ELSEVIER

Enriched with multifunctionality, hollow and porous inorganic nanomaterial can potentially revolutionize the area of sensing, imaging, diagnosis and therapy, leading to ideal medical nanodevices.

Inorganic hollow nanoparticles and nanotubes in nanomedicine Part 2: Imaging, diagnostic, and therapeutic applications

Sang Jun Son[1,2], Xia Bai[1] and Sang Bok Lee[1]

[1] Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA
[2] Gachon BioNano Research Institute, Kyungwon University, San 65, Bokjeong-Dong, Sugeong-Gu, Seongnam-Si, Kyeonggi-Do 461-701, Republic of Korea

Inorganic nanoparticles, such as carbon nanotubes, quantum dots and gold nanoshells, have been adopted for biomedical use, due to their unique optical and physical properties. Compared to conventional materials, inorganic nanomaterials have several advantages such as simple preparative processes and precise control over their shape, composition and size. In addition, inorganic porous nanomaterials are fundamentally advantageous for developing multifunctional nanomaterials, due to their distinctive inner and outer surfaces. In this review, we describe recent developments of hollow and porous inorganic nanomaterials in nanomedicine, especially for imaging/diagnosis and photothermal therapy.

The application of nanotechnology to nanomedicine research has enormous potential in modern biomedical research, disease diagnosis and therapy. Organic fluorophore-based detection techniques have been widely used as imaging and signal transduction tools for the detection of trace levels of analytes. The photostability of the fluorophores, however, limits their application in complex environments, such as living bio-systems where degradation or photobleaching can occur. The potential of nanoparticles with various sizes, shapes and materials as the carriers of fluorophores or imaging agents has been studied as a possible solution to this problem.

Quantum dots (QDs) are one of the most intriguing fluorescent probes, due to their unique optical properties. QDs have high quantum yields, high molar extinction coefficients (~10–100 times than that of organic dyes) and broad absorption with narrow, symmetric photoluminescence spectra from UV to near infrared [1,2]; however, the bioconjugation of QDs is somewhat challengeable and is relatively inflexible [1].

Hollow and mesoporous nanoparticles, such as nanotubes, nanoshells and hollow spheres can be loaded with a large amount of imaging molecules, which will considerably enhance signal and sensitivity. The detection of trace amounts of biomolecules, critical for early imaging and diagnosis of cancer, will be facilitated by the imaging molecule-dense nanoparticles. Additionally, the photostability of doped imaging molecules is increased by the protective carriers. Compared with polymer nanoparticles, the advantages of silica nanoparticles lie in well-developed silane chemistry, easy preparation and biocompatibility [3,4]. In addition to functioning as imaging agents, hollow and mesoporous nanoparticles with distinct inner and outer surfaces simultaneously have potential as drug carriers. To be able to detect, treat and report the success of therapy with a single injection will eventually be a future and challenging medical application of nanotechnology.

Some other inorganic nanoparticles, such as magnetic nanoparticles and gold nanoparticles, can be used for imaging diagnosis as well as hyperthermal therapy, due to their unique physical properties. The addition of hyperthermal therapy to chemotherapy can enhance the sensitivity of cells to drugs and this synergic effect was intensely reviewed by van der Zee [5]. In this review, we describe recent developments of hollow and porous inorganic nanomaterials in the aspect of: (1) imaging and diagnosis applications and (2) photothermal and photosensitizing therapy applications.

Corresponding author: Lee, S.B. (slee@umd.edu)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.06.012

Imaging and diagnosis applications

Carbon nanotube (CNT)

Since the electrical conductance of carbon nanotubes (CNTs) is sensitive to the environment and also changes with the surface adsorption of various molecules, CNTs have been proposed as a sensing array element for detecting biological molecules such as cancer markers in fluids [6–8]. Chen *et al.* reported a highly specific electronic biomolecular detector based on single-walled carbon nanotubes (SWNTs) [9]. Their devices are composed of SWNTs grown on a quartz substrate and Ti/Au electrodes bridged by SWNTs. Using quartz crystal microbalance (QCM) and electronic transport measurements, they revealed that as-grown SWNTs exhibit a high degree of nonspecific binding with proteins. Non-covalent functionalizations with Tween 20 or triblock copolymer chains on the SWNT surface prevented the non-specific binding of proteins and also immobilized specific probe molecules that can bind to specific target proteins. The detection can be monitored electronically in real time without the need for labeling. Using SWNTs conjugated with Tween 20 and U1A (a marker protein for autoimmune response), 10E3 (an antibody associated with human autoimmune diseases) was successfully detected at relatively low concentrations (less than 1 nM), compared to a conventional fluorescence-based detection method of immobilized antigens on planar arrays [10].

