Biotechnology and Genetic Engineering Reviews - Vol. 25, 93-112 (2008)

Prospects for Gold Nanorod Particles in Diagnostic and Therapeutic Applications

DAKRONG PISSUWAN[1], STELLA M. VALENZUELA[2], AND MICHAEL B. CORTIE[1*]

[1]Institute for Nanoscale Technology, University of Technology Sydney, Australia and [2]Department of Medical and Molecular Biosciences, University of Technology Sydney, Australia

Abstract

Rod-shaped gold nanoparticles ('nanorods') have recently attracted widespread attention due to their unique optical properties and facile synthesis. In particular, they can support a longitudinal surface plasmon, which results in suspensions of them having a strong extinction peak in the upper visible or near-infrared parts of the spectrum. The position of this peak can be readily tuned by controlling the shape of the rods. In addition, the surface of the nanorods can be functionalized by a very wide variety of molecules. This has led to interest in their use as selective biomarkers in biodiagnostics or for selective targeting in photothermal thearapeutics. Here, we review the recent advances in the use of gold nanorods in these applications. Additionally, the information available regarding their biocompatibility is discussed.

Introduction

Nanoparticles of gold offer a number of properties which make them suitable for use in biological applications. In particular they have a strong optical extinction peak that can be varied by control of particle morphology, they are 'electron dense' and

*To whom correspondence may be addressed (michael.cortie@uts.edu.au)

Abbreviations: PSS, polystyrene sulfonate; SERS, surface enhanced Raman spectroscopy; GFP, growth factor receptor; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; CXCR4, chemokine receptor 4; HEK293, kidney mammalian cell line; GFP, green fluorescent protein; CTAB, hexadecyltrimethylammonium bromide; BSA, bovine serum albumin; PEI, polyethylenimine; PEG, polyethyleneglycol; mPEG-SH, thiol-terminated methoxy-poly(ethylene glycol); TEM, transmission electron microscopy; NIR, near infrared.

radiopaque, their surface chemistry allows for easy attachment of organic molecules tailored to specific needs, and they manifest a low level of toxicity when introduced into biological systems. In addition, current interest in nanotechnology has stimulated a number of studies on the use of a variety of shapes of gold nanoparticles in biological applications. The morphologies of interest include nanospheres, nanoshells, nanocages, and nanorods (Daniel and Astruc, 2004; Dietricha *et al.*, 2005; Hirsch *et al.*, 2003; Kelly *et al.*, 2003; Liao and Hafner, 2005; Pissuwan *et al.*, 2006; Skrabalak *et al.*, 2007; Wu *et al.*, 2005). Nanoparticles of these shapes can be produced by wet chemical means, or by physical vapour deposition onto a suitable template.

Interest in gold nanorods, in particular, has recently soared, both because their optical properties are well-matched for exploitation in diagnostic and therapeutic applications, and because of significant improvements to the wet chemical process by which they can be produced (Jana *et al.*, 2001; Perez-Juste *et al.*, 2004). Background information on gold nanorods is available in some excellent reviews (Murphy *et al.*, 2005; Perez-Juste *et al.*, 2005); here we will provide only the information essential to appreciate the possible role of these particles in biotechnological applications.

The rod-like shape of these gold nanoparticles causes them to have a strong surface plasmon absorption and, if they are big enough, an enhanced capability to scatter light. The first attribute is useful in the development of a selective therapeutic agent and the second for imaging and diagnostics. Actually, gold nanorods have two surface plasmon resonance modes: transverse and longitudinal. The transverse surface plasmon resonance, which is due to an electronic oscillation across the width of the rod, is effectively of the same nature as the plasmon resonance of simple gold nanospheres. It peaks at about ~520 nm (*i.e.* at the wavelength of green light) and is comparatively weak. However, the longitudinal mode provides a much larger extinction coefficient and is due to oscillation of electrons in the long direction of the rod. It occurs at longer wavelengths than the transverse resonance (*i.e.* it is 'red-shifted' relative to the transverse mode) (Kelly *et al.*, 2003). When compared with other shapes of gold nanoparticles such as nanoshells and nanospheres, gold nanorods also provide superior competence of light absorption at their longitudinal plasmon resonance (Harris *et al.*, 2008; Jain *et al.*, 2006).

The wavelength of light at which the longitudinal resonance occurs is controlled for the most part by the aspect ratio of the rods (*Figure 1*), and gold nanorods can now be synthesized in aspect ratios of up to 20:1 (Kim *et al.*, 2002; Murphy *et al.*, 2005; Perez-Juste *et al.*, 2005), although yields drop off steeply as the aspect ratio increases above 4:1. The minimum absorption of light in most human tissue occurs between wavelengths of 700 nm and 900 nm, a feature sometimes referred to as the NIR tissue 'window' (Simpson *et al.*, 1998; Weissleder, 2001). Therefore, gold nanorods with aspect ratios of between 3:1 and 4:1, which have a peak extinction in this 'window', should be useful for *in vivo* therapy since their use would minimize the risk of damage to healthy cells from light extinction by intrinsic chromophores. The present review focuses on the diverse ways in which these properties of gold nanorods are being explored in a biological context, especially in respect of diagnostic and therapeutic applications.

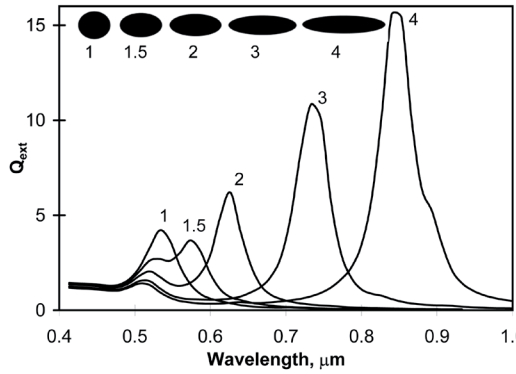

Figure 1. Calculated optical extinctions of typically-sized gold nanorods of varying aspect ratios in water. The volume of these rods is fixed at 33,500 nm3. Reproduced with permission from Advanced Functional Materials (Xu and Cortie, 2006); copyright Wiley-VCH Verlag GmbH&Co. KGaA.

Gold nanorods in diagnosis

Living cells contain various substances that can absorb light. These include haemoglobin, oxy-haemoglobin, melanin and water. Additionally, tissue scattering of light also results from the cell membrane and intracellular structures such as the nucleus and mitochondria. Optical imaging techniques designed to visualize tissue utilise this ability of tissue to absorb and scatter light. The contrast that results between different tissue types allows for their identification, as well as discrimination between pathological and non-pathological tissue. However, endogenous tissue contrast and light interaction with tissue are often inadequate or not specific to a pathology, therefore there is a need to use contrast agents in order to optimize tissue visualization (Rayavarapu *et al.*, 2007; Richards-Kortum and Sevick-Muraca, 1996). Gold nanoparticles with strong light absorption and scattering can be used as imaging labels and contrast agents for biomedical diagnosis. This is particularly true of gold nanorods, the optical absorption efficiencies of which are 20 times or more greater than for gold nanospheres of the same volume (Copland *et al.*, 2004). Scattering coefficients per micron of gold nanorods are also an order of magnitude higher compared to those of nanoshells and nanospheres (Jain *et al.*, 2006). In addition, gold nanorods have a strong binding affinity to thiol groups allowing them to be efficiently conjugated with numerous bio-molecules after stabilizing with ethylene glycol (Liao and Hafner, 2005) or polystyrene spheres (Huang *et al.*, 2007a). Functionalization of the gold nanorods with antibodies (Pissuwan *et al.*, 2007b) or other biomolecules (Copland *et al.*, 2004; Huff *et al.*, 2007b) allows their specific attachment to any target cell. This is a useful attribute for biomedical diagnostic applications.

