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Optical imaging technique has strong potential for sensitive cancer diagnosis, particularly at the early stage of cancer development. This is a sensitive, non-invasive, non-ionizing (clinically safe) and relatively inexpensive technique. Cancer imaging with optical technique however greatly relies upon the use of sensitive and stable optical probes. Unlike the traditional organic fluorescent probes, fluorescent nanoparticle probes such as dye-doped nanoparticles and quantum dots (Qdots) are bright and photostable. Fluorescent nanoparticle probes are shown to be very effective for sensitive cancer imaging with greater success in the cellular level. However, cancer imaging in an *in vivo* setup has been recently realized. There are several challenges in developing fluorescent nanoparticle probes for *in vivo* cancer imaging applications. In this review, we will discuss various aspects of nanoparticle design, synthesis, surface functionalization for bioconjugation and cancer cell targeting. A brief overview of *in vivo* cancer imaging with Qdots will also be presented.

Importance of Diagnostic Cancer Imaging

According to the American Cancer Society's annual report (1), about 570,280 people are expected to die of various cancers in the year 2005 in the United States of America (USA). Early cancer diagnosis, in combination with the precise cancer therapies could eventually save millions of lives. The diagnosis of cancer at the early stage is extremely challenging and has been an active research area these days. Existing diagnostic non-invasive imaging techniques such as Computed Tomography (CT), Magnetic Resonance (MR), Positron Emission Tomography (PET), Single Photon emission CT (SPECT), and Ultrasound (US) are suitable for the diagnosis of abnormalities in the macroscopic level. However, sensitive imaging of abnormalities in the microscopic level has been very challenging. Substantial research efforts are being made for the development of better cancer imaging techniques in the microscopic level. Several imaging techniques such as, SPECT, PET, and optical techniques have shown great promises (2, 3) in the microscopic level. Using fluorescent nanoparticle probes, the feasibility of developing optical imaging technique for the sensitive detection of cancer has been recently demonstrated (4, 5).

Nanoparticle Probes for Cancer Tissue Imaging

Like normal tissues, cancer tissues can interact with light photons by absorption, scattering, and reflection. It is, therefore, expected that the optical image would be somewhat distorted. Again, most tissues autofluoresce (6) upon interaction with the light in the UV and visible spectrum. To develop a robust optical imaging system it is thus important to address all sorts of light interaction with tissue as well as tissue autofluorescence. Again, effective loading of nanoparticle probes to cancer target is important prior to imaging. The concentration of

nanoparticles per unit volume of target tissue would determine the signal strength. Therefore, higher nanoparticle loading is always desirable for better image resolution. Prior to developing nanoparticle probes for cancer tissue imaging the following things should be considered.

Firstly, nanoparticles with the excitation and emission band maxima in the near-infrared (NIR) range (650 nm to 900 nm) are highly preferable for deep tissue imaging. The NIR light is capable of penetrating much deeper (up to several centimeters) into the soft tissues and bones (7, 8). This is due to the relatively low absorption of tissue components (water and hemoglobin) in the NIR spectral range. Secondly, nanoparticles should have high extinction coefficient for effective absorption and high quantum yield for obtaining strong fluorescence signal. Using such nanoparticles, the sensitivity of the optical imaging could be greatly improved. Fluorescent quantum dots (Qdots) that emit in the visible range have been successfully used in cancer imaging (4, 9-30). However, the excitation and emission of Qdots in the UV range and visible range, respectively, are not desirable and such Qdots will have very limited *in vivo* applications. To overcome this problem, NIR Qdots have recently been introduced, showing a great promise for *in vivo* imaging (31-33). The dye-doped nanoparticles (NPs) such as, dye-doped silica (34-37) and dye-doped polymer particles (38-40), present another class of materials for sensitive cancer detection. Thirdly, nanoparticles should be photostable. Photostable nanoparticles will allow non-invasive and real-time monitoring of cancer progression (*e.g.*, monitoring cancer growth and metastasis) and also monitoring the effect of cancer drugs during cancer therapy. Fourthly, nanoparticles should be appropriately surface modified so that they are hydrophilic and an aqueous based formulation can be easily made. Also, they can be attached to appropriate cancer specific delivery systems (*e.g.*, antibodies, peptides, folates, *et cetera*) for targeting. Lastly, it is also desirable to integrate multiple imaging modalities into a single nanoparticle probe [also called multifunctional nanoparticles (41-50)], making them suitable for cancer imaging using multiple modalities (such as fluorescence, X-ray, MRI, *et cetera* (48, 51, 52)). This would have great importance for pre-operative cancer diagnosis and intra-operative surgical guidance for cancer tissue resection.