Another type of biosensor based on CNTs was reported by Li *et al.* [11]. In this study, SWNTs were integrated into a biosensor for the detection of prostate cancer. As seen in Figure 1, an active channel bridging the source/drain electrodes was made up of n-type In_2O_3 nanowires (NWs) and p-type carbon nanotubes and the silicon substrate was used as a gate. For the detection of PSA, the outer surface of a NW or SWNT was functionalized with anti-PSA monoclonal antibodies (PSA-AB), a specific ligand for the PSA protein. Li *et al.* investigated the chemical gating effect of PSA on the devices including both individual NWs and individual semiconducting SWNTs. The devices were incubated in a solution containing PSA for 15 hours and then measuring the electrical properties of the devices, including both current–voltage (I–V_{ds}) and current–gate voltage (I–V_g) characteristics. Enhanced conductance for NW devices was consistently observed after PSA incubation, whereas the group observed reduced conductance for SWNT devices. They proposed that this complementary response in conductance could be ascribed to the fact that In_2O_3 NWs are n-type and SWNTs are p-type semiconductors. In addition, for the real-time detection of PSA, the electric current was monitored over time and Li *et al.* successfully detected PSA with a sensitivity of 5 ng/ml, a level used for clinical diagnosis of prostate cancer [12].

Silica nanoshell

A fundamental limitation of molecular imaging is the signal to background ratio caused by non-specific adhesion of bio-imaging agents to surfaces [13] making it difficult to identify a low intensity signal. A molecular imaging agent that only lights up following a particular molecular event is ideal for *in vivo* tissue pathology. With well-developed silane chemistry, various surface modifications of silica nanoparticles can easily be realized and are widely performed. Silica nanoparticle-based bio-imaging agents are promising in nanomedicine as bioimaging agents [14].

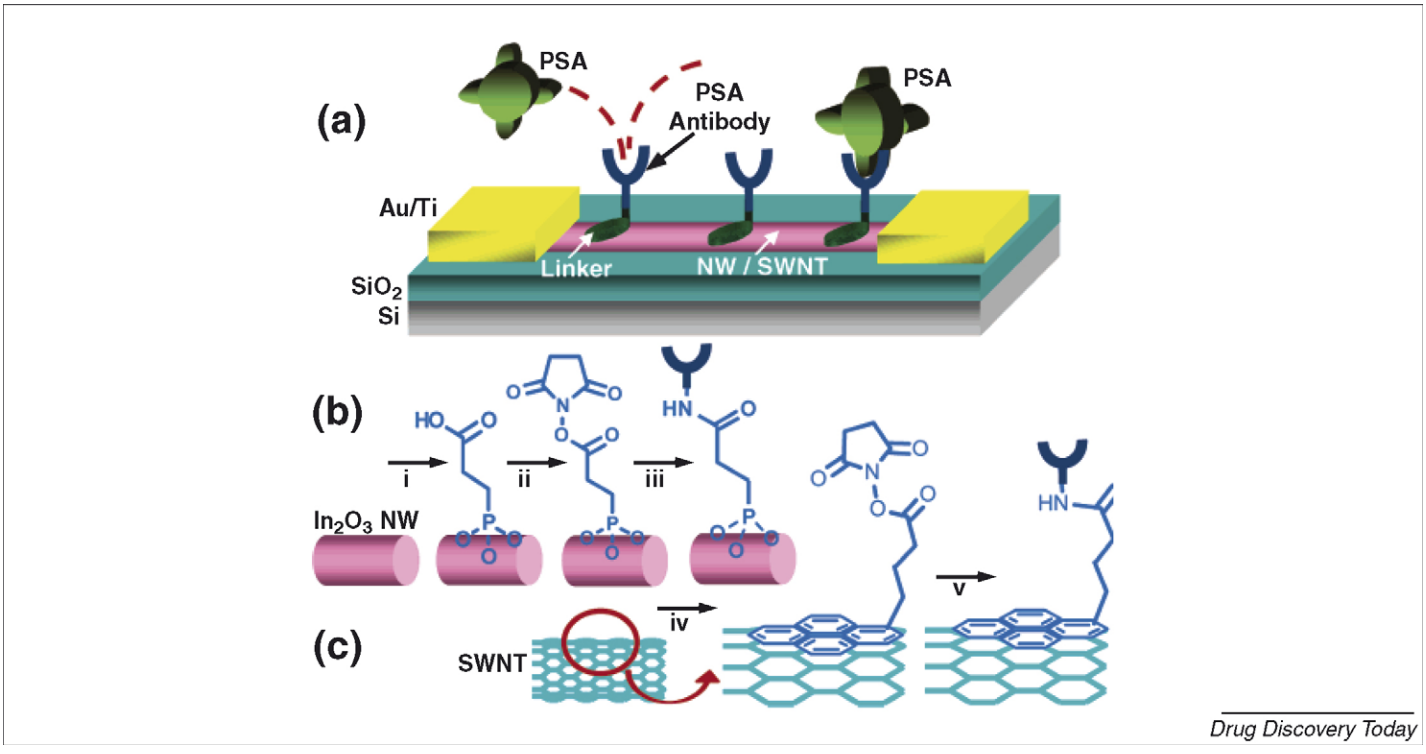

FIGURE 1

Complementary detection of prostate-specific antigen using In_2O_3 nanowires and carbon nanotubes, as reported By Li *et al.* [11]. **(a)** Schematic diagram of the nanosensor. PSA-Abs are anchored to the NW/SWNT surface and function as specific recognition groups for PSA binding. **(b)** Reaction sequence for the modification of In_2O_3 NW: (i), deposition of 3-phosphonopropionic acid; (ii), DCC and *N*-hydroxysuccinimide activation; (iii), PSA-AB incubation. **(c)** Reaction sequence for the modification of SWNT: (iv), deposition of 1-pyrenebutanoic acid succinimidyl ester; (v), PSA-AB incubation.