GOLD NANORODS AS DIAGNOSTIC MARKERS FOR IMAGING

Optical imaging technologies have been used in the diagnosis of diseases such as cancer. The results can be enhanced if some highly visible marker molecule or na-

noparticle is available that will attach selectively to a characteristic cellular feature of the disease. Some organic fluorophores have been used in this role but there are problems due to interference caused by, for example, photodecomposition by the intense illuminating light in fluorescence microscopy and sensitivity to quenchers (Yguerabide and Yguerabide, 1998). More recently, the use of quantum dots has been explored for cell imaging. These are resistant to the photobleaching which normally occurs with conventional fluorescent dyes (Chan and Nie, 1998). However, quantum dots have been shown to be cytotoxic, both *in vitro* and *in vivo*, and therefore careful consideration of their use in humans is necessary (Hardman, 2006).

In contrast, gold nanorods are biocompatible (see later), do not suffer from photobleaching or decomposition, and are highly visible in both optical and electron microscopy. They are therefore also a candidate for use in diagnosis. For example, Huang *et al.* (Huang *et al.*, 2007a) functionalised gold nanorods by conjugating them with monoclonal anti-epidermal growth factor receptor antibodies (anti-EGFR). EGFR is known to be over-expressed in many malignant epithelial tumour cells. The anti-EGFR gold conjugations were incubated with a normal skin cell line and an oral cancer cell line. The cancer cells were brightly stained under dark field mode microscopy (*Figure 2*) due to the strong scattering of red light by the bound gold nanorod-anti EGFR conjugates located on their surfaces. This type of staining represents a new method by which to identify cancer cells. Huang *et al.* also used Raman spectroscopy and found that bioconjugated gold nanorods attached to the cancer cells showed a greatly enhanced Raman spectrum, due evidently to an amplification of the Raman effect by the closely packed nanorods present on the cancer cells. Other instances of cell imaging that exploit gold nanorods have also been reported (Huang *et al.*, 2006; Rayavarapu *et al.*, 2007).

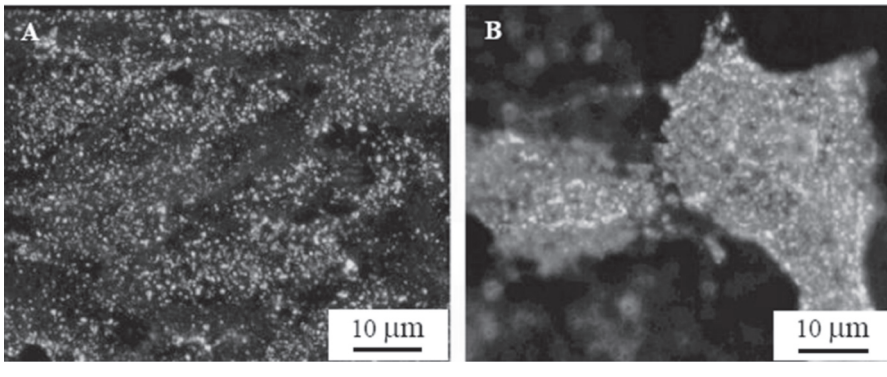

Figure 2. Normal cells (A) and cancer cells (B) labelled with gold nanorod-anti-EGFR antibody conjugates, viewed using dark field optical imaging. Reprinted with permission from *Nano Letters* (Huang *et al.*, 2007a) ©2007 American Chemical Society.

In addition, gold nanorods have been used as photoluminescence agents owing to their efficient two-photon enhanced luminescence which is extremely bright compared to the two-photon fluorescence achieved from single dye molecules. (Wang *et al.*, 2005). Recent work using gold nanorods as a bright contrast agent for two-photon luminescence imaging to detect cancer cells located in deep tissue has been carried out by Durr and colleagues (Durr *et al.*, 2007).

Optoacoustic or photoacoustic tomography is another new technique used for imaging cells and deep tissue and the use of gold nanorods can improve the results here too. This form of tomography employs a combination of optical illumination and ultrasonic detection. Irradiation of the tissue with a strong laser pulse of very short duration will produce a sudden and localized increase in temperature around optically absorptive regions. There is an associated acoustic pulse generated by the sudden thermal expansion of the material effected, which can be detected by appropriate ultrasonic technology. This can in turn be used to reconstruct a 3D acoustic image (Andreev *et al.*, 2003; Haisch and Niessner, 2002). Characteristic endogenous variations in optical contrast around a tumour (Salomatina *et al.*, 2006) due, for example, to abnormal vascular development, might be sufficient to produce an image, however detection of malignant tumours in regions such as the breast requires high resolution and high contrast imaging with specific molecular labelling of the cancer cells in order for their detection *in situ*. This can reportedly be achieved using optoacoustic tomography (Copland *et al.*, 2004) but results can be improved and earlier detection achieved by using an exogenous contrast agent such as gold nanospheres (Copland *et al.*, 2004; Mallidi *et al.*, 2007). Of course, it is necessary that a means be found of selectively concentrating the gold particles in the target tissue.

Gold nanospheres are strong and biocompatible absorbers, with four to six orders of magnitude stronger absorption compared to conventional fluorescent markers (Zharov *et al.*, 2006). Unfortunately, their peak absorption is in the mid-visible region of light, and outside of the tissue window (Cerussi *et al.*, 2001; Eghtedari *et al.*, 2007). Therefore they are not the most suitable contrast enhancing agent for *in vivo* optoacoustic imaging. Gold nanorods, on the other hand, can now be readily synthesized with peak absorption in the tissue window and so there is currently keen interest in their use in optoacoustic tomography. For example, Li and colleagues (Li *et al.*, 2007) used a mixture of gold nanorods of two different aspect ratios (3.7 and 5.9, with longitudinal plasmon modes at 785 and 1000 nm respectively) for the early detection of cancer cells by optoacoustic imaging (*Figure 3*). Two monoclonal antibodies, anti-HER2 and anti-CXCR4, were separately conjugated to the short and long gold nanorods respectively. Laser irradiation at a wavelength tuned to the longitudinal plasmon resonance of one or other of the two kinds of nanorods allowed selective imaging of two different sites in the same sample. In another study, Kim and colleagues (Kim *et al.*, 2007) conjugated cell-specific antibodies to gold nanorods modified with polyacrylic acid. The optoacoustic image of stimulated cells with bound bioconjugated gold nanorods showed a significantly higher intensity compared to unstimulated cells (*Figure 4*). These results also confirm that gold nanorods can be excellent contrast agents for the *in vitro* detection of early stage inflammatory responses.

In vivo detection of gold nanorods using a laser optoacoustic imaging system also has been studied. A 25 μl suspension of PSS-coated gold nanorods at 1.25 pM Au was injected into the lower abdominal region of a mouse. A laser optoacoustic imaging system was used to generate an image of the distribution of gold nanorods *in vivo*. Under optoacoustic imaging a mouse that had been injected with gold nanorods displayed bright features at the injected area because of the high optical contrast between gold nanorods and normal tissues. In contrast, little detail was visible in an optoacoustic image of a non-injected mouse (Eghtedari *et al.*, 2007). Once again the conclusion was drawn that the use of suitably functionalized gold nanorods would permit the detection of target cells *in vivo*.

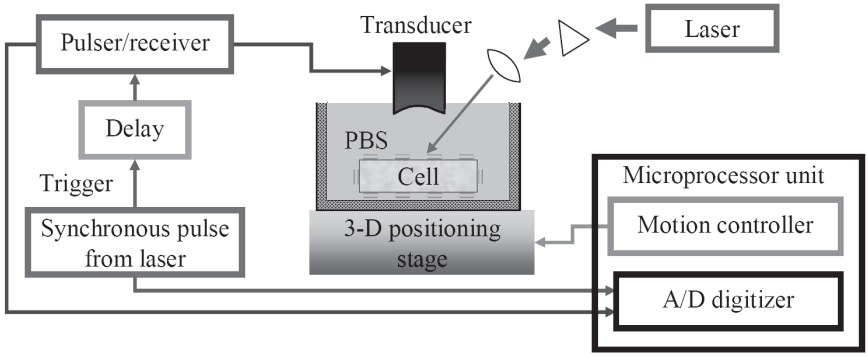

Figure 3. Diagram illustrating the process of optoacoustic imaging and ultrasound employing gold nanorods. Redrawn with permission from *Optics Express* (Mallidi *et al.*, 2007).

