano-materials) and development of in-house metal oxide (TiO_2 , ZrO_2 , Fe_2O_3 etc.) using simple chemistry-based processes for catalytic, environmental and biological applications.

Rachel Cheng Yi Wan is an undergraduate from the School of Materials Science and Engineering, Nanyang Technological University, Singapore. She is assisting in this research work on the synthesis of gold nanoshells as part of her final year project.

Jing Song is an undergraduate from the School of Materials Science and Engineering, Nanyang Technological University, Singapore. She is assisting in this research work on the synthesis of gold nanoshells as part of her final year project.

Timothy White is currently Associate Professor at the School of Materials Science and Engineering and Director of the Facility for Advanced Characterisation, Testing & Simulation (FACTS). He has over twenty years of experience in the design and demonstration of advanced materials for environmental, superconducting, ionic conductivity and hydrogen storage applications. His particular interests lie in tailoring ceramics at the atomic scale to develop or enhance particular properties. These studies have been supported and facilitated through the use and deep knowledge of advanced characterization methods, including atomic resolution electron microscopy, crystal refinement using X-ray and neutron diffraction, and synchrotron-based surface analysis.

Subodh Mhaisalkar is an Associate Professor in the School of Materials Science & Engineering at the Nanyang Technological University (NTU), Singapore. He is also the Director of NTU Nanoscience & Nanotechnology Cluster and Head of Materials Technology Division. Subodh received his Bachelors' degree from IIT-Bombay and his MS/PhD degrees from The Ohio State University. He has over 10 years of research and engineering experience in the Microelectronics industry where he held Engineering and R&D positions in ST Assembly & Test Services, National Semiconductor, and the Singapore Institute of Manufacturing Technology. His areas of expertise and research interests include Organic & Molecular Electronics, Nanobioelectronics including biosensors.

Iman Bte Ahmad graduated with a degree in B (Materials Eng.)(Hons) from Nanyang Technological University, Singapore. She worked for over a year as a Research Engineer for the National University of Singapore, focusing on techniques to fabricate gold-SiO₂

nanostructures for optical imaging. She also developed atomically-flat gold substrates embedded with self-assembled SiO₂ nanoparticle monolayers for SERS. She is currently pursuing her M.Sc. at the Nanyang Technological University and studying PVD and Nitriding techniques for steel hardening.

Colin Sheppard is Professor and Head of the Division of Bioengineering at National University of Singapore. Professor Sheppard received the MA and PhD degrees from Cambridge University, and a DSc from Oxford University. He was also Professor of Physics and Head of the Physical Optics Department at the University of Sydney for 14 years. He has served as Vice-President of the International Commission for Optics (ICO) and President of the International Society for Optics Within Life Sciences (OWLS), and is Editor-in-Chief of Journal of Optics A: Pure and Applied Optics. Professor Sheppard played a major role in the development of the confocal microscope, including launching the first commercial confocal microscope, and has received several awards, including a Humboldt Research Award for invention of the two-photon microscope.

Malini Olivo is currently Principal Investigator of the Photodynamic Treatment and Diagnosis and Biophotonics Laboratories in the Singapore National Cancer Centre and Singapore Health Research Facilities, Head of Bio-optical Imaging at the Singapore Bioimaging Consortium and she also holds an Adjunct appointment in the Department of Pharmacy at the National University of Singapore. She has a PhD in Bio-Medical Physics. She has pioneered the area of clinical application of Photodynamic Diagnosis and Treatment in Cancer in Singapore and has initiated several collaborative projects in nanophotonics imaging applications in biomedical research. Her research interests include nanophotonics, optical bioimaging and photomedicine/photobiology.

Acknowledgments

This work was supported by a grant from the Singapore Bioimaging Consortium, A*STAR. The authors would also like to thank the staff of the Tan Chin Tuan Laboratory of Optical Imaging and Photodynamic Therapy of Cancer, National Cancer Centre Singapore as well as the staff of the Electron Laboratory X-Ray Diffraction Laboratory, Nanyang Technological University where the work was jointly carried out, with special thanks to Ms. Samantha Lim and Ms. Guo Jun for her assistance with the TEM imaging. J.C.Y. Kah would like to acknowledge the support from Division of Bioengineering, National University of Singapore through the award of a postgraduate research scholarship.