The two general synthetic approaches to prepare dye-doped silica nanoparticles are the Stöber method and a microemulsion process. Ow *et al.* [3] synthesized multifunctional silica-based fluorescent nanoparticles using the Stöber method. The encapsulation process first involves covalent attachment of the dye molecules (tetramethylrhodamine isothiocyanate: TRITC) to an amine-containing silica precursor (3-aminopropyltriethoxysilane: APS) to form a dye-rich core, followed by co-condensation of APS with sol–gel monomer (tetraethylorthosilicate: TEOS) in a mixture of water, ammonia, and ethanol to form a denser silica network around the core. This approach enables the incorporation of a variety of dye molecules into the silica nanoparticles. The hydrodynamic radius and the brightness of the nanoparticles have been characterized by fluorescence correlation spectroscopy (FCS). These nanoparticles are monodisperse in solution with a diameter of 30 nm. The fluorescent silica nanoparticles are 20 times brighter than the constituent dye. Ow *et al.* [3] proposed that the protection of the fluorophore from the solvent by the silica shell increases the photostability and, hence, contributed to enhancement of brightness. Potential application of these nanoparticles as biomarkers has been demonstrated. The rat basophilic leukemia (RBL) mast cells were successfully labeled with antibody-adsorbed silica nanoparticles with minimal nonspecific interaction between the silica nanoparticles and the cell surface [3].

The microemulsion process, the dye-doped silica nanoparticles are synthesized by hydrolyzing TEOS in a reverse micelle or water-in-oil microemulsion system. The dye molecules are encapsulated physically in the silica matrix. Zhao *et al.* [15] synthesized these nanoparticles for bioanalysis applications as labeling reagents. The dye molecule, tetramethylrhodamine (TMR) was linked to a dextran molecule before the reverse microemulsion process. In this way, the organic dye molecules become more water soluble, are more easily entrapped inside silica pores and have reduced leakage due to the increased size as compared to TMR alone. In addition, the dye molecules have stronger physisorption under acidic conditions during synthesis because of the electrostatic attraction. Effective entrapment of a large amount of dye molecules to each silica nanoparticle makes ultra-sensitive detection in bioanalysis feasible.

Gold nanoshell and nanorod

Gold nanoparticles have been reported to have negligible cytotoxicity *in vivo* and *in vitro* [16]. Nanoshells have a high scattering optical cross-section and thus can also work as contrast agents for photonics-based imaging modalities, such as reflectance confocal microscopy and optical coherence tomography. The scattering of light in the NIR spectral range can provide optical signals for cancer detection whereas biological tissue has low inherent scattering. Loo *et al.* [17] have designed immunotargeted nanoshells to simultaneously provide both scattering and absorption at specific frequencies in the near infrared spectral region for dual imaging/therapy applications. They successfully detected and destroyed breast carcinoma cells that overexpress HER2 with the antibody-conjugated nanoshells.

Metal oxide hollow sphere

Titirici *et al.* have recently reported a simple one-pot synthesis of hollow spheres of crystalline metal oxide via a hydrothermal approach [18]. Various metal salts were dissolved together with carbohydrates in water and then heated at 180 °C in an autoclave. Hollow spheres of various metal oxides, such as Fe_2O_3, Ni_2O_3, Co_3O_4, CeO_2, MgO, and CuO, can be obtained.

Template-free syntheses of water soluble and amine-functionalized magnetic hollow nanospheres have been developed by Wang *et al.* [19]. The hollow nanospheres were prepared by dissolving 1,6-hexadiamine and sodium acetate in $FeCl_3{\cdot}6H_2O$ in ethylene glycol. The mixture was then incubated at a high temperature (around 200 °C), for 6 h. By controlling the amount of the ligand, 1,6-hexadiamine and keeping the reaction temperature between 190 and 205 °C, nanospheres of 100–200 nm were obtained. *In vivo* magnetic resonance imaging experiments were performed as well. Magnetite solid nanoparticles were obtained with a higher concentration of 1,6-hexadiamine and a smaller amount of anhydrous sodium acetate. The saturation magnetizations of the hollow spheres with a diameter of 150 nm and the nanoparticles with tunable size from ~15 to ~50 nm were determined to be 71.2 and 41.3–59.8 emu/g at 25 °C. *In vivo* magnetic resonance imaging in live mice was performed with 25 nm amine-functionalized magnetic nanoparticles as a contrast agent. The MRI signal decreased greatly from 293.52 to 169.49 and no acute fatal toxicity was observed.