Nanoparticle Probe Development

Most of the fluorescent nanoparticles (quantum dots, dye-doped silica, *et cetera*) that have been successfully applied for bioimaging applications such as labeling cancer cells, tissues *et cetera*, typically possess a core-shell structure (4, 9-30, 34-37) where the core is fluorescent (Figure 1). The nanoparticle shell is to protect the core from photobleaching and to improve nanoparticle dispersibility in aqueous medium. The shell structure can also be designed to obtain appropriate surface functional groups for attaching biomolecules. In some cases (35, 48, 51, 53), the core is encapsulated by multiple shell structures. In the following sections a brief overview of the development of Qdots and dye-doped silica nanoparticles are given.

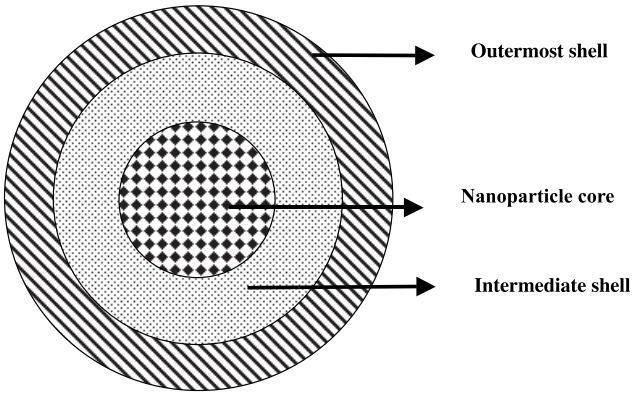

Figure 1: Schematic representation of a typical core-shell nanoparticle design. In some cases, the nanoparticle core is protected just by a single shell (*i.e.*, there is no intermediate shell structure).

Quantum Dots

Semiconductor Qdots have been extensively used for targeting cancer cells. Qdots are in the size range between 2 and 8 nm. They have broad absorption band with narrow and symmetric emission band (full-width at half-maximum ~25·40 nm) and Qdots typically emit in the visible to NIR spectral range (5, 54, 55). Qdot emission is due to a radiative recombination of an exciton (an electron-hole pair), which is characterized by a long lifetime (56) (>10 ns) leading to the emission of a photon in a narrow and symmetric energy band. In comparison to traditional fluorescent molecules (fluorophores) or fluorescent proteins (*e.g.*, GFP), Qdots have several attractive optical features that are desirable for long-term, multi-target, and highly sensitive bioimaging applications. Some of the major optical features of Qdots are described below.

Firstly, Qdots have large molar extinction coefficient value. Unlike organic fluorescent compounds, Qdots have very large molar extinction coefficient value (57), typically in the order of 0.5-5 $\times$ 10^6 $M^{-1}cm^{-1}$ which means that Qdots are capable of absorbing excitation photons very efficiently (the absorption rate is approximately 10-50 times faster than organic dyes). The higher rate of absorption is directly correlated to the Qdot brightness and it has been found that Qdots are approximately 10-20 times brighter than organic dyes (58-60), allowing highly sensitive fluorescence imaging. Secondly, Qdots are extremely photostable. Approximately Qdots are several thousand times more photostable than organic dyes. This feature allows real-time

monitoring of biological processes over a long period of time. Thirdly, Qdots have much longer lifetime, making them suitable for time-correlated lifetime imaging spectroscopy due to the longer excited state lifetime of Qdots (about one order of magnitude longer than that of organic dyes). This allows effective separation of Qdot fluorescence from the background fluorescence, resulting in improvement in the image contrast by reducing the signal-to-noise ratio dramatically (61, 62) in the time-delayed data acquisition mode. Fourthly, Qdots have large Stokes shift value. Unlike in organic dyes, the excitation and the emission spectrum of Qdots are well separated (*i.e.*, large Stokes shift value; typically 300-400 nm depending on the wavelength of the excitation light), allowing further improvement of sensitivity of the detection by reducing the high autofluorescence background often seen in biological specimens (4). Lastly, Qdots have multiple targeting capabilities. The wavelength of Qdot emission is size dependent. The broad Qdot absorption coupled with size dependent emission allows excitation of many sized Qdot particles simultaneously. Unlike organic fluorescent dyes, this unique feature of Qdot materials allows imaging and tracking of multiple targets simultaneously using a single excitation source. This is particularly important in tracking a panel of disease specific molecular biomarkers simultaneously (63). The development of Qdot based fluorescent probes involves a multi-step process, synthesis, surface capping, and bioconjugation. Each of these steps is described below in details.

