Emerging use of nanoparticles in diagnosis and treatment of breast cancer

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The biological application of nanoparticles is a rapidly developing area of nanotechnology that raises new possibilities in the diagnosis and treatment of human cancers. In cancer diagnostics, fluorescent nanoparticles can be used for multiplex simultaneous profiling of tumour biomarkers and for detection of multiple genes and matrix RNA with fluorescent in-situ hybridisation. In breast cancer, three crucial biomarkers can be detected and accurately quantified in single tumour sections by use of nanoparticles conjugated to antibodies. In the near future, the use of conjugated nanoparticles will allow at least ten cancer-related proteins to be detected on tiny tumour sections, providing a new method of analysing the proteome of an individual tumour. Supermagnetic nanoparticles have exciting possibilities as contrast agents for cancer detection in vivo, and for monitoring the response to treatment. Several chemotherapy agents are available as nanoparticle formulations, and have at least equivalent efficacy and fewer toxic effects compared with conventional formulations. Ultimately, the use of nanoparticles will allow simultaneous tumour targeting and drug delivery in a unique manner. In this review, we give an overview of the use of clinically applicable nanoparticles in oncology, with particular focus on the diagnosis and treatment of breast cancer.

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Introduction

Nanobiotechnology, defined as biomedical applications of nano-sized systems, is a rapidly developing area within nanotechnology. Nanomaterials, which measure 1–1000 nm, allow unique interaction with biological systems at the molecular level. They can also facilitate important advances in detection, diagnosis, and treatment of human cancers and have led to a new discipline of nano-oncology.[1,2] Nanoparticles are being actively developed for tumour imaging in vivo, biomolecular profiling of cancer biomarkers, and targeted drug delivery. These nanotechnology-based techniques can be applied widely in the management of different malignant diseases.

Some breast cancers express protein biomarkers (eg, oestrogen receptor, progesterone receptor, and ERBB2) on which therapeutic decisions are made. Semiconductor fluorescent nanocrystals, such as quantum dots, have been conjugated to antibodies, allowing for simultaneous labelling and accurate quantification of these target proteins in one breast tumour section (figure 1).[3] The use of nanoparticles—not only quantum dots of different sizes and emission spectra, but also gold-containing nanoparticles (ie, Raman probes)—will allow the simultaneous detection and quantification of several proteins on small tumour samples, which will ultimately allow the tailoring of specific anticancer treatment to an individual patient's specific tumour protein profile.[4] The ability to detect molecular targets simultaneously on individual tumour samples could allow correlation between gene products and proteins in real time.[5] In addition, the effects of an individual treatment on expression of the target protein can be monitored before and after treatment, and provide a rapid method to measure the efficacy of a targeted therapy.

Nanotechnological approaches (eg, nanocantilevers and nanoprobes) are being actively investigated in cancer imaging.[6] Nanoparticles coupled with cancer-specific targeting ligands can be used to image tumours and detect peripheral metastases.[7] Supermagnetic nanoparticles that have a metal core and are bioconjugated with antibodies against ERBB2 have shown promising results for simultaneous imaging and targeting of breast cancers therapeutically in vivo.[8] Moreover, nanoparticles conjugated to cancer-specific ligands could be used in early identification of tumours, allowing early intervention with a chemopreventive agent.

Several nanotechnological approaches have been used to improve delivery of chemotherapeutic agents to cancer cells with the goal of minimising toxic effects on healthy tissues while maintaining antitumour efficacy.