Photothermal and photosensitizing therapy applications

NIR-absorbing gold nanostructures

Photothermal therapy that employs a local application of heat to the target is a well-known concept in cancer therapy and has been widely studied [20]. However, due to limited accessibility of the heat source to the target tissue, the development of this therapeutic strategy remains at an early state [20]. In an attempt to solve this problem, near infrared (NIR)-absorbing materials have been used to produce localized heat after they are internalized in the target tissue. NIR light in the 700–1100-nm NIR window can penetrate tissue at depths of more than 1 cm with no observable damage, because most tissue has almost no absorbance in NIR region [21]. Although organic dyes have been used for the NIR-absorbing material, the dyes exhibited a photobleaching problem and relatively low NIR-absorbing efficiency [22]. Recently, SWCNs and gold nanostructures such as nanoshells, nanorods and nanoparticles have been reported to have higher NIR-absorbing efficiency and do not have this photobleaching problem [21,23,24]. The main advantage of using nanomaterial as a NIR-absorbing material is that the surface of nanomaterial can be easily functionalized with a target-specific group, such as antibodies, to decrease non-specific cell death. In addition, the absorbance wavelength can be controlled by tuning various aspects of the nanomaterial such as gold layer thickness, aspect ratio, size and shape.

Gold nanoshell

Nanoshells are a novel type of optically tunable nanoparticles consisting of a dielectric core, surrounded by a thin metallic shell of gold or silver. Gold-dielectric core-shell nanoparticles have several attractive properties in terms of therapeutic applications. They are biocompatible and stable. By varying the dimensions of the core and the shell, the nanoshells exhibit unique tunable optical properties. Additionally, biomolecules such as antibodies can be readily conjugated to the surface of gold nanoparticles for

tumor specific targeting. Hirsch *et al.* [21] have reported the synthesis and application of these nanoshells for cancer imaging and therapy. The optical resonance of these nanoshells can be precisely and systematically varied over a broad region ranging from the near UV to the mid infrared. These particles can be activated by NIR light where absorption by biomatter is low. In this study, human breast cancer cells were incubated with the nanoshells. As seen in Figure 2, upon exposure to NIR light, the tumor cells, monitored by *in situ* magnetic resonance temperature imaging, underwent an average temperature increase of 38 °C at a depth of 2.5 mm beneath the dermal surface, Irreversible thermal damage was observed and confined to the tumor area.

Gold nanoparticle

Souza *et al.* have developed biologically active gold (Au)-phage networks consisting of bacteriophages directly assembled with gold nanoparticles [25]. In addition to targeting cells, the networks can function as signal reporters. Taking advantages of gold optical properties, the networks can be used as labels for enhanced fluorescence and dark-field microscopy, surface-enhanced Raman scattering (SERS) detection and NIR photon-to-heat conversion. The surface plasmon absorption wavelengths can be modulated by the phage input and the existence of a nanoparticle complexing agent such as imidazole. The Au-phage shows loose, dispersed structures, compared to the dense network of Au-phage-imid as a result of imidazole-induced aggregation of Au nanoparticles. The agglomeration of Au nanoparticles will cause a red shift and a broadening of the surface plasmon absorption peak. The more compact networks have a relatively larger red shift in the extinction spectrum with increased absorption in the NIR wavelength region. A substantial temperature change has been observed due to the efficient photon-to-heat conversion. Melanoma cells with over-expressed surface receptor for a well-characterized phage displaying the peptide CDCRGDCFC (RGD-4C) were used for the *in vitro* study. Au-RGD-4C networks show the capability of targeting and receptor-mediated internalization. Compared to RGD-4C networks, the targeted Au-RGD-4C networks show enhanced fluorescence, explained by the synergy between receptor-mediated phage internalization and electromagnetically induced surface enhancement of the Au nanoparticles. Souza *et al.* [25,26] have reasoned that the Au-phage networks can serve as sensitive reporters in localizing and evaluating ligand binding and receptor-mediated internalization events with the Au nanoparticles can work as contrast agents for dark-field microscopy. Cells treated with Au-RGD-4C phage can be differentiated from the untreated cells with NIR-SERS spectra. In other words, the Au nanoparticle-phage complex can work as a cell-targeting agent and a SERS-based label for high-throughput molecular and biological detection schemes [25].

Gold nanorod

Au nanorods of various aspect ratios enable tunable absorption wavelengths in the NIR region. It is predicted that surface plasmon field enhancement of gold nanorod absorption is the strongest of all the different shapes of gold and silver nanoparticles [27]. As seen in Figure 3, the nanorod extinction cross section at 780 nm was estimated to be 1.3×10^{-15} m^2 [28]. In comparison, nanocages and nanoshells were reported to have a cross section of 1.3×10^{-14} m^2 [29] and $\sim 4 \times 10^{-14}$ m^2 [17] respectively at SPR wavelengths near 800 nm. Huang *et al.* [30] have conjugated gold nanorods with an aspect ratio of 3.9 to antiepidermal growth factor receptor (anti-EGFR) antibodies for selective photothermal therapy in the NIR region. The gold nanorods were incubated in