Synthesis, Surface Modification and Bioconjugation of Qdots: High quality Qdots are highly crystalline, monodispersed (size distribution 8-11%) and usually synthesized in high boiling point non-polar organic solvents/ligands (mixture of trioctyl phosphine/trioctyl phosphine oxide, TOP/TOPO) at elevated temperature (64). A combination of tri-n-octylphosphine oxide (TOPO) and hexadecylamine has also been reported (55). The mixture serves as a robust reaction medium and also coordinates with unsaturated metal atoms on the Qdot surface to prevent the formation of bulk semiconductors. Following the similar synthesis strategy, Qu *et al*. (65) has reported the formation high quality CdSe nanocrystals having fluorescence quantum yields as high as 85% at room temperature. The reverse micelle synthesis route also produce high quality CdS:Mn/ZnS core-shell Qdots (48, 51, 52, 66-68). Reverse micelles [also called water-in-oil (W/O) microemulsion system] is an isotopic, thermodynamically stable homogeneous mixture of oil, water, and surfactant molecules. The surfactant capped water droplets that are dispersed in bulk oil serve as a nano-reactor for the synthesis of Qdots. This method does not require any extreme reaction conditions such as high temperature. Yang *et al*. (52, 66, 67) reported the synthesis of manganese doped cadmium sulfide core and zinc sulfide shell (CdS:Mn/ZnS) Qdots using AOT (dioctylsulfosuccinate sodium salt, a surfactant)/heptane (an oil)/water reverse micelle system. The bright yellow emitting CdS:Mn/ZnS Qdots are small (average Qdot size was 3.2 nm) and highly photostable.

The surface passivation of Qdot nanocrystal core are done with a wide bandgap semiconductor materials (shell) such as ZnS (58, 69) to effectively passivate the surface defects (58, 66, 70, 71), protecting the core from photooxidation. As a result of effective surface passivation, Qdot quantum yield increases. According to Derfus *et al*. (71), adding a shell of 1-2 monolayers of ZnS produced Qdots less susceptible to oxidation, but did not fully eliminate the cytotoxicity induced by 8 hours of UV photooxidation. The toxicity of Qdots is still an open research question. Surface passivation with silica has been shown to be effective for the CdSe core (52, 72, 73) nanocrystals. The TOP/TOPO-capped Qdots are hydrophobic and for biological applications, however, it is necessary to obtain aqueous dispersible Qdots. This is usually done by the surface functionalization with hydrophilic ligands. There are three major routes of surface functionalization. Firstly, the "cap exchange" route that involves replacement of TOP/TOPO caping with bifunctional ligands. The bifunctional ligands (60, 74-77) have two functional moieties, Qdot surface anchoring (*e.g.*, thiol) and hydrophilic moieties (*e.g.*, hydroxyl, carboxyl). Secondly, the formation of hydrophilic silica shell (48, 51, 52, 59, 66, 78) that encapsulates the Qdot. Lastly, the overcoating of TOP/TOPO-capped Qdots with amphiphilic 'diblock' and 'triblock' copolymers and phospholipids (4, 29, 79-83). Other than these three major surface functionalization routes, Qdot surface has also been capped with mono-mercapto ligands (84), polydentate thiolated ligands (containing more than one thiol groups) (75, 77, 84) and biocompatible hybrid silica shell (48, 51, 52). Longer Qdot stability is observed with polydentate thiolated ligands and hybrid silica surface capping.