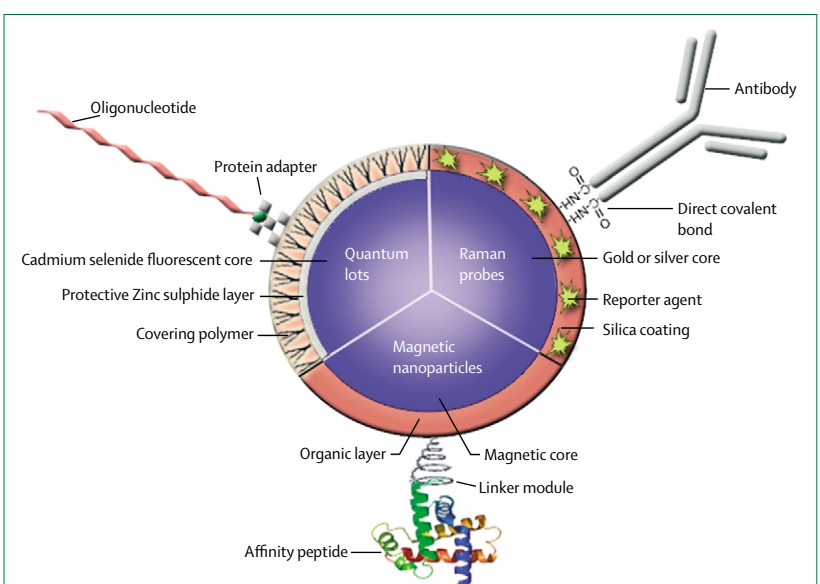

Figure 1: **Basic structure of inorganic nanoparticles**

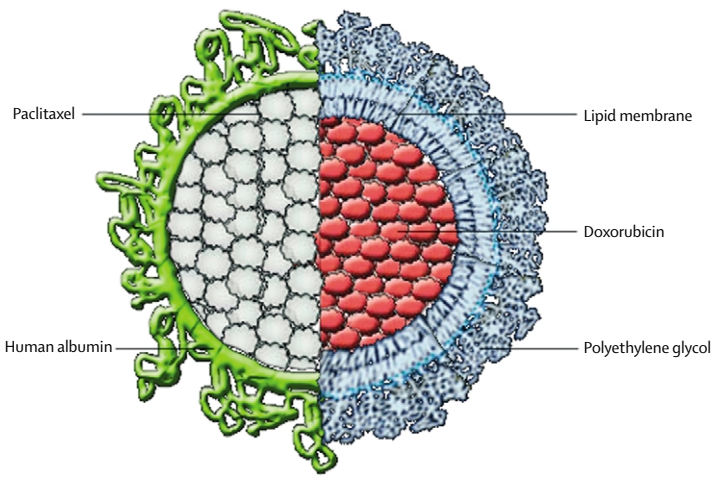

Figure 2: **Basic structure of organic nanoparticles**

Doxorubicin has been formulated with a liposome delivery system into nanoparticle size (figure 2), which maintains the efficacy of the drug and decreases cardiac toxic effects.[9,10] One of these delivery systems, pegylated liposomal doxorubicin, is approved for treatment of refractory ovarian cancer and Kaposi's sarcoma in the USA. Nanoparticle albumin-bound (NAB) paclitaxel also has greater efficacy than conventional castor-oil-based paclitaxel with an improved safety profile,[11,12] and is approved in the USA for treatment of metastatic breast cancer.

The use of nanotechnology in cancer encompasses many nanotechnological approaches, and it would be impossible to cover these in a single review. We have therefore focused this review on the emerging use of nanoparticles in breast cancer.

Types of biomedical nanoparticles

Although the number of different types of nanoparticles is increasing rapidly, most can be classified into two major types: particles that contain organic molecules as a major building material (figure 1) and those that use inorganic elements, usually metals, as a core (figure 2). Liposomes, dendrimers, carbon nanotubes, emulsions, and other polymers are a large and well-established group of organic particles. Use of these organic nanoparticles has already produced exciting results.[13–19] Liposomes are being used as vehicles for drug delivery in different human tumours, including breast cancer.[13,14] Dendrimers, used in MRI as contrast agents, have aided visualisation of various pathological processes.[15,16] Conjugated with pharmacological agents and targeting molecules, organic nanovectors are potent vehicles for drug delivery and selective imaging of different human cancers.[15–19] The structure, function, and biomedical applications of these organic nanoparticles have been reviewed (table).[13–19]

	Structure	Applications	Ref
Liposomes	Self-assembled closed colloid structures composed of lipid layers	Drug delivery: anthracyclines, taxanes, vinca alkaloids, platinums, camphothecins; immunoliposomes: antiERBB2 conjugates	13,14
Dendrimers	Globular macromolecules for which all bonds emerge radially from a central focal point with regular branching pattern and repeated units	Drug delivery: fluorouracil, methotrexate, doxorubicin, oestrogen; MRI; gene delivery	15,16
Carbon nanoparticles	Carbon-containing nanotubes	Drug delivery; sentinel-node visualisation	17,18