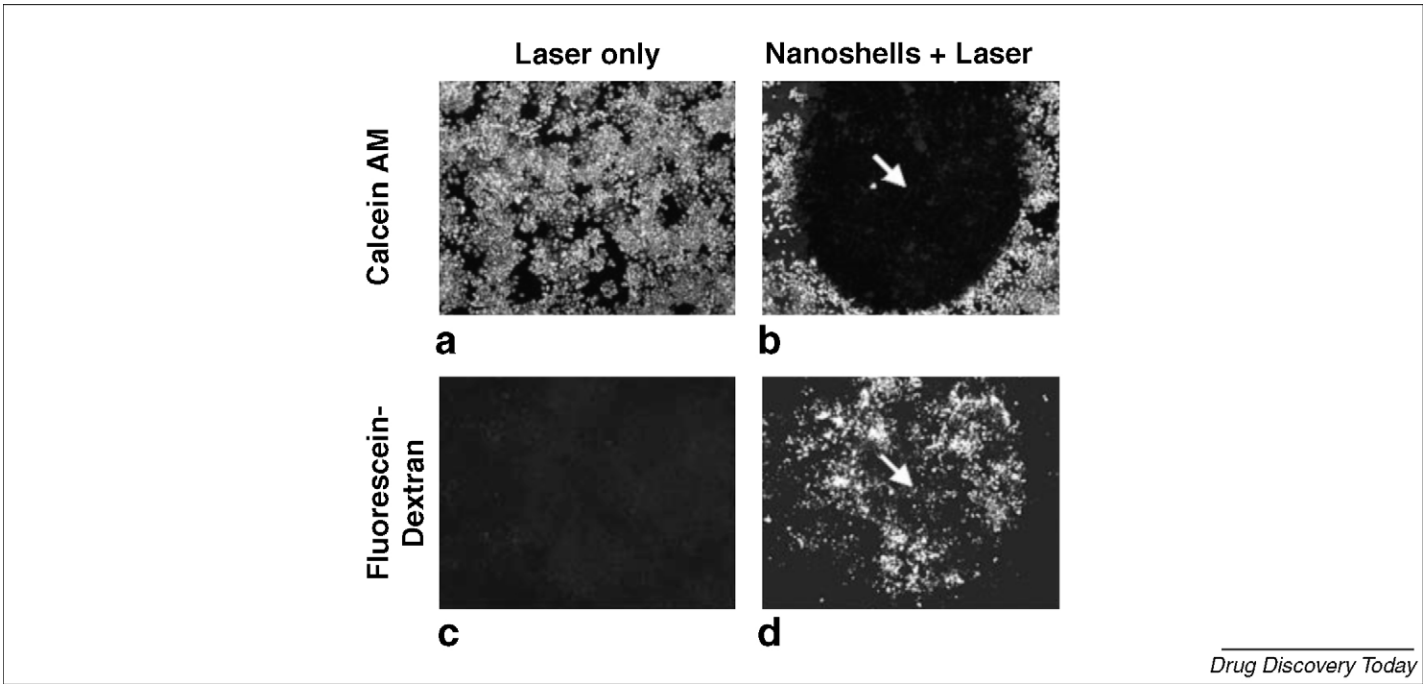

FIGURE 2

Localized photothermal destruction of carcinoma cells by NIR-absorbing gold nanoshell, as reported by Hirsch *et al.* [21]. Calcein AM viability staining reveals that only gold nanoshell-treated samples underwent photothermal destruction within the laser spot **(a, b)**. Fluorescein-dextran that is impermeable to healthy cells was clearly detected in the cells treated with gold nanoshell and laser together **(c, d)**.