The bioconjugation represents the attachment of biomolecules (*e.g.*, proteins, antibodies, peptides, DNA, *et cetera*) to the nanoparticle surface, forming a hybrid structure interfacing both the inorganic and the biological materials, for targeting to biological systems such as cells, tissues, *et cetera*, either specifically or non-specifically. There are three major avenues to attach proteins to Qdot surface. Firstly, using carbodiimide (*e.g.*, EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) coupling chemistry carboxylated Qdots are covalently conjugated to the protein molecules *via* the formation of stable amide bond. Secondly, peptides can be directly attached to the Qdot surface via the disulfide bond formation between Qdot surface sulfur atoms (from ZnS surface) and peptides containing cysteine residues (85, 86). Histidine expressing proteins (87) or peptides containing polyhistidine residues (88-90) can also be directly attached

to the Zn atom on the Qdot surface. Lastly, engineered proteins containing positively charged domains can be non-covalently adsorbed onto the negatively charged Qdot surface *via* electrostatic interaction (75, 91, 92).

Cancer Imaging with Quantum Dots: Surface-functionalized quantum dots have been used to image various tumor cells and tissues both in *in vitro* and *in vivo* experiments. Some of the different cell lines that have been used are human mammary epithelial tumor (MDA-MB-231) (93), human breast cancer [MDA-MB-435S (94)] [(MDA-MD-435 (86) MCF 7 (94) and SK-BR-3 (29)], human prostate cancer (4), squamous carcinoma (95, 96), B16 melanoma (skin cancer) (19), human neuro-blastoma (SK-N-SH) (97), colon tumor (SW480) (94), lung tumor (NCI H1299 (94), and bone tumor (Saos-2) (94) cells. Parak and co-workers used water-soluble (78) siloxane coated quantum dots of two different core sizes (2.8 nm with 554 nm emission and 4.1 nm with 626 nm emission) to label human mammary epithelial tumor cells (MDA-MB-231) (93). Confocal microscopic images verified the presence of nanocrystals ingested rapidly inside the cells, and not on the surface. The quantum dot crystals were found in the perinuclear region of the cells even after a week. Akerman and coworkers (86) incubated human breast carcinoma MDA-MD-435 cells with peptide coated quantum dots. These cells were then injected into mice to create tumor grafts and the mice were imaged 8-12 weeks after tumor inoculation. The found that intravenously injected Qdots were accumulated in the MDA-MB-435 breast carcinoma xenograft tumors. Wu *et al*. (29) used streptavidin conjugated commercial Qdots, QD 560 (emission maximum 560 nm), and QD 608 (emission maximum 608 nm) to detect Her2 cancer markers on the surface of human breast cancer cells (SK-BR-3). The nanocrystals effectively labeled the cancer cells with negligible affinity to normal cells. Shuming Nie and coworkers (4) have developed multifunctional nanoparticle probes (2.5 nm radius core protected by a 1 nm TOPO cap with 2 nm polymer coating and 5 nm PEG/affinity ligand shell) for imaging. The quantum dots were used for *in vivo* imaging to target to tumor sites either through a slow passive targeting process or a more efficient active targeting process. Quantum dots have also been used for *in vivo* imaging to map sentinel lymph nodes in rats and pigs (98-100). It is known that the presence of lymph node metastases is an early warning signal for breast and lung cancer. Near infrared (NIR) nanocrystals with oligomeric phosphine coating (for solubility in aqueous buffers) was used to guide a surgeon during cancer surgery.

Free cadmium ion is toxic. Qdots can generate free cadmium ions if continuously exposed to excitation source. Most of the current research reports (54, 101) have addressed Qdot toxicity in the cellular level and in animal models. It appears that Qdots are safe both *in vitro* and *in vivo* if they are appropriately surface coated.