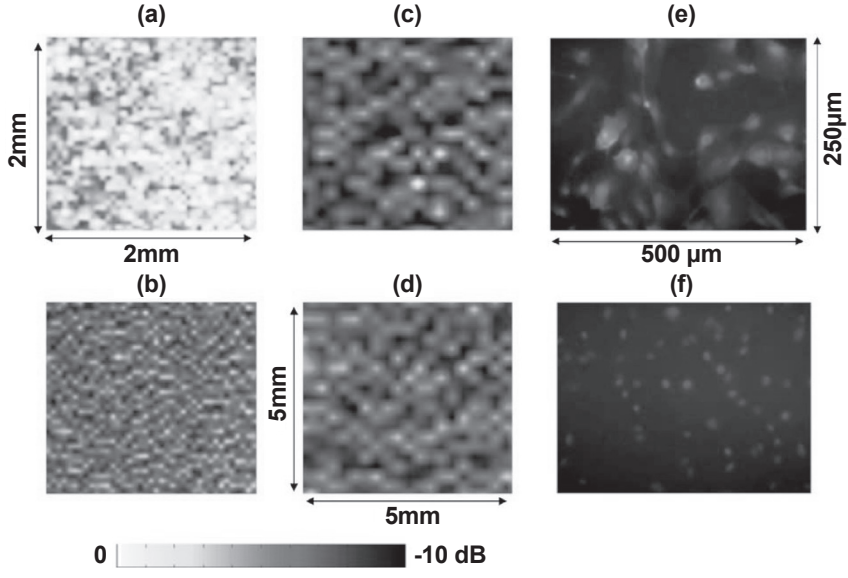

Figure 4. Photoacoustic images of stimulated and non-stimulated endothelial cells. The top images are stimulated endothelial cells. The bottom images are non-stimulated endothelial cells. Cells with gold nanorod-antibody conjugates are shown in (a) and (b). Cells with unconjugated gold nanorods are shown in (c) and (d). Fluorescent images of stimulated and non-stimulated endothelial cells are shown as (e) and (f) respectively. Reprinted with permission from *Applied Physics Letters* (Kim *et al.*, 2007).

GOLD NANORODS IN IMMUNOASSAY AND BIOSENSING APPLICATIONS

Gold nanorods have also been incorporated into immunoassays and biosensors for molecular diagnostics. Detection of human IgG (hIgG) has been carried out using a dot-immunogold assay, where gold nanorods have been conjugated with protein that binds to hIgG. A blue- grey spot developed on the membrane after incubation with the bioconjugated gold nanorods, due to the interaction between hIgG molecules on the membrane and the bioconjugated gold nanorods (Alekseeva *et al.*, 2005). In another example, Li and co-workers studied the fluorescence properties of gold nanorods. They

found that gold nanorods of high aspect ratio offer a higher fluorescence intensity. This property will encourage the development of techniques using gold nanorods in fluorescent probe microarray assays and optical biosensor applications for DNA analysis (Li *et al.*, 2005). In a related vein, El-Sayed and co-workers (Oyelere *et al.*, 2007) have shown that gold nanorods could be used as optical sensors for Raman-based intracellular biosensing. Gold nanorods were conjugated to a simian virus nuclear localisation sequence (NLS) peptide via a linker of thioalkyl-triazole resulting in a gold nanorod-peptide conjugate. The conjugates were incubated with normal cells and cancer cells. The measured Raman signals were found to be different for cancer cells and normal cells, with some additional peaks observed in the case of the cancer cells. The differences between these spectra might be useful for cancer diagnosis and other diagnostic biomedical applications (Oyelere *et al.*, 2007).

There have also been developments in respect of using gold nanorods in refractometric biomolecular sensors. For example, Chen and colleagues (Chen *et al.*, 2007) have shown the potential of such a sensor by studying how the optical properties of a glass slide coated with gold nanorods varies with the refractive index of the surrounding medium. They found that gold nanorods offer much higher sensitivity (366 nm per refractive index unit) than gold nanospheres (76.4 nm per refractive index unit) (Chen *et al.*, 2007; Nath and Chilkoti, 2002). They also studied the response of the longitudinal surface plasmon band of gold nanorod/biotin conjugates to streptavidin at different concentrations, and showed that the wavelength at which the optical extinction peaks shifts linearly as the concentration of the streptavidin is changed. In another recent paper, Yu and Irudayaraj provided a demonstration of using gold nanorods as a biosensor for detecting multiple biological targets. Three different aspect ratios of gold nanorods were used, each targeted to a specific biomolecule (Yu and Irudayaraj, 2007). These studies indicate that the longitudinal surface plasmon mode of gold nanorods provides a basis on which efficacious refractometric biosensors could be developed.

Gold nanorods in therapeutic applications

GOLD NANORODS IN GENE DELIVERY

Gene delivery is a type of therapy for treating and controlling diseases in which a viral or nonviral vector is used to transport foreign genes into somatic cells to increment defective genes there or provide supplementary biological functions (Luo and Saltzman, 2000; Roy *et al.*, 1999; Salem *et al.*, 2003). However, the methodology for DNA delivery by viral vector-mediated systems has some disadvantages. These include issues relating to toxicity, limited targeting of specific cell types and DNA carrying capacity, inability of viral vector to infect non-dividing cells, and problems of production and packing (Crystal, 1995; Luo and Saltzman, 2000; Rayavarapu *et al.*, 2007; Zhang and Godbey, 2006). Nonviral delivery systems, which include chemical and physical methods, provide several potential benefits and have low toxicity compared with viral delivery systems. Therefore, nonviral delivery systems have become attractive in research laboratories and clinical practice (Luo and Saltzman, 2000). However, these methods have been limited by poor specific targeting and low transfection efficiency due to the difficulty of controlling the processes at the nanoscale

(Mehier-Humberta and Guy, 2005; Salem *et al*., 2003). Gene gun technology or the particle bombardment method is one of the non-viral techniques used to insert DNA plasmid into target cells by using an accelerated particle carrier. This technology has been considered to be the most competent DNA vaccination method in terms of the number of plasmids needed (Mehier-Humberta and Guy, 2005). Gold nanoparticles can be used in conjunction with gene gun technology on account of their chemical and physical properties. DNA-coated gold particles have been used for intradermal genetic immunization by particle bombardment (Yang and Sun, 1995). Nevertheless, there are some limitations to this approach including the relatively low DNA binding capacity of the gold nanoparticles and the shallow depth of their penetration (Rabussay *et al.*, 2003).

Salem and colleagues (Salem *et al.*, 2003) have described the use of multifunctional nanorods for gene delivery. In their work, gold-nickel nanorods were fabricated by electrodeposition into porous alumina membranes. Nanorods were functionalized with the linker molecule 3-[(2-aminoethyl) dithio] propionic acid resulting in the carboxylate end-group binding to the nickel segment of nanorod particle. This enabled the binding of DNA onto the nickel segment. The protein transferrin was then coupled to the gold segment of particle. This protein was used in order to encourage cellular uptake of the particle via a receptor-mediated pathway. An *in vitro* transfection experiment of the human embryonic kidney mammalian cell line (HEK293) with genes encoding the green fluorescent protein (GFP) and luciferase reporting were carried out using these nanorods. The uptake of gold-nickel nanorods by HEK293 cells into intracellular vesicles or cytoplasm were confirmed by transmission electron microscopy. This indicates that the plasmids were released or cleaved from the gold-nickel nanorods before entering to the nucleus. A schematic diagram of the transfection process using gold-nickel nanorods is shown in *Figure 5*. The percentage of HEK293 cells expressing GFP and luciferase was higher after application of the gold-nickel nanorod transfection process than in control samples of HEK293 cells transfected with naked DNA.