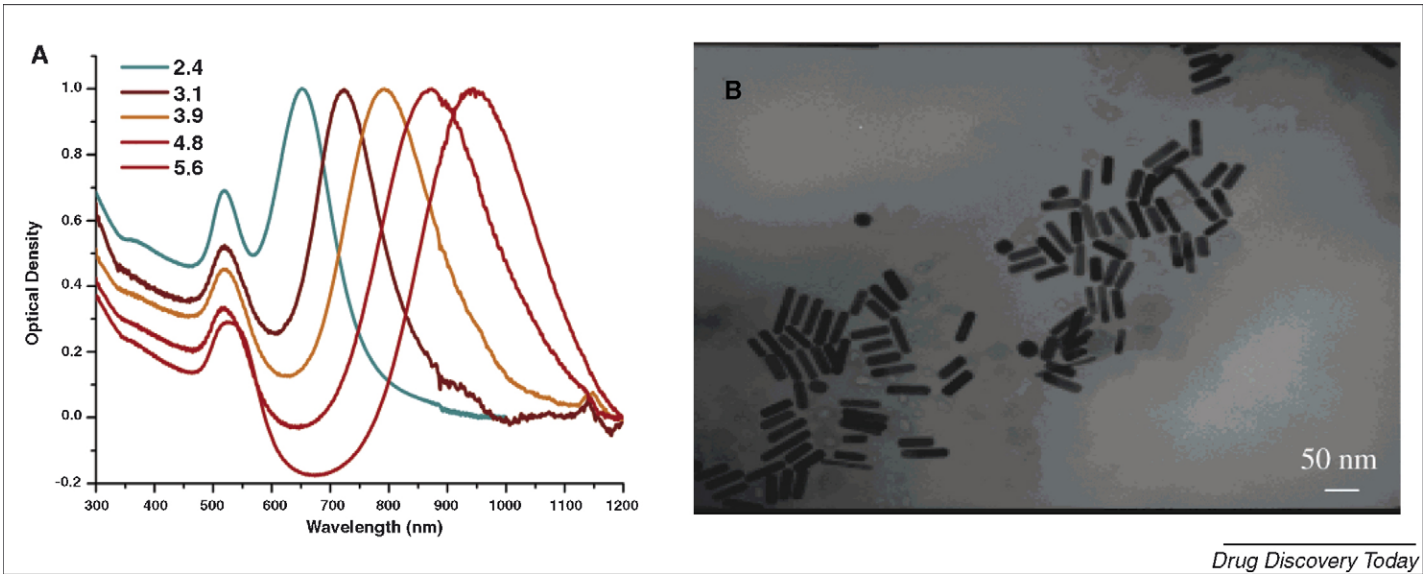

FIGURE 3

Optical property of gold nanorods, as reported by Huang *et al.* [30]. **(A)** Surface plasmon absorption spectra of gold nanorods of different aspect ratios, showing the sensitivity of the strong longitudinal band to the aspect ratios of the nanorods. **(B)** TEM images of nanorods of aspect ratio of 3.9, the absorption spectrum of which is shown as the orange curve in panel **(A)**.

cell cultures with a non-malignant epithelial cell line (HaCaT) and two malignant oral epithelial cell lines (HOC 313 clone 8 and HSC 3). The absorption spectra of the three types of cells reveal that the amount of nanorods on the two malignant cells is over two times higher than that of the non-malignant cells. Malignant cell death occurs with less than half the laser energy required to kill the normal cells, due to the overexpression of the EGFR on the surface of malignant cells. Compared to the core-shell particles, in this study, the threshold energy needed to kill the cancer cells is lower, with a value of 10 W/cm^2. In addition to the fact that gold nanorods have a higher absorption cross section than nanospheres, Huang *et al.* have proposed that the difference can be due to a higher affinity constant for binding of the gold nanoparticles to the antibody, or a higher affinity constant for binding of the antibody to the cancer cell surface [30].

Gold nanorods have also been studied for targeted drug delivery. In addition to the unique optical properties, nanorods undergo shape transformation to spherical nanoparticles upon NIR irradiation. The shape transformation can be used for controlled release of the conjugated biomoleucles. Chen *et al.* [31] have designed an approach to remote control gene delivery in specific cells. Gold nanorods were conjugated with the gene of enhanced green fluorescence protein (EGFP) via Au–S bond. The Au–S bond is broken upon exposure to NIR light and the EGFP gene is released.

Single-walled carbon nanotube (SWNT)

Although most applications of carbon nanotubes have been focused on microelectronic devices, due to their unique electronic and physical properties [32–34], carbon nanotubes have shown some attractive properties for the biomedical use, including easy translocation across cell membranes and relatively low toxicity [35,36]. It has recently been discovered that SWNTs have strong optical absorbance in the NIR region where biological systems are known to be highly transparent [22,23,37]. In addition to the biocompatibility of carbon nanotubes, the ability of SWNTs to absorb NIR makes it possible for them to be used for photothermal cancer therapy by causing irreversible thermal cellular destruction.

Kam *et al.* showed that SWNTs prepared from high-pressure CO conversion (Hipco) can be used to destroy cancer cells selectively upon irradiation with NIR [37]. To achieve this, Kam *et al.* functionalized SWNTs noncovalently with folic acid (FA), which can selectively bind to the common tumor marker folate receptor (FR) and incubated them with FR-overexpressed HeLa cells and normal cells. After NIR irradiation, the group observed extensive cell death for the FR-overexpressed HeLa cells, whereas no significant cell morphology change was observed in the normal cells. Kam *et al.* showed that SWNTs have a higher optical absorbance than NIR-absorbing Au nanoshells. As a result, lower laser power and shorter radiation times are expected to be necessary for cancer cell destruction, which may help reduce unwanted normal cell destruction.