Table: **Clinical applications of organic nanoparticles in breast cancer**

Most inorganic nanoparticles share the same basic structure—a central core that defines the fluorescence, optical, magnetic, and electronic properties of the particle, with a protective organic coating on the surface (figure 1). This outside layer protects the core from degradation in a physiologically aggressive environment and can form electrostatic or covalent bonds, or both, with positively charged agents and biomolecules that have basic functional groups such as amines and thiols. Several research groups have successfully linked fluorescent nanoparticles to peptides, proteins, and oligonucleotides (figure 1).[5,7,8,20,21]

Quantum dots are fluorescent nanoparticles with sizes of 2–10 nm that contain a core of hundreds to thousands of atoms of group II and VI elements (eg, cadmium, technetium, zinc, and selenide) or group III (eg, tantalum) and V elements (eg, indium).[22,23] Quantum dots containing a cadmium selenide core and a zinc sulphide shell, surrounded by a coating of a coordinating ligand and an amphiphilic polymer, are most commonly used for biological application (figure 1).[7,23] This structure enables quantum dots to emit powerful fluorescence that differs in nature from organic dyes. Quantum dots can be tuned to emit at between 450 nm and 850 nm (ie, from ultraviolet to near infrared) by changing the size or chemical composition of the nanoparticle. This so-called quantum confinement effect produces many quantum-dot colours, which can be visualised simultaneously with one light source. Quantum dots emit narrow symmetrical emission peaks with minimum overlap between spectra, allowing unique resolution of their spectra and measurement of fluorescent intensity from several multicolour fluorophores by real-time quantitative spectroscopy. These key advantages make it possible to label multiple molecular targets simultaneously by use of quantum dots both in vitro and in vivo.[3,7,23–25] However, use of quantum dots in imaging and therapeutics in vivo is limited by the toxic effects of the heavy-metal core.[26]

Surface-enhanced Raman scattering is another sensitive method for spectroscopic detection of multiple targets.[27] Modern surface-enhanced Raman scattering probes typically contain a metal core of silver or gold for optical enhancement, a reporter molecule for

spectroscopic signature, and a silica shell for protein conjugation (figure 1). When illuminated with a laser beam, the reporter dye molecule produces a unique shift in the electromagnetic spectrum, which manifests as several sharp peaks and give the characteristic fingerprint of the reporter.[28] Colloidal gold nanoparticles with a size range of 55–60 nm can be optimised for surface enhancement at 632–647 nm excitation. The benefit of using surface-enhanced Raman scattering and nanoparticles in terms of selectivity and sensitivity has previously been shown by the detection of ultra-low concentrations (ie, 10^{-4} mol/m³) of amfetamine sulfate in colloidal suspension.[29]

Supermagnetic nanoparticles contain a metal core (eg, iron, cobalt, or nickel) that is magnetically active, and are used as contrast enhancement agents to improve the sensitivity of MRI. Magnetic particles, when coated with an organic outer layer, can also be conjugated to biomolecules and used as site-specific drug-delivery agents for cancer treatment (figure 2). Iron-oxide-based magnetic materials have been used widely in clinical practice as magnetic resonance agents and in studies of gene expression, angiogenesis imaging, and cellular trafficking.[30,31] Metal nanoparticles in combination with fluorescent active molecules can be used for combined optical and magnetic imaging.[32]

Diagnosis and imaging of breast cancer

Profiling of biomarkers

With the increasing use of targeted therapies in oncology, it is imperative that methods of molecular profiling are optimised. The success of many targeted treatments depends on the expression of specific proteins or genes present in cancer cells. For example, in breast cancers, the level of hormone-receptor expression correlates directly with the benefit of endocrine treatments, and the presence of HER2 protein overexpression or gene amplification, or both, is a prerequisite for benefit from the monoclonal antibody, trastuzumab.[33–36] Immunohistochemistry is the standard method of determining the expression of hormone receptors or HER2. Although immunohistochemical methods combined with automated image analysis can quantify precisely the expression of these biomarkers in clinical breast-cancer specimens, these systems are not widely available.[37] Furthermore, the use of immunohistochemistry to detect proteins simultaneously on single tumour specimens can be difficult for several reasons, including the need to use antibodies needing different antigen retrieval methods. An assay that could accurately quantify several cancer-related proteins simultaneously on single tumour sections or small tumour specimens could offer clear advantages over standard immunohistochemical methods.