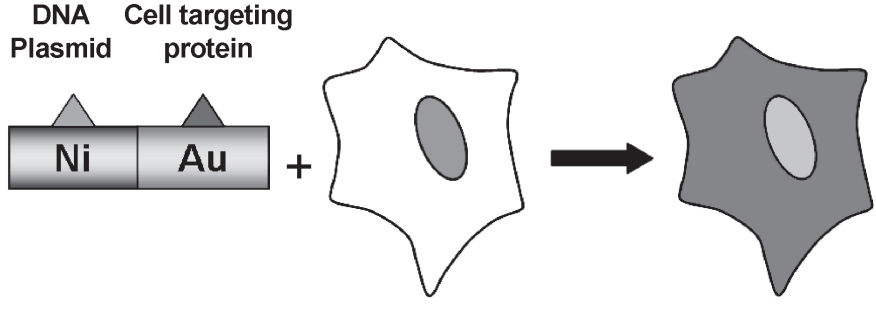

Figure 5. Schematic illustration of gene delivery using two-segment nanorods to carry GFP plasmid into the target cell.

Salem and co-workers also studied genetic immunization by particle bombardment of gold-nickel segment nanorods *in vivo*. Two-segment nanorods functionalized with the model antigen ovalbumin and DNA plasmid were delivered into mice via gene gun bombardment to stimulate the immune response. A strong antibody response was observed in the blood stream and a strong CD8+T-cell response detected in the

spleen. There was virtually no response of the CD8+T-cells when only DNA bound to segmented nanorods was delivered into the mice. In contrast, delivery of segmented nanorods with DNA and ovalbumin provided a strong CD8+T-cell response. The results suggested that further optimization of this process to deliver immuno-stimulants to cells might facilitate genetic immunization (Salem *et al.*, 2005).

The combination of photodynamic therapy along with traditional gene therapy is a novel approach that may prove useful as a technique for efficient delivery of genes into cells (Umeda *et al.*, 2005). Recent studies investigating the feasibility of this approach are yielding positive results. For example, Takahashi and co-workers (Takahashi *et al.*, 2005) have investigated the use of modified gold nanorods for releasing plasmid DNA. They used gold nanorods modified with phosphatidylcholine and conjugated with plasmid DNA, and exposed these complexes to a laser light in the NIR at different powers, while simultaneously subjecting the sample to gel electrophoresis. In the absence of laser irradiation all plasmid DNA remained in the well without any migration. This was evidently due to the formation of modified gold nanorod / DNA complexes. However, the electrophoretic migration of supercoiled DNA through the agarose gel was observed after irradiation of the gold nanorod-DNA complex at relatively high energies of pulsed-laser light (160, 180, and 200 mJ/pulse, *Figure 6*). Laser irradiation of this intensity was also found to have changed the shape of the nanoparticles from rod-like to spherical. Evidently, either the shape change or temperature excursion was responsible for freeing the DNA from gold nanorod-DNA complexes. A similar study using gold nanorods, this time targeting HeLa cells, was published by Chen and colleagues (Chen *et al.*, 2006). These studies also indicated that the release of plasmid DNA from the surface of gold nanorods can be obtained by the application of infrared irradiation.

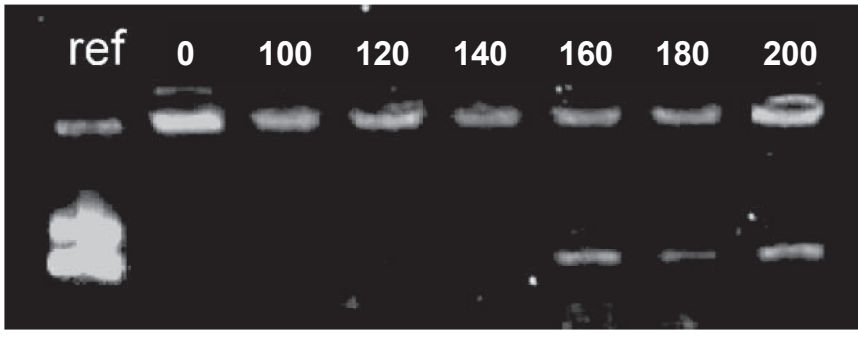

Figure 6. Gel electrophoretic patterns of gold nanorods DNA complexes with and without laser irradiation. The laser intensities used were 100, 120, 140, 160, 180, and 200 mJ/pulse. The first lane is a reference lane showing agarose gel electrophoresis of plasmid DNA. The second lane is gold nanorod DNA complexes without a laser. Reprinted with permission from *Chemical Communications* (Takahashi *et al.*, 2005).

GOLD NANORODS IN PHOTOTHERMAL THERAPY

Application of heat to inhibit or destroy specific cells is a well-known concept for the treatment of cancer and other conditions. Generally, a non-invasive method to destroy tumour cells by heat is referred to as thermotherapy or hyperthermia (Zee, 2002). The increase in temperature in the target zone may be generated with a variety

of heating sources, including infrared lamps, ultrasound, radio frequency sources and lasers. (Jolesz and Hynynen, 2002; Mirza *et al.*, 2001; Philipp *et al.*, 1995; Seki *et al.*, 1999). The processes mentioned, however, have the problem of not limiting the heat generated to a defined and specific area of target tissue (Jolesz and Hynynen, 2002). In photodynamic therapy greater specificity is obtained by ensuring that the target tissue, for example a tumour, is infiltrated with a photoabsorbing dye, and then laser irradiation is used to illuminate it. Unfortunately, the residual dye molecules can migrate to normal tissue such as skin too, causing side effects such as photo-sensitivity in patients (El-Sayed *et al.*, 2006; Salata, 2004). Gold nanoparticles offer a potential alternative to photoabsorbing dyes, with much of the current interest traceable to a series of ground-breaking publications and patents by the Halas group on the use of gold nanoshells, e.g.(Hirsch *et al.*, 2003). We have previously reviewed the state-of-the-art with respect to gold spheres and shells (Pissuwan *et al.*, 2006). Since then, however, a number of publications describing the potential use of gold nanorods in photothermal therapeutic applications have appeared and these studies now seem to outnumber those based on gold nanoshells.

As mentioned earlier, gold nanorods have properties which make them very attractive candidates for photothermal therapy. Although spherical gold nanoparticles do exhibit plasmon resonance, they have several limitations: not only is the efficiency of heating comparatively low (compared to nanorods or nanoshells) (Zharov and Galitovsky, 2003) but the wavelength at which the resonance mode occurs is in the mid-visible and therefore outside of the 'tissue window'. These limitations can be overcome by the use of gold nanorods since these are more efficient converters of light to thermal energy (Chou *et al.*, 2005; El-Sayed *et al.*, 2006) and of course the wavelength at which the plasmonic heating is a maximum can be 'tuned' to the 'tissue window' (O'Neal *et al.*, 2004; Weissleder, 2001). Furthermore, the longitudinal resonance of gold nanorods exhibits a narrower plasmon absorption bandwidth than any other known shape of nanoparticle (Link and El-Sayed, 2000). In combination, these properties should decrease the damage to healthy cells during photothermal therapy. These principles have recently been put to the test in the form of *in vitro* studies in which the nanorods have been functionalized with specific targeting molecules, attached to target cells, and then irradiated.

For example, Huff and co-workers conjugated folate ligands with oligoethyleneglycol onto gold nanorods by *in situ* dithiocarbamate (DTC) formation (Huff *et al.*, 2007b). The folate conjugated gold nanorods were selectively bound to KB cancer cells (a tumor cell line derived from oral epithelium) which led to photothermal damage on cell membranes following laser irradiation. Another study from the same group showed that membrane blebbing that occurred as a result of the irradiation is due to the influx of Ca^{2+} into the cell (Tong *et al.*, 2007). Huang *et al.* (Huang *et al.*, 2006) have also recently described the photothermal destruction of cancer cells using biofunctionalized gold nanorods. The nanorods were conjugated to anti-epidermal growth factor receptor (anti-EGFR); the specific antibody to the malignant cell types used, and then incubated with a nonmalignant epithelial cell line (HaCat) and two malignant oral epithelial cell lines (HOC313 clone8 and HSC3). Following laser irradiation, malignant cells were destroyed at about half the laser fluence needed to kill the nonmalignant cells. The efficient destruction of the malignant cells was evidently due to the preferential attachment of the anti-EGFR-gold nanorod conjugates to the over-expressed EGFR on the surface of the malignant cell.