Porous silica nanoparticles embedded with a photosensitizing agent

The water-insoluble photosensitizing anticancer drug 2-deviny-2-(1-hexyloxyethyl) pyropheophorbide (HPPH), has been entrapped in the nonpolar core of micelles formed from the hydrolysis of triethoxyvinylsilane [38], as shown schematically in Figure 4. The HPPH-doped nanoparticles are quite stable and disperse well in aqueous solutions. Upon irradiation with visible light, singlet oxygen is generated and diffuses out of the porous nanoparticle walls, and causes irreversible destruction of impregnated cells. Singlet oxygen production was confirmed directly by luminescence spectroscopy and indirectly by spectrophotometry with a disodium salt of 9,10-anthracenedipropionic acid as a sensor. Roy *et al.* [38] have demonstrated that the loss of fluorescence from the entrapped photosensitizing drug was largely prevented in aqueous media as compared to the free drug. Cellular uptake was studied with two tumor cell lines, UCI-107 and HeLa. The confocal images confirm the interval of the nanoparticles. Cell phototoxicity was

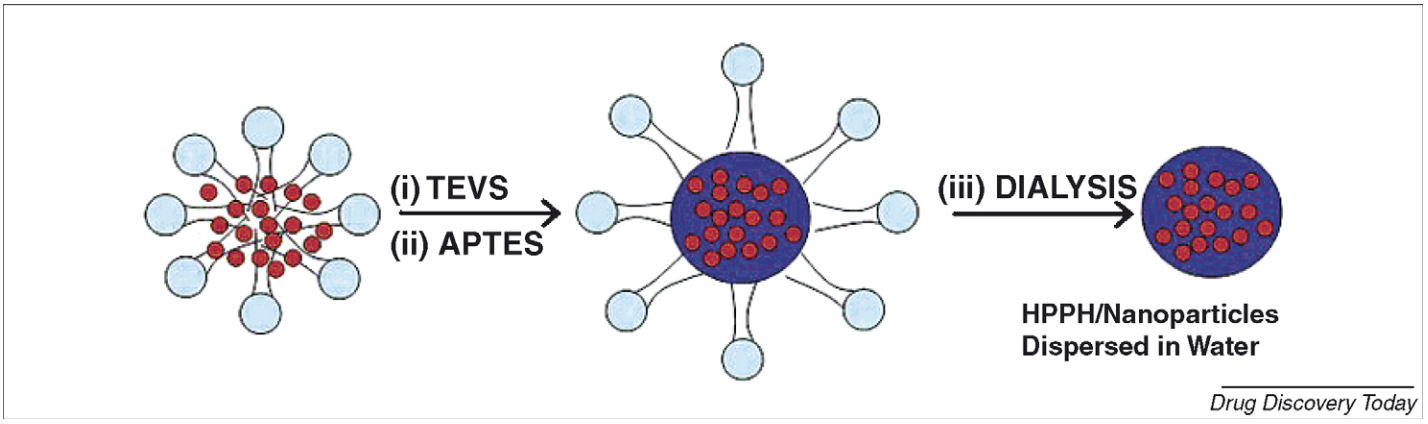

FIGURE 4

Scheme depicting the synthesis and purification of HPPH-doped silica-based nanoparticles in a micellar medium, as reported by Roy *et al.* [37].

studied with void nanoparticles as a control. The data show that HPPH-doped nanoparticles result in significant cell death. These observations illustrate the potential of the system for photodynamic therapy.

Conclusion

In this article, we have reviewed hollow and porous inorganic nanomaterials in nanomedicine, focusing on imaging/diagnosis, and photothermal therapy. Proof-of-concept studies have shown that inorganic nanoparticles with hollow structure and well-controlled shape and dimensions have great promise in biomedical applications. A variety of inorganic material with inherent chemical, optical, magnetic, or electronic properties, can have simultaneous potential applications for diagnosis and therapy that is not possible with the traditional imaging, delivery, and sensing devices. More detailed studies are needed in the interactions of nanoparticles with tumor cells and physiological components, however, before these preliminary results can be employed clinically. Safety is one of the most important issues. Fortunately, although long-term safety studies have to be thoroughly examined, recent studies show that the toxicity of inorganic nanomaterials can be alleviated by suitable environmental-friendly surface functionalization [36,39,40]. Reliable pharmacological profiles of administered nanoparticles *in vivo* can be obtained with the well-defined physical and chemical properties. Besides the ability of nano-sized materials to avoid biobarriers, unique optical and electronic properties of inorganic nanomaterials enable the smart multifunctionalized nanomaterials, which are in high demand by modern biomedical applications. Hollow and porous inorganic nanomaterial can potentially revolutionize medical sensing, imaging, diagnosis and therapy. Future studies will determine the opportunities as well as the limitations.