Dye-doped Silica Nanoparticles

Unlike Qdots, silica does not have inherent strong fluorescence property that can be exploited for sensitive imaging applications. However, silica nanoparticles can be made fluorescent by incorporating fluorescent dye molecules inside the silica matrix (dye-doping). There are several attractive features in silica-based nanoparticles. For example, silica is water dispersible, resistant to microbial attack. The size of silica particles remains unchanged by changing solvent polarity (*i.e*., resistant to swelling). Silica matrix is optically transparent that allows excitation and emission light to pass through the silica matrix efficiently. Moreover, fluorescent dyes can be effectively entrapped inside the silica particles. The spectral characteristics of the dye molecules remain almost intact. Silica encapsulation provides a protective layer around dye molecules, reducing oxygen molecule penetration (that causes photodegradation of dye molecules) both in air and in aqueous medium (in this case dissolved oxygen). As a result, photostability of dye molecules increases substantially in comparison to bare dyes in solution. However, the photostability of the encapsulated dyes depends on how well dyes are encapsulated by the silica layer (thickness of the silica layer) and whether or not the silica layer is hydrated. In general, better dye stability is observed with thicker and less hydrated silica encapsulation. The dye photostability increased at least by a factor of 100 in the solution state when particles were post-coated with a pure silica shell of about 10 nm thick. Amorphous silica appears to be a biocompatible (22) and non-toxic (23) material, and have strong potential biological applications. The surface of silica particle can be easily modified to attach biomolecules such as proteins, peptides, antibodies, oligonucleotides, *et cetera*, using conventional silane based chemistry. For example, carboxylated silica nanoparticles can be covalently attached to the amine groups of proteins, antibodies, *et cetera*, *via* the formation of stable amide bond (102). Peptides containing cysteine residue (*via*, S-H group) can be attached to the aminated silica nanoparticles (51) through (SPDP) coupling chemistry.

Synthesis, Surface Modification and Bioconjugation of Dye-doped Silica Nanoparticles: A general synthesis strategy of fluorescent silica nanoparticles is the incorporation of organic or metallororganic dye molecules inside the silica matrix (34, 103-109). For example, a metallorganic dye, tris(2,2′-bipyridyl)dichloro ruthenium (II) (Rubpy) has been entrapped inside the silica nanoparticles using a reverse microemulsion based synthesis approach (34) where the positively charged Rubpy molecules were electrostatically bound to the negatively charged silica matrix. The

dye-doped silica based imaging probes are non-isotopic, sensitive, and relatively photostable in the physiological environment. Additionally, the interaction potential of the silica surface can be easily manipulated to facilitate the interaction with cells (110-112).

There are two synthesis routes reported in the literature for dye doped silica nanoparticle synthesis, Stobers' sol-gel method and reverse microemulsion or water-in-oil (W/O) microemulsion method. In a typical Stobers' method, alkoxysilane compounds (*e.g.*, tetraethyl orthosilicate (TEOS), tetramethylorthosilicate (TMOS), a variety of TEOS, or TMOS derivatives, *et cetera*) undergo base-catalysed hydrolysis and condensation reaction in ammonia-ethanol-water mixture, forming a stable alcosol. This method has been widely used for synthesizing both pure and hybrid (when more than one silane compound are used, such as dye-doped silica particles) silica nanoparticles with particle diameter ranging from a few tens of nanometers to several hundreds of nanometers (sub-micron size). Following the Stobers' protocol with a slight modification, the synthesis of fairly monodisperse organic dye (such as fluorescein isothiocyanate, FITC) doped fluorescent silica nanoparticles has been reported (35, 53). The microemulsion method is used for the synthesis of pure silica, as well as inorganic and organic dye-doped silica nanoparticles. The W/O microemulsion is an isotropic, single-phase system that consists of surfactant, oil (as the bulk phase), and water (as nanosize droplets). Each surfactant-coated water droplets that are stabilized in the oil phase serve as an individual nanoreactor for the synthesis of silica nanoparticles. This is a multi-step process that involves the synthesis of dye-doped silica nanoparticle core followed by the formation of pure silica intermediate shell and the outermost shell. The dye-doped nanoparticle core is formed by the hydrolysis and condensation reaction of tetraethylorthosilicate (TEOS) in the presence of ammonium hydroxide (NH_4OH) catalyst. Fluorescent dyes are doped during the synthesis of silica core. It is expected that some of the dye molecules will remain of the surface of the core. A pure silica coating (intermediate silica shell) is then applied. The intermediate silica core protects fluorescent dye molecules from photobleaching and also from adverse biological environment that sometimes alters the spectral characteristics of the dye molecules. For bioconjugation, it is necessary to obtain suitable surface functional groups such as carboxyl, thiol, amine, *et cetera*, on the nanoparticle surface. Also, it is important to consider nanoparticle aqueous dispersibility. A hybrid silica shell (outermost shell) is then formed (53) by the hydrolysis and co-condensation reaction of TEOS, 3-aminopropyltriethoxysilane (APTS, an amine containing silane reagent), and (3-trihydroxysilyl)propyl methylphosphonate (THPMP, a negatively charged silane reagent that improves nanoparticle aqueous dispersibility). The fluorescence brightness of dye-doped silica nanoparticles can be improved by incorporating high-quantum yield organic dyes having large absorption coefficient values. Dye-doped silica nanoparticles in the size range between 30 nm and 400 nm have been reported in the literature (104, 113).