Not only cancer cells can be destroyed in this manner. We have previously described (Pissuwan *et al.*, 2007b) how a specific macrophage cell line could be selectively targeted by conjugating gold nanorods with the CD11b antibody, followed by their destruction using laser irradiation. In a more recent paper by our group (Pissuwan *et al.*, 2007a), gold nanorods were conjugated with a specific antibody against the pathogenic parasite *Toxoplasma gondii*. Following laser exposure, it was shown that about 80% of the organism's tachyzoites were destroyed at a laser power density of ~51 W/m^2.

These exercises indicate that there is considerable scope for the use of selectively attached and plasmonically heated gold nanorods in therapeutic applications.

Cytotoxicity of gold nanorods

SURFACE OF GOLD NANORODS

As we have shown, there are a number of potential applications for gold nanorods in the biomedical arena. However, it is important to consider the possible biocompatibility of gold particles too. Normally, gold has two oxidation states as Au^{I+} and Au^{III+}. Gold Au^{I+} associates with 'soft' ligands, for example sulphur donor ligands (Colacio *et al.*, 1996) and gold Au^{III+} prefers to complex with 'hard' ligands such as oxygen or nitrogen donors (Pearson, 1963). Both Au^{I+} and Au^{III+} can be easily reduced to form Au^0, often in a nanoscale colloidal form. There have been reviews on the biological activity of gold Au^I and Au^{III} compounds (Merchant, 1998), and some of them are quite useful in a medical sense, with gold(I) thiolates, for example, serving with some success to mitigate arthritis (Shaw, 1999). However, due to its chemical nobility, it is generally expected that particles of Au^0 are innocuous when in living organisms. For example, Shukla and co-workers suggested that colloidal gold nanospheres do not show any cytotoxic effect up to 100μM concentration, even after incubation for up to 72 hours with RAW264.7 macrophage cells (Shukla *et al.*, 2005). There are at present only a few other publications considering the possible cytotoxicity of gold nanoparticles, and even fewer that specifically address gold nanorods.

Nevertheless, the production of gold nanorods requires the use of hexadecyltrimethylammonium bromide (CTAB); a cationic surfactant that is also known to be a detergent suitable for the degradation of biomembranes and peptides. The CTAB is a stabilizing agent for the rods (Niidome and Niidome, 2006; Nikoobakht and El-Sayed, 2001) and is evidently present after synthesis, both in the supernatant, and as a bi-layer on the flanks of the rods themselves. Free CTAB is certainly toxic to human cells; but it can largely be removed from preparation of gold nanorods by double centrifugal washing of the suspensions with Milli Q water. The residual CTAB bi-layer bound to surface of the gold nanorods is evidently not particularly toxic to those cells types tested so far (*e.g.* the K562 leukemia cell line, (Connor *et al.*, 2005; Cortesi *et al.*, 1996), RAW 264.7 murine macrophage cells (Pissuwan *et al.*, 2007b) or the tachyzoites of the parasite *Toxoplasma gondii* (Pissuwan *et al.*, 2007a)), but there is evidently still some statistically important reduction of cell viability due to the nanorods, particularly if at higher concentration (Takahashi *et al.*, 2006).

Clearly, some process to remove at least the excess CTAB from suspensions of gold nanorods is essential in order to produce biocompatible gold nanorods, but removal of

all the CTAB would be even better. This is because the CTAB bi-layers on a typical nanorod surface are certainly not statically bound, so there will always be a degree of desorption of CTAB molecules into the surrounding medium. Unfortunately, removal of all bound CTAB, in the absence of the substitution of some other stabilizing agent, is undesirable as it will cause aggregation of the nanorods (Niidome and Niidome, 2006). Therefore, to prevent the risk of toxicity from CTAB and to prevent aggregation of the nanorods, some groups have exchanged it, after synthesis of the rods, for a more innocuous stabilizing molecule. For example, chloroform extraction has been used to remove the bound CTAB and replace it with phosphatidylcholine, a common phospholipid contained in mainly biomembranes (Niidome *et al.*, 2007; Takahashi *et al.*, 2006). Incubation of live cells with these modified nanorods was associated with a higher level of cell viability compared with unmodified gold nanorods that had been only been centrifuged two times to remove excess CTAB (Takahashi *et al.*, 2006). Nanorods can be further functionalized with bovine serum albumin (BSA) and polyethylenimine by the 'layer-by-layer' technique. In this case they have proven to be proficient and non-toxic transfection vectors into HeLa cells (Niidome *et al.*, 2007). This could become the basis of a new and safer method of gene delivery in the future.

Polyethyleneglycol (PEG); a hydrophilic polymer, has also been used to modify the surface of gold nanorods. PEG is a biocompatible polymer frequently used in drug and DNA delivery (Liu *et al.*, 1999; Luo *et al.*, 2002), and surface modification of gold nanorods with it also provides a degree of biocompatibility. Naturally, antibodies can be added on top of the PEG layer too (Liao and Hafner, 2005). A detailed assessment of PEG-modified gold nanorods was recently provided by Niidome and co-workers (Niidome *et al.*, 2006). The nanorods were coated by mixing the original CTAB-stabilized particles with thiol-terminated methoxy-poly(ethylene glycol): mPEG-SH. There was no aggregation after the treatment, even though the modification had changed the surface charge from a cationic surface to almost neutral. The cytotoxicity of such PEG-modified gold nanorods was checked using HeLa cells. Cell viability for the test conditions used was around 90% in the presence of 0.05 mM Au of PEG-coated nanorods and only about 20% for CTAB-stabilized rods. These results confirm that elimination of excess CTAB and modification with PEG will remove any residual cytotoxicity of gold nanorods.

Another way to modify gold nanorods involves the application of PSS polyelectrolyte; a novel, non-cytotoxic antimicrobial contraceptive agent. This converts the surface charge of the nanorod from positive to negative (Durr *et al.*, 2007; Garg *et al.*, 2005). PSS coated gold nanorods can be conjugated with antibodies without any aggregation and are expected to be biocompatible (Caruso *et al.*, 1997; Huang *et al.*, 2007b). The antibodies are adsorbed on PSS layer by non-specific interactions (Huang *et al.*, 2007b). A schematic diagram illustrating the coating of gold nanorods with PSS and antibodies is shown in *Figure 7*.

CELLULAR UPTAKE OF GOLD NANORODS

Cellular uptake of gold nanoparticles is another issue that should be considered when considering their use in diagnostic and therapeutic applications. In general, nanoparticles can go deeper into tissue than larger particles and often penetrate the

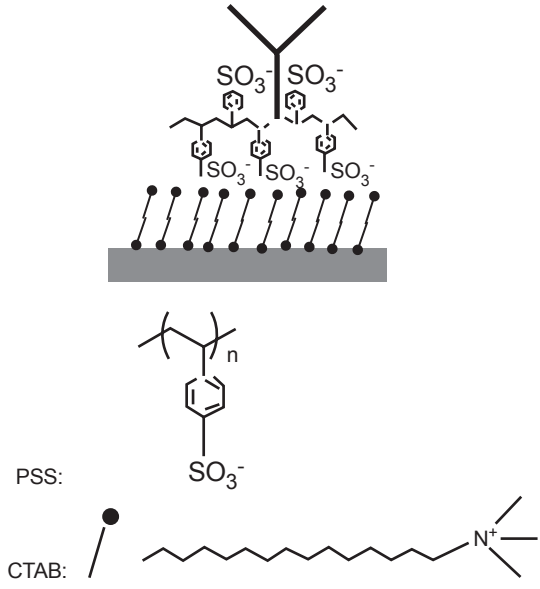

Figure 7. Schematic illustration of the bioconjugation of PSS coated gold nanorods with antibodies. Reprinted with permission from *Lasers in Medical Science* (Huang *et al.*, 2007b).