References

1 Medintz, I.L. *et al.* (2005) Quantum dot bioconjugates for imaging, labeling and sensing. *Nat. Mater.* 4, 435–446
2 Michalet, X. *et al.* (2005) Quantum dots for live cells, *in vivo* imaging, and diagnostics. *Science* 307, 538–544
3 Ow, H. *et al.* (2005) Bright and stable core-shell fluorescent silica nanoparticles. *Nano Lett.* 5, 113–117
4 Luo, D. (2004) A new solution for improving gene delivery. *Trends Biotechnol.* 22, 101–103
5 van der Zee, J. (2002) Heating the patient: a promising approach? *Annal. Oncol.* 13, 1173–1184
6 Kong, J. *et al.* (2000) Nanotube molecular wires as chemical sensors. *Science* 287, 622–625
7 Collins, P.G. *et al.* (2000) Extreme oxygen sensitivity of electronic properties of carbon nanotubes. *Science* 287, 1801–1804
8 Shim, M. *et al.* (2001) Polymer functionalization for air-stable n-type carbon nanotube field-effect transistors. *J. Am. Chem. Soc.* 123, 11512–11513
9 Chen, R.J. *et al.* (2003) Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4984–4989
10 Haab, B.B. *et al.* (2001) Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biol.* 2, R4
11 Li, C. *et al.* (2005) Complementary detection of prostate-specific antigen using In_2O_3 nanowires and carbon nanotubes. *J. Am. Chem. Soc.* 127, 12484–12485
12 Li, X. *et al.* (2005) Gene therapy for prostate cancer by controlling adenovirus E1a and E4 genes expression with PSES enhancer. *Cancer Res.* 65, 1941–1951
13 Shaffer, C. (2005) Nanomedicine transforms drug delivery. *Drug Discov. Today* 10, 1581–1582
14 Wang, L. *et al.* (2006) Watching silica nanoparticles glow in the biological world. *Anal. Chem.* 78, 646–654
15 Zhao, X.J. *et al.* (2004) Development of organic-dye-doped silica nanoparticles in a reverse microemulsion. *Adv. Mater.* 16, 173–176
16 Shukla, R. *et al.* (2005) Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: A microscopic overview. *Langmuir* 21, 10644–10654
17 Loo, C. *et al.* (2005) Immunotargeted nanoshells for integrated cancer imaging and therapy. *Nano Lett.* 5, 709–711
18 Titirici, M.M. *et al.* (2006) A generalized synthesis of metal oxide hollow spheres using a hydrothermal approach. *Chem. Mater.* 18, 3808–3812
19 Wang, L. *et al.* (2006) One-pot synthesis and bioapplication of amine functionalized magnetite nanoparticles and hollow nanospheres. *Chem. Eur. J.* 12, 6341–6347
20 Pissuwan, D. *et al.* (2006) Therapeutic possibilities of plasmonically heated gold nanoparticles. *Trends Biotechnol.* 24, 62–67
21 Hirsch, L.R. *et al.* (2003) Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proc. Natl. Acad. Sci. U. S. A.* 100, 13549–13554
22 O'Neal, D.P. *et al.* (2004) Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles. *Cancer Lett.* 209, 171–176
23 O'Connell, M.J. *et al.* (2002) Band gap fluorescence from individual single-walled carbon nanotubes. *Science* 297, 593–596
24 Daniel, M.C. and Astruc, D. (2004) Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem. Rev.* 104, 293–346

25 Souza, G.R. *et al.* (2006) Networks of gold nanoparticles and bacteriophage as biological sensors and cell-targeting agents. *Proc. Natl. Acad. Sci. U. S. A.* 103, 1215–1220
26 Souza, G.R. *et al.* (2006) *In vivo* detection of gold-Imidazole self-assembly complexes: NIR-SERS signal reporters. *Anal. Chem.* 78, 6232–6237
27 Hao, E. *et al.* (2004) Synthesis and optical properties of anisotropic metal nanoparticles. *J. Fluoresc.* 14, 331–341
28 Oldenburg, A.L. *et al.* (2006) Plasmon-resonant gold nanorods as low backscattering albedo contrast agents for optical coherence tomography. *Opt. Express* 14, 6724–6738
29 Cang, H. *et al.* (2005) Gold nanocages as contrast agents for spectroscopic optical coherence tomography. *Opt. Lett.* 30, 3048–3050
30 Huang, X. *et al.* (2006) Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *J. Am. Chem. Soc.* 128, 2115–2120
31 Chen, C.C. *et al.* (2006) DNA-gold nanorod conjugates for remote control of localized gene expression by near infrared irradiation. *J. Am. Chem. Soc.* 128, 3709–3715
32 Fan, S. *et al.* (1999) Self-oriented regular arrays of carbon nanotubes and their field emission properties. *Science* 283, 512–514
33 Kong, J. *et al.* (1998) Synthesis of individual single-walled carbon nanotubes on patterned silicon wafers. *Nature (Lond.)* 395, 878–881
34 Zhou, C. *et al.* (2000) Modulated chemical doping of individual carbon nanotubes. *Science* 290, 1552–1555
35 Bianco, A. and Prato, M. (2003) Can carbon nanotubes be considered useful tools for biological applications? *Adv. Mater.* 15, 1765–1768
36 Bianco, A. *et al.* (2005) Biomedical applications of functionalized carbon nanotubes. *Chem. Comm.* 571–577
37 Kam, N.W.S. *et al.* (2005) Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc. Natl. Acad. Sci. U. S. A.* 102, 11600–11605
38 Roy, I. *et al.* (2003) Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: A novel drug-carrier system for photodynamic therapy. *J. Am. Chem. Soc.* 125, 7860–7865
39 Singh, R. *et al.* (2006) Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. *Proc. Natl. Acad. Sci. U. S. A.* 103, 3357–3362
40 Sayes, C.M. *et al.* (2006) Functionalization density dependence of single-walled carbon nanotubes cytotoxicity *in vitro*. *Toxicol. Lett.* 161, 135–142