For bioimaging (*e.g.*, cancer imaging), it is highly desirable that dye-doped silica nanoparticles are appropriately surface modified with cancer targeting molecules such as cancer specific antibodies, folates. This surface modification involves with a few steps. Firstly, the particle surface should be modified to obtain appropriate functional groups such as, amines (35), carboxyls (114), thiols (115), *et cetera*. Secondly, using suitable coupling reagents, nanoparticles are attached to the bio-recognition molecules (such as antibodies, folates, *et cetera*). Nanoparticles have also been coated with avidin molecules that are shown to specifically bind biotinalated molecules such as antibodies, proteins, *et cetera* (116). The surface hydroxyl groups of silica nanoparticles can also be activated by the reactive cyanogens bromide, forming a reactive -OCN derivative. The OCN derivative then readily reacts with proteins (*via* amine groups), forming a "zero-length" bioconjugate as there is no spacer in between the particles surface and the protein molecule (34). Lastly, bioconjugated particles are targeted to cancers. Note that all these steps are usually carried out in aqueous based solutions.

Cancer Imaging With Dye-doped Silica Nanoparticles: Dye-doped silica nanoparticles have been used for *in vitro* imaging of cancer cells. Researchers have demonstrated the use of these nanoparticles to label human leukemia (34, 104, 117), HepG liver cancer (36), human oral carcinoma (35), and lung carcinoma cells (53, 118). Tan and coworkers have used 60 nm dye-doped silica nanoparticles, doped with Rubpy dye to label human leukemia cells (34, 104, 117). Similarly, He and coworkers (36) have reported a method to recognize HepG liver cancer cells using FITC-APTS doped silica nanoparticles. Recently, our group has developed FITC-doped silica nanoparticles, containing primary amine groups on the particle surface. The FITC-doped silica nanoparticles were then covalently attached to folic acid molecules by a carbodiimide coupling reaction. The affinity of folate immobilized conjugates for folate receptors on the cancer cell surface were utilized for imaging (35). It is known that many cancer cells such as human oral carcinoma (35) and lung carcinoma cells (53, 118, 119) overexpress folate receptors on the cell surface. Figure 2 showed the effectiveness of folate conjugated nanoparticles for targeting human lung cancer cells. The nanoparticle loaded cells were imaged using a confocal microscope. Control experiments with amine functionalized nanoparticles (without folic acid) and with human dermal fibroblast cells did not result effective cell labeling, suggesting that folate conjugated nanoparticle can be used for labeling A549 cells. This technique could be extended to label other cancer cells.

Multimodal silica nanoparticle: Multimodal nanoparticles with optical and magnetic properties could help in the preoperative diagnosis and the intraoperative surgical resection of tumors (such as brain tumors, breast cancers *et cetera*). The synthesis of similar nanoparticles for dual (fluorescence and magnetic) imaging was reported by others (49, 50), in which organic fluorescent dyes were used. The use of organic dyes for tumor imaging might be limited as these dyes often cleared rapidly and a large quantity of dyes are needed for optical imaging. Our research group has recently developed single-core multiple-shell novel Rubpy:Gd (III)/SiO_2 nanoparticles that are fluorescent, radio-opaque, and paramagnetic. These nanoparticles might be suitable as a multipurpose imaging probe and can be visualized under CT, MRI, and diffuse optical tomography. The surface reactive groups of this probe can be modified to contain both ligands and antibodies, allowing for the detection of cellular events *in vivo*.