cell itself (Leslie-Pelecky, 2007). The reader is referred to a recent review, which summarises the situation for many types of nanoparticle, including those of gold (Lewinski *et al.*, 2008). In general, gold nanoparticles, including rods, enter cells by a non-specific process of endocytosis and concentrate in endosomes. The available data indicates that they do not cross over into the nucleus unless they are specifically functionalised to do so. The size and shape of the particle certainly have an influence on the outcome. For example, Chithrani *et al.* (Chithrani *et al.*, 2006) studied the effects of size, shape, concentration, and incubation time on kinetics of uptake of nanorods and nanospheres of different sizes into mammalian (HeLa) cells. Curiously, the fastest uptake in the case of spheres was for comparatively large particles of 50 nm diameter. Actually, a similar conclusion was drawn by Osaki *et al.* (Osaki *et al.*, 2004) for semiconductor nanoparticles, where once again it was the larger particles that more readily entered cells by receptor-mediated endocytosis. Intracellular uptake of gold nanoparticles into cells has been confirmed by imaging in a TEM. This showed that gold nanoparticles in the range of 14 to 100 nm were located inside cells and trapped in vesicles inside the cytoplasm, and external to the nucleus. Furthermore, the balance of evidence so far is that there is no cytotoxicity associated with the presence of the gold nanoparticles. It is possible that the uptake of the gold nanoparticles might have been facilitated by the non-specific absorption of serum proteins from the culture medium, which might have stimulated receptor-mediated endocytosis (Chithrani *et al.*, 2006; Huff *et al.*, 2007a). Some studies (Chithrani *et al.*, 2006) have suggested that gold nanospheres are more readily taken up than rods, but of course the difference may simply be due to the differing surface chemistries of the two forms. For example, CTAB-stabilized rods are positively charged, while citrate-stabilized nanospheres are negatively charged. This might also influence the degree of aggregation of the nanoparticles during experiments (Chithrani *et al.*, 2006; Limbach *et al.*, 2005). As

far as nanorods of different aspect ratios are concerned, cellular uptake of the low aspect ratio (shorter) rods is higher than of longer rods. The reason could be that the high aspect ratio gold nanorods have a larger contact area with the cell membrane receptors than the small aspect ratio gold nanorods and spherical gold nanoparticles at similar size, however it seems likely that the various factors that control endocytosis of gold nanorods are not yet fully understood.

Recently, a comparison has been published (Huff *et al.*, 2007a) of the cellular uptake of gold nanorods as a function of surface modification. The uptake of CTAB-coated nanorods, CTAB-coated rods modified with methylated poly(ethyleneglycol) conjugated dithiocarbamate (mPEEG-DTC) and gold nanorods coated with bis(*p*-sulfonatophenyl) phenyphosphine (BSP) in KB cells was assessed. The cells incubated with CTAB-coated gold nanorods grew to confluence over 5-day period, demonstrating that CTAB-coated gold had little or no effect on the growth of KB cells. The excretion of CTAB-coated gold nanorods by the KB cells could not be detected; however, some gold nanorods were compartmentalized inside cells in an aggregated form. On the other hand, the extent of non-specific uptake of the CTAB-coated gold nanorods was about 20 times is higher than for the mPEG-DTC-coated gold nanorods. The accumulation of gold nanorods inside cells has both positive and negative outcomes for biomedical applications. The positive outcome is that they can serve as multifunctional imaging and therapeutic agents for specific targeted cells (Huang *et al.*, 2006; Pissuwan *et al.*, 2007b). However, there is also a potential problem in so far as non-specific uptake of gold nanorods might cause interference. Therefore, it appears important to avoid non-specific uptake and accumulation of gold nanorods. The surface coating of gold nanorods by mPEG chains and dithiocarbamate formation is one effective example of a technology to prevent this.

Biodistribution of gold nanorods

It is obviously useful to develop an understanding of where and how gold nanorods distribute themselves when they are introduced into a living system. As we have already shown, this is certainly going to be influenced by the nature of any surface modification. Some recent studies by Niidome *et al*. (Niidome *et al.*, 2006) (Niidome *et al.*, 2007) have looked at this in greater detail. Both CTAB-stabilized and PEG-treated nanorods were introduced into mice by intravenous injection and their distribution studied. About a third of the ordinary nanorods were rapidly concentrated in the animal's liver, but PEG-treated rods stayed in the bloodstream for far longer (54% still present after 0.5 hours) before a third finally reported to the liver. A small amount of PEG-coated gold was found in the lung, spleen and kidney. However, the fate of the remaining two thirds or so of the gold was not identified in this study. In any event, these results show gold nanorods can be hidden to some extent from a mammal body's normal processes for sequestrating foreign materials, a process which these and other authors have described as imparting a 'stealth' character to the particles. The use of 'stealthy' gold nanorods, modified with ligands such as antibodies, has been identified as being a promising basis for the development of new targeted delivery systems (Niidome *et al.*, 2006; Visaria *et al.*, 2006).

Conclusion

Gold nanorods have a variety of real or potential applications in medical diagnosis and therapeutic treatments as a result of their particularly attractive combination of optical, physical and chemical properties. These applications include optical microscopy, optoacoustic imaging, immuno-assaying, gene therapy and hyperthermia. Specificity of effect and selective targeting can be achieved, generally by functionalization of the gold nanorods with a biomolecule. In principle, for example, antibody-conjugated gold nanorods can be used to target specific cell lines, or even invading organisms, either for diagnostic of therapeutic purposes. Nevertheless, these are early days and continued exploration of the field, and especially the completion of more *in vivo* clinical trials, is required.

References

Alekseeva, A.V., Bogatyrev, V.A., Dykman, L.A. *ET AL.* (2005). Preparation and optical scattering characterization of gold nanorods and their application to a dot-immunogold assay. *Applied Optics* **44**, 6285-95.

Andreev, V.G., Karabutov, A.A. and Oraevsky, A.A. (2003). Detection of ultrawide-band ultrasound pulses in optoacoustic tomography. *IEEE: Transactions on Ultrasonics Ferroelectrics and Frequency Control* **50**, 1383-90.

Caruso, F., Niikura, K., Furlong, D.N. and Okahata, Y. (1997). Assembly of alternating polyelectrolyte and protein multilayer films for immunosensing. *Langmuir* **13**, 3427-33.

Cerussi, A.E., Berger, A.J., Bevilacqua, F. *ET AL.* (2001). Sources of absorption and scattering contrast for near-infrared optical mammography. *Academic Radiology* **8**, 211-8.

Chan, W.C.W. and Nie, S. (1998). Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* **281**, 2016-8.

Chen, C.-C., Lin, Y.-P., Wang, C.-W. *ET AL.* (2006). DNA-gold nanorod conjugates for remote control of localized gene expression by near infrared irradiation. *Journal of the American Chemical Society* **128**, 3709-15.

Chen, C.-D., Cheng, S.-F., Chau, L.-K. and Wang, C.R.C. (2007). Sensing capability of the localized surface plasmon resonance of gold nanorods. *Biosensors and Bioelectronics* **22**, 926-32.

Chithrani, B.D., Ghazani, A.A. and Chan, W.C.W. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Letters* **6**, 662-8.

Chou, C.-H., Chen, C.-D. and Wang, C. (2005). Highly efficient, wavelength-tunable, gold nanoparticle based optothermal nanoconvertors *Journal of Physical Chemistry B* **109**, 11135-8.

Colacio, E., Cuesta, R., Gutiérrez-Zorrilla, J.M. *ET AL.* (1996). Gold(I)-purine interactions: synthesis and characterization of cyclic and open chain polynuclear gold(I) complexes containing xanthine derivatives and bis(phosphine) as bridging ligands. Crystal structures of $[Au_2(\mu\text{-}HX)(\mu\text{-}dmpe)].3H_2O$ and $[Au_2(\mu\text{-}TT)(\mu\text{-}dmpe)].H_2O$ (H_3X = xanthine; H_2TT) 8-mercaptotheophylline). *Inorganic Chemistry* **35**, 4232-8.

Connor, E.E., Mwamuka, J., Gole, A., Murphy, C.J. and Wyat, M.D. (2005). Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* **1**, 325-7.

Copland, J.A., Eghtedari, M., Popov, V.L. *ET AL.* (2004). Bioconjugated gold nanoparticles as a molecular based contrast agent: implications for imaging of deep tumors using optoacoustic tomography. *Molecular Imaging and Biology* **6**, 341-9.