References

- J. G. Fujimoto, et al., *Optical biopsy and imaging using optical coherence tomography*. Nat Med, 1995. **1**(9): p. 970-2
- A. Hoffman, et al., *Confocal laser endomicroscopy: technical status and current indications*. Endoscopy, 2006. **38**(12): p. 1275-83
- A. P. Gibson, J. C. Hebden, and S. R. Arridge, *Recent advances in diffuse optical imaging*. Phys Med Biol, 2005. **50**(4): p. R1-43
- S. A. Wickline, and G. M. Lanza, *Molecular imaging, targeted therapeutics, and nanoscience*. J Cell Biochem Suppl, 2002. **39**: p. 90-7
- K. Sokolov, et al., *Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles*. Cancer Res, 2003. **63**(9): p. 1999-2004
- R. A. Alvarez-Puebla, et al., *Surface-enhanced Raman scattering on nanoshells with tunable surface plasmon resonance*. Langmuir, 2005. **21**(23): p. 10504-10508
- C. Loo, et al., *Nanoshell-enabled photonics-based imaging and therapy of cancer*. Technol Cancer Res Treat, 2004. **3**(1): p. 33-40
- L. R. Hirsch, et al., *Metal nanoshells*. Ann Biomed Eng, 2006. **34**(1): p. 15-22
- S. J. Oldenburg, et al., *Nanoengineering of optical resonances*. Chemical Physics Letters, 1998. **288**(2-4): p. 243-247
- J. L. West, and N. J. Halas, *Applications of nanotechnology to biotechnology: Commentary*. Current Opinion in Biotechnology, 2000. **11**(2): p. 215-217
- E. E. Connor, et al., *Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity*. Small, 2005. **1**(3): p. 325-7
- C. Loo, et al., *Gold nanoshell bioconjugates for molecular imaging in living cells*. Opt Lett, 2005. **30**(9): p. 1012-4
- A. Agrawal, et al., *Quantitative evaluation of optical coherence tomography signal enhancement with gold nanoshells*. J Biomed Opt, 2006. **11**(4): p. 041121
- L. R. Hirsch, et al., *Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance*. Proc Natl Acad Sci USA, 2003. **100**(23): p. 13549-54
- C. Loo, et al., *Immunotargeted nanoshells for integrated cancer imaging and therapy*. Nano Lett, 2005. **5**(4): p. 709-11
- S. J. Oldenburg, et al., *Surface enhanced Raman scattering in the near infrared using metal nanoshell substrates*. Journal of Chemical Physics, 1999. **111**(10): p. 4729-4735
- C. Graf and A. van Blaaderen, *Metallo-dielectric colloidal core-shell particles for photonic applications*. Langmuir, 2002. **18**(2): p. 524-534
- T. Pham, et al., *Preparation and characterization of gold nanoshells coated with self-assembled monolayers*. Langmuir, 2002. **18**(12): p. 4915-4920
- W. L. Shi, et al., *Gold nanoshells on polystyrene cores for control of surface plasmon resonance*. Langmuir, 2005. **21**(4): p. 1610-1617
- D. G. Duff, A. Baiker, and P. P. Edwards, *A New Hydrosol of Gold Clusters. 1. Formation and Particle-Size Variation*. Langmuir, 1993. **9**(9): p. 2301-2309
- D. G. Duff, et al., *A New Hydrosol of Gold Clusters. 2. A Comparison of Some Different Measurement Techniques*. Langmuir, 1993. **9**(9): p. 2310-2317
- T. H. Ji, et al., *Preparation, characterization, and application of Au-shell/polystyrene beads and Au-shell/magnetic beads*. Advanced Materials, 2001. **13**(16): p. 1253-1256
- J. H. Zhang, et al., *Facile methods to coat polystyrene and silica colloids with metal*. Advanced Functional Materials, 2004. **14**(11): p. 1089-1096
- Y. Kobayashi, V. Salgueirino-Maceira, and L. M. Liz-Marzan, *Deposition of silver nanoparticles on silica spheres by pretreatment steps in electroless plating*. Chemistry of Materials, 2001. **13**(5): p. 1630-1633
- Y. T. Lim, O. O. Park, and H. T. Jung, *Gold nanolayer-encapsulated silica particles synthesized by surface seeding and shell growing method: near infrared responsive materials*. Journal of Colloid and Interface Science, 2003. **263**(2): p. 449-453
- H. H. Kung, M. C. Kung, and C. K. Costello, *Supported Au catalysts for low temperature CO oxidation*. Journal of Catalysis, 2003. **216**(1-2): p. 425-432
- L. Prati and G. Martra, *New gold catalysts for liquid phase oxidation*. Gold Bulletin, 1999. **32**(3): p. 96-101
- M. Haruta, *Nanoparticulate gold catalysts for low-temperature CO oxidation*. Journal of New Materials for Electrochemical Systems, 2004. **7**(3): p. 163-172
- F. Moreau, G. C. Bond, and A. O. Taylor, *Gold on titania catalysts for the oxidation of carbon monoxide: control of pH during preparation with various gold contents*. Journal of Catalysis, 2005. **231**(1): p. 105-114
- S. Ivanova, et al., *Preparation of alumina supported gold catalysts: Gold complexes genesis, identification and speciation by mass spectrometry*. Applied Catalysis A: General, 2006. **298**: p. 203-210
- W. Stober, A. Fink, and E. Bohn, *Controlled growth of monodisperse silica spheres in the micron size range*. Journal of Colloid and Interface Science, 1968. **26**(1): p. 62-69
- A. L. Aden and M. Kerker, *Scattering of Electromagnetic Waves from Two Concentric Spheres*. Journal of Applied Physics, 1951. **22**(10): p. 1242 - 1246
- P. B. Johnson and R. W. Christy, *Optical Constants of the Noble Metals*. Physical Review B, 1972. **6**(12): p. 4370 - 4379
- C. K. Chang, Y.-J. Chen, and C.-t. Yeh, *Characterizations of alumina-supported gold with temperature-programmed reduction*. Applied Catalysis A: General, 1998. **174**(1-2): p. 13-23
- Z. Xu, Q. Liu, and J. A. Finch, *Silanation and stability of 3-aminopropyl triethoxy silane on nanosized superparamagnetic particles: I. Direct silanation*. Applied Surface Science, 1997. **120**(3-4): p. 269-278
- P. J. Murphy and M. S. LaGrange, *Raman spectroscopy of gold chloro hydroxy speciation in fluids at ambient temperature and pressure: a re evaluation of the effects of pH and chloride concentration*. Geochimica et Cosmochimica Acta, 1998. **62**(21-22): p. 3515-3526
- A. Dokoutchaev, et al., *Colloidal metal deposition onto functionalized polystyrene microspheres*. Chemistry of Materials, 1999. **11**(9): p. 2389-2399
- A. Wolf and F. Schuth, *A systematic study of the synthesis conditions for the preparation of highly active gold catalysts*. Applied Catalysis A: General, 2002. **226**(1-2): p. 1-13