In order to demonstrate multimodal imaging capability, the following *in vitro* tests have been performed on Rubpy:Gd (III)/SiO_2 nanoparticles (120). Nanoparticles were first conjugated to folate using carbodiamide coupling chemistry. Upon incubation with human lung cancer cells (A-549) for a couple of hours, nanoparticles were internalized by the cancer cells by the receptor-mediated endocytosis process. About one million of nanoparticle loaded A-549 cells were then embedded in 2% agarose gel. Both the fluorescence and MRI images of the gel were then recorded. Figure 3 clearly showed effective loading of nanoparticles into A-549 cell and multimodal bioimaging capability of these nanoparticles. Moreover, clusters of labeled cells were optically and MR visible.

Gold Nanoparticles and Their Applications for Cancer Imaging

For more than 30 years, nanometer sized gold particles have primarily been used to stain cells and tissue samples for electron microscopy. However, the use of gold nanoparticles for cancer targeting has been realized very recently (121-123). The basic principle of interactions between gold particles and biomolecules, like proteins, has been well studied for immunocytochemical staining applications. Gold nanoparticles do not fluoresce but effectively scatter light, exhibiting a range of intense colors in the visible and NIR spectral regions. This is known as surface plasmon resonance (124) which is caused by the collective oscillation of the conduction electrons induced by the incident electric field (light).

In the solution state, gold nanoparticles are primarily synthesized from gold precursors (*e.g.*, hydrochloroauric acid, $HAuCl_4$) using appropriate reducing agents, such as citrate (125-128), sodium borohydride (129), ascorbic acid (130). Appropriate capping agents are used to control the particle size and size distribution, prevent particle aggregation and stabilize particle solution (such as in aqueous based medium). The citrate reduction of the gold (III) ions has been widely used. While sodium citrate reduces ($AuCl_4^-$) ions in hot aqueous solution, it forms a colloid. The reported average particle size is about 20 nm. Both the citrate ions and its oxidation products (*e.g.*, acetone dicarboxylate) act as capping agents (125-127). Microemulsions (131-133), copolymer micelles (134), reversed micelles (133), surfactant, membranes, and other amphiphiles have also been used for the synthesis of stabilized gold nanoparticles.

Gold bioconjugates have been used for vital imaging of precancerous and cancerous cells by researchers for *in vitro* and *in vivo* experiments. The unique optical property of the metal in the nanosized range has been used for detecting breast carcinoma cells (SK-BR-3) (121) breast cancer markers like HER2, oral epithelial live cancer cells (HOC 313 clone 8 and HSC 3) (122), and neoplastic cervical biopsies (123).

Potential of Dye-doped Silica, Quantum Dots, and Gold Nanopaticles

Quantum dots have been successfully used for imaging cancer cells and tissues. Since Qdots are small (<10 nm), bright, highly photostable, they might be useful for sensitive real-time monitoring of various intracellular processes in cancer cell. The large Stokes shift value of Qdots is certainly an advantage in reducing the background signal, permitting sensitive detection. The multiplexing capability of Qdots will allow sensitive detection of more than one cancer specific surface receptors at a time using a single excitation. This multiplexing feature might enhance our ability to diagnose cancer at the early stage.

Gold nanoparticles are optically stable. They scatter light strongly and therefore photobleaching does not occur with gold nanoparticles. Unlike Qdots, gold nanoparticles are likely non-toxic. Other than bioimaging, gold nanoparticles can be used for cancer therapy *via* nanophotothermolysis (135). The size of gold nanoparticles can be varied from a few nanometers to a several tens of nanometers.

Dye doped silica nanoparticles are comparatively larger than Qdots, typically particle size varies from a few tens of nanometers to a couple of hundreds of nanometers. Silica is a biocompatible host matrix for fluorescent dye molecules and it is likely non-toxic in its native form. Dye doped silica are optically more stable than bare dyes. Like Qdots and gold nanoparticles, dye-doped silica nanoparticles are also suitable for diagnostic cancer imaging.

Conclusions and Perspectives

Nanoparticle based contrast agents such as quantum dots, gold nanoparticles, dye-doped silica nanoparticles have shown great promise for highly sensitive optical imaging of cancers. As described in this manuscript that most of the successful experiments were conducted *in vitro* using various cancer cell lines. Only a few animal experiments have been performed so far using quantum dots that demonstrate that quantum dots have strong potential for *in vivo* cancer imaging. Fluorescent nanoparticle based cancer imaging has strong potential for non-invasive diagnostic cancer imaging at the early stage. However, there are several challenges to overcome for development of highly sensitive and photostable nanoparticles. Nanoparticle surface modification is a critical step for protecting the fluorescent core, improving aqueous dispersibility, and obtaining appropriate surface functionality for bioconjugation. Specific targeting of cancers is important to reduce nonotoxicity related side effects. The use of multifunctional nanoparticles (such as fluorescent and paramagnetic) would have advantage for pre-operative tumor assessment and intra-operative surgical guidance for the tumor tissue resection. Again, the development of nanoparticles with near-infrared excitation and emission would be extremely helpful for deep tissue cancer imaging.