Cortesi, R., Esposito, E., Menegatti, E., Gambari, R. and Nastruzzi, C. (1996). Effect of cationic liposome composition on *in vitro* cytotoxicity and protective effect on carried DNA. *International Journal of Pharmaceutics* **139**, 69-78.

Crystal, R.G. (1995). Transfer of genes to humans: early lesson and obstacles to success. *Science* **270**, 404-10.

Daniel, M.-C. and Astruc, D. (2004). Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical Reviews* **104**, 293-346.

Dietricha, H.R.C., Younga, I.T. and Garinia, Y. (2005). Gold nanoparticles: a novel application of spectral imaging in proteomics-preliminary results. *Proceedings of SPIE (Spectral Imaging: Instrumentation, Applications, and Analysis III)* **5694**, 82-9.

Durr, N.J., Larson, T., Smith, D.K. *ET AL.* (2007). Two-photon luminescence imaging of cancer cells using molecularly targeted gold nanorods. *Nano Letters* **7**, 941-5.

Eghtedari, M., Oraevsky, A., Copland, J.A. *ET AL.* (2007). High sensitivity of *in vivo* detection of gold nanorods using a laser optoacoustic imaging system. *Nano Letters* **7**, 1914-8.

El-Sayed, I.H., Huang, X. and El-Sayed, M.A. (2006). Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles. *Cancer Letters* **239**, 129-35.

Garg, S., Vermani, K., Garg, A. *ET AL.* (2005). Development and characterization of bioadhesive vaginal films of sodium polystyrene sulfonate (PSS), a novel contraceptive antimicrobial agent. *Pharmaceutical Research* **22**, 584-95.

Haisch, C. and Niessner, R. (2002). Light and sound photoacoustic spectroscopy. *Spectroscopy Europe* **14**, 10-5.

Hardman, R. (2006). A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environmental Health Perspectives* **114**, 165-72.

Harris, N., Cortie, M.B., Ford, M.J. and Mulvaney, P. (2008). Plasmon absorption in gold nanoparticles: shells versus rods. *Gold Bulletin*, in press.

Hirsch, L.R., Stafford, R.J., Bankson, J.A. *ET AL.* (2003). Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 13549-54.

Huang, X., El-Sayed, I.H., Qian, W. and El-Sayed, M.A. (2006). Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *Journal of the American Chemical Society* **128**, 2115-20.

Huang, X., El-Sayed, I.H., Qian, W. and El-Sayed, M.A. (2007a). Cancer cells assemble and align gold nanorods conjugated to antibodies to produce highly enhanced, sharp, and polarized surface Raman spectra: a potential cancer diagnosis marker. *Nano Letters* **7**, 1591-7.

Huang, X., K.Jain, P., El-Sayed, I.H. and El-Sayed, M.A. (2007b). Plasmonic photothermal therapy (PPT) using gold nanoparticles. *Lasers in Medical Science*, DOI 10.1007/s10103-007-0470-x.

Huff, T.B., Hansen, M.N., Zhao, Y., Cheng, J.-X. and Wei, A. (2007a). Controlling the cellular uptake of gold nanorods. *Langmuir* **23**, 1596-9.

Huff, T.B., Tong, L., Zhao, Y. *ET AL.* (2007b). Hyperthermic effects of gold nanorods on tumor cells. *Nanomedicine* **2**, 125-32.

Jain, P.K., Lee, K.S., EL-Sayed, I.H. and EL-Sayed, M.A. (2006). Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *Journal of Physical Chemistry B* **110**, 7238-48.

Jana, N.R., Gearheart, L.A. and Murphy, C.J. (2001). Seed-mediated growth approach for shape-controlled synthesis of spheroidal and rod-like gold nanoparticles using a surfactant template. *Advanced Materials* **13**, 1389-93.

Jolesz, F.A. and Hynynen, K. (2002). Magnetic resonance image-guided focused ultrasound surgery. *Cancer Journal* **8**, S100-S12.

Kelly, K.L., Coronado, E., Zhao, L.L. and Schatz, G.C. (2003). The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. . *Journal of Physical Chemistry B* **107**, 668-77.

Kim, F., Song, J.H. and Yang, P. (2002). Photochemical synthesis of gold nanorods. *Journal of the American Chemical Society* **124**, 14316-7.

Kim, K., Huang, S.-W., Ashkenazi, S. *ET AL.* (2007). Photoacoustic imaging of early inflammatory response using gold nanorods. *Applied Physics Letters* **90**, DOI: 10.1063/1.2743752.

Leslie-Pelecky, D.l. (2007). Nanotoxicology. In *Biomedical Applications of Nanotechnology* eds. V. labhasetwar and D. L. leslie-Pelecky, pp 227-234. New Jersey: Wiley-Interscience.

Lewinski, N., Colvin, V. and Drezek, R. (2008). Cytotoxicity of nanoparticles. *Small* **1**, 26-49.

Li, C.-Z., Male, K.B., Hrapovic, S. and Luong, J.H.T. (2005). Fluorescence properties of gold nanorods and their application for DNA biosensing. *Chemical Communications*, 3924-6, DOI: 10.1039/b716113a.

Li, P.-C., Wei, C.-W., Liao, C.-K. *ET AL.* (2007). Photoacoustic imaging of multiple targets using gold nanorods. *IEEE: Transactions on Ultrasonics Ferroelectrics and Frequency Control* **54**, 1642-7.

Liao, H. and Hafner, J.H. (2005). Gold nanorod bioconjugates. *Chemistry of Materials* **17**, 4636-41.

Limbach, L.K., Li, Y.u., Grass, R.N. *ET AL.* (2005). Oxide nanoparticle uptake in human lung fibroblasts: effects of particle size, agglomeration, and diffusion at low concentrations. *Environmental Science and Technology* **39**, 9370-6.

Link, S. and El-Sayed, M.A. (2000). Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals. *International Reviews in Physical Chemistry* **19**, 409-53.

Liu, M., Kono, K. and Fre'Chet, J.M.J. (1999). Water-soluble dendrimer–poly(ethylene glycol) star like conjugates as potential drug carriers. *Journal of Polymer Science Part A: Polymer Chemistry* **37**, 3492-503.

Luo, D. and Saltzman, W.M. (2000). Synthetic DNA delivery systems. *Nature*

Biotechnology **18**, 33-7.

Luo, D., Haverstick, K., Belcheva, N., Han, E. and Saltzman, W.M. (2002). Poly(ethylene glycol)-conjugated PAMAM dendrimer for biocompatible, high-efficiency DNA delivery. *Macromolecules* **35**, 3456-62.

Mallidi, S., Larson, T., Aaron, J., Sokolov, K. and Emelianov, S. (2007). Molecular specific optoacoustic imaging with plasmonic nanoparticles. *Optics Express* **15**, 6583-8.

Mehier-Humberta, S. and Guy, R.H. (2005). Physical methods for gene transfer improving the kinetics of gene delivery into cells. *Advanced Drug Delivery Reviews* **57**, 733-53.

Merchant, B. (1998). Gold, the noble metal and the paradoxes of its toxicology. *Biologicals* **26**, 49-59.

Mirza, A.N., Fornage, B.D., Sneige, N. and Kuerer, H.M. (2001). Radio frequency ablation of solid tumors. *The Cancer Journal* **7**, 95-102.

Murphy, C.J., Sau, T.K., Gole, A.M. *ET AL.* (2005). Anisotropic metal nanoparticles: synthesis, assembly, and optical applications. *Journal of Physical Chemistry B* **109**, 13857-70.

Nath, N. and Chilkoti, A. (2002). A colorimetric gold nanoparticle sensor to interrogate biomolecular interactions in real time on a surface. *Analytical Chemistry* **74**, 504-9.

Niidome, T., Yamagata, M., Okamoto, Y. *ET AL.* (2006). PEG-modified gold nanorods with a stealth character for *in vivo* applications. *Journal of Controlled Release* **114**, 343-7.

Niidome, Y. and Niidome, T. (2006). Gold nanorods: application to bioscience and medicine. In *Nano Biophotonics* eds.H. Masuhara, S. Kawata and F. Tunaka, pp 297-307. Oxford: Elsevier.

Niidome, Y., Honda, K., Higashimoto, K. *ET AL.* (2007). Surface modification of gold nanorods with synthetic cationic lipids. *Chemical Communications*, 3777-9, DOI: 10.1039/b706671f.

Nikoobakht, B. and El-Sayed, M.A. (2001). Evidence for bilayer assembly of cationic surfactants on the surface of gold nanorods. *Langmuir* **17**, 6368-74.

O'Neal, D.P., Hirsch, L.R., Halas, N.J., Payne, J.D. and West, J.L. (2004). Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles. *Cancer Letters* **209**, 171-6.

Osaki, F., Kanamori, T., Sando, S., Sera, T. and Aoyama, Y. (2004). A quantum dot conjugated sugar ball and its cellular uptake on the size effects of endocytosis in the subviral region. *Journal of the American Chemical Society* **126**, 6250-1.

Oyelere, A.K., Chen, P.C., Huang, X., El-Sayed, I.H. and El-Sayed, M.A. (2007). Peptide-conjugated gold nanorods for nuclear targeting. *Bioconjugate Chemistry* **18**, 1490-7.

Pearson, R.G. (1963). Physical and inorganic chemistry: hard and soft acids and bases. *Journal of the American Chemical Society* **85**, 3533-9.

Perez-Juste, J., Pastoriza-Santos, I., Liz-Marzan, L.M. and Mulvaney, P. (2005). Gold nanorods: synthesis, characterization and applications. *Coordination Chemistry Reviews* **249**, 1870-901.

Perez-Juste, J., Liz-Marzan, L.M., Carnie, S., Chan, D.Y.C. and Mulvaney, P. (2004). Electric-field-directed growth of gold nanorods in aqueous surfactant solutions.

Advanced Functional Materials **14**, 571-9.

Philipp, C.M., Rohde, E. and Berlien, H.-P. (1995). Nd:YAG Laser procedures in tumor treatment. *Seminars in Surgical Oncology* **11**, 290-8.

Pissuwan, D., Valenzuela, S.M. and Cortie, M.B. (2006). Therapeutic possibilities of plasmonically heated gold nanoparticles. *Trends in Biotechnology* **24**, 62-7.

Pissuwan, D., Valenzuela, S.M., Miller, C.M. and Cortie, M.B. (2007a). A golden bullet? Selective targeting of *Toxoplasma gondii* tachyzoites using antibody-functionalized gold nanorods. *Nano Letters* **7**, 3808-12.

Pissuwan, D., Valenzuela, S.M., Killingsworth, M.C., Xu, X. and Cortie, M.B. (2007b). Targeted destruction of murine macrophage cells with bioconjugated gold nanorods. *Journal of Nanoparticle Research* **9**, 1109-24.

Rabussay, D., Dev, N.B., Fewell, J. *ET AL.* (2003). Enhancement of therapeutic drug and DNA delivery into cells by electroporation. *Journal of Physics D: Applied Physics* **36**, 348-63.

Rayavarapu, R.G., Petersen, W., Ungureanu, C. *ET AL.* (2007). Synthesis and bioconjugation of gold nanoparticles as potential molecular probes for light-based imaging techniques. *International Journal of Biomedical Imaging* **2007**, doi:10.1155/2007/29817.

Richards-Kortum, R. and Sevick-Muraca, E. (1996). Quantitative optical spectroscopy for tissue quantitative optical spectroscopy for tissue diagnosis. *Annual Review of Physical Chemistry* **47**, 555-606.

Roy, K., Mao, H.-Q., huang, S.-K. and Leong, K.W. (1999). Oral gene delivery with chitosan–DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nature Medicine* **5**, 387-91.

Salata, O. (2004). Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology* **2**, Paper 3.

Salem, A.K., Searson, P.C. and Leong, K.W. (2003). Multifunctional nanorods for gene delivery. *Nature Materials* **2**, 668-71.

Salem, A.K., Hung, C.F., Kim, T.W. *ET AL.* (2005). Multi-component nanorods for vaccination applications. *Nanotechnology* **16**, 484-7.

Salomatina, E., Jiang, B., Novak, J. and Yaroslavsky, A.N. (2006). Optical properties of normal and cancerous human skin in the visible and near-infrared spectral range. *Journal of Biomedical Optics* **11**, 064026-9.

Seki, T., Wakabayashi, M., Nakagawa, T. *ET AL.* (1999). Percutaneous microwave coagulation therapy for patients with small hepatocellular carcinoma: comparison with percutaneous ethanol injection therapy. *Cancer* **85**, 1694-702.

Shaw, C.F. (1999). Gold-based therapeutic agents. *Chemical Reviews* **99**, 2589-600.

Shukla, R., Bansal, V., Chaudhary, M. *ET AL.* (2005). Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: a microscopic overview. *Langmuir* **21**, 10644-54.

Simpson, C.R., Kohl, M., Essenpreis, M. and Copey, M. (1998). Near-infrared optical properties of ex vivo human skin and subcutaneous tissues measured using the Monte Carlo inversion technique. *Physics in Medicine and Biology* **43**, 2465-78.

Skrabalak, S.E., Chen, J., Au, L. *ET AL.* (2007). Gold nanocages for biomedical applications. *Advanced Materials* **19**, 3177-84.

Takahashi, H., Niidome, Y. and Yamada, S. (2005). Controlled release of plasmid DNA from gold nanorods induced by pulsed near-infrared light. *Chemical Communications*,

2247-9, DOI: 10.1039/b500337g.

Takahashi, H., Niidome, Y., Niidome, T. *ET AL.* (2006). Modification of gold nanorods using phosphatidylcholine to reduce cytotoxicity. *Langmuir* **22**, 2-5.

Tong, L., Zhao, Y., Huff, T.B. *ET AL.* (2007). Gold nanorods mediate tumor cell death by compromising membrane integrity. *Advanced Materials* **19**, 3136-41.

Umeda, M., Harada-Shiba, M., Uchida, K. and Nakayama, Y. (2005). Photo-control of the polyplexes formation between DNA and photo-cation generatable water-soluble polymers. *Current Drug Delivery* **2**, 207-14.

Visaria, R.K., Griffin, R.J., Williams, B.W. *ET AL.* (2006). Enhancement of tumor thermal therapy using gold nanoparticle–assisted tumor necrosis factor-A delivery. *Molecular Cancer Therapeutics* **5**, 1014-20.

Wang, H., Huff, T.B., Zweifel, D.A. *ET AL.* (2005). *In vitro* and *in vivo* two-photon luminescence imaging of single gold nanorods. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 15752-6.

Weissleder, R. (2001). A clearer vision for *in vivo* imaging. *Nature Biotechnology* **19**, 316–7.

Wu, C., Liang, X. and Jiang, H. (2005). Metal nanoshells as a contrast agent in near-infrared diffuse optical tomography. *Optics Communications* **253**, 214-21.

Xu, X. and Cortie, M.B. (2006). Shape change and color gamut in gold nanorods, dumbbells and dog-bones. *Advanced Functional Materials* **16**, 2170-6.

Yang, N.S. and Sun, W.H. (1995). Gene gun and other nonviral approaches for cancer gene-therapy. *Nature Medicine* **1**, 481-3.

Yguerabide, J. and Yguerabide, E.E. (1998). Light-scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications. *Analytical Biochemistry* **262**, 137-56.

Yu, C. and Irudayaraj, J. (2007). Multiplex biosensor using gold nanorods. *Analytical Chemistry* **79**, 572-9.

Zee, J.V.D. (2002). Heating the patient: a promising approach? *Annals of Oncology* **13**, 1173-84.

Zhang, X. and Godbey, W.T. (2006). Viral vectors for gene delivery in tissue engineering. *Advanced Drug Delivery Reviews* **58**, 515-34.

Zharov, V.P. and Galitovsky, V. (2003). Photothermal detection of local thermal effects during selective nanophotothermolysis. *Applied Physics Letters* **83**, 4897-9.

Zharov, V.P., Galanzha, E.I. and Tuchin, V.V. (2006). *In vivo* photothermal flow cytometry: imaging and detection of individual cells in blood and lymph flow. *Journal of Cellular Biochemistry* **97**, 916-32.