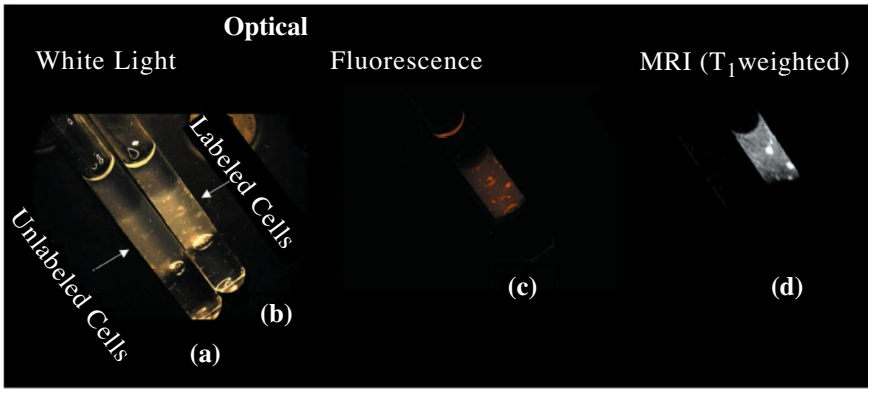

Figure 3: Demonstration of cancer cell imaging using multimodal nanoparticles (NP). Two 5 mm NMR tubes loaded with 2% agarose containing (**a**) 1×10^{-6} unlabeled A549 cells. (**b**) NP labeled 1×10^{-6} A549 cells visualized with white light illumination (**c**) labeled cells visualized using reflectance fluorescence imaging, (**d**) labeled cells detected by MRI on T1 weighted MRI scans.

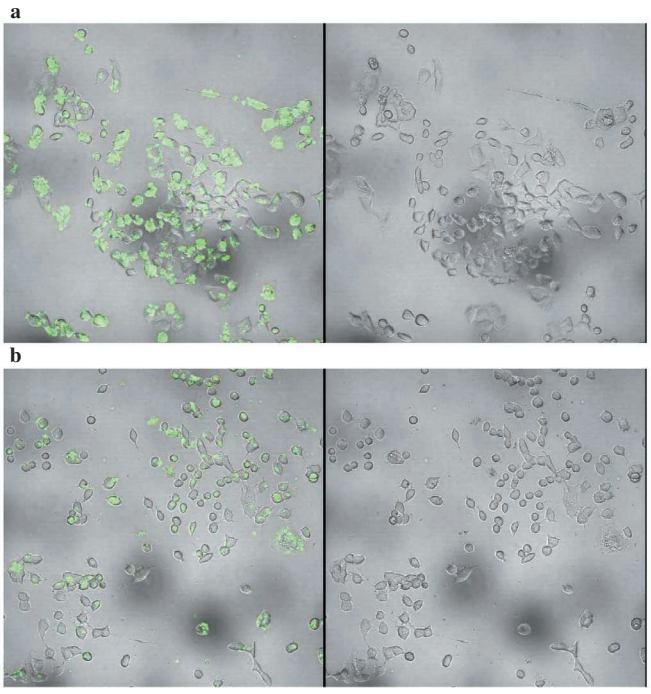

Figure 2: Fluorescence (left) and transmission (right) images of human lung cancer (A549) cells incubated with 200 nm size folate-conjugated (**a**) and amine-functionalized (**b**) fluorescein isothiocyanate (FITC) doped silica nanoparticles. Extra-cellular nanoparticle concentration was 200 □g/ml and the incubation time was 24 hours. This experiment showed more nanoparticle uptake with folate-conjugated nanoparticles, suggesting folate receptor over-expression in A-549 cells. This labeling technique could be extended to label other cancer cells that over-express folate receptors.

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