Although several, even colocalised, targets can be visualised by use of immunofluorescent staining methods with spectra-separation systems, the use of organic dye molecules such as fluorescent tags for antibodies has important limitations.[38] Quantum dots have unique optical properties that can overcome some drawbacks associated with conventional methods of biomolecular labelling. They have exceptional photostability, allowing the emission of fluorescent light over a long time without a rapid decline in emission (ie, photobleaching).[7,20,39] The unique fluorescent emission peaks of quantum dots can be easily detected and quantified with spectrometry. Since their emission spectrum depends on size, the peak wavelength of every colour is known. Individual quantum dots can be linked to different antibodies targeted to specific proteins, allowing spectra from multiple quantum dots conjugated to different proteins to be detected simultaneously by spectroscopy.

The level of fluorescent emission from these conjugated nanoparticles correlates with expression of the protein.[3] The bright fluorescence of quantum dots enables identification of targets in low levels in cancer cells, resulting in increased sensitivity.[24,25] In addition, several studies[7,20,40] have shown exceptional specificity of quantum dots for labelling of molecular targets. Giepmans and colleagues[41] used multiple quantum dots to detect molecular targets with high sensitivity and specificity. They showed that quantum dots targeted to microtubules in fibroblasts suggested colocalisation with the cytoskeleton, which was confirmed by electron microscopy. Because quantum dots have fluorescent properties and are electron dense, they can be discriminated optically by their emission wavelength and physically by size during electron microscopy. These findings will allow quantum dots to be used as probes for light microscopy and simultaneous

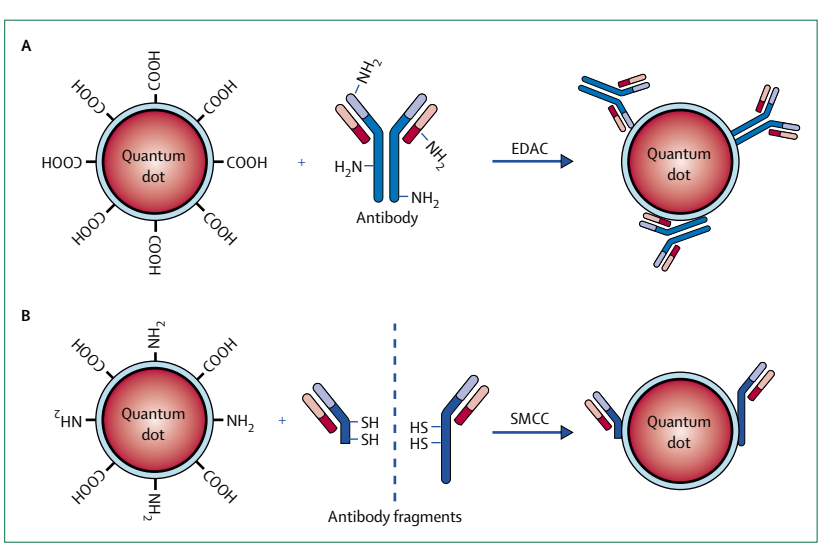

***Figure 3*: Methods for conjugating quantum dots to biomolecules**
EDAC=ethyl-3-dimethyl-amino-propyl-carbodiimide. SMCC=succinimidyl-4-N-maleimidomethyl-cyclohexane carboxylate. COOH=carboxyl group. NH2= amine group. SH=sulfhydryl group. (A) Traditional covalent crosslinking chemistry with EDAC as catalyst. (B) Conjugation of antibody fragments to quantum dots via reduced sulphhydryl-amine coupling. Reproduced with permission from ref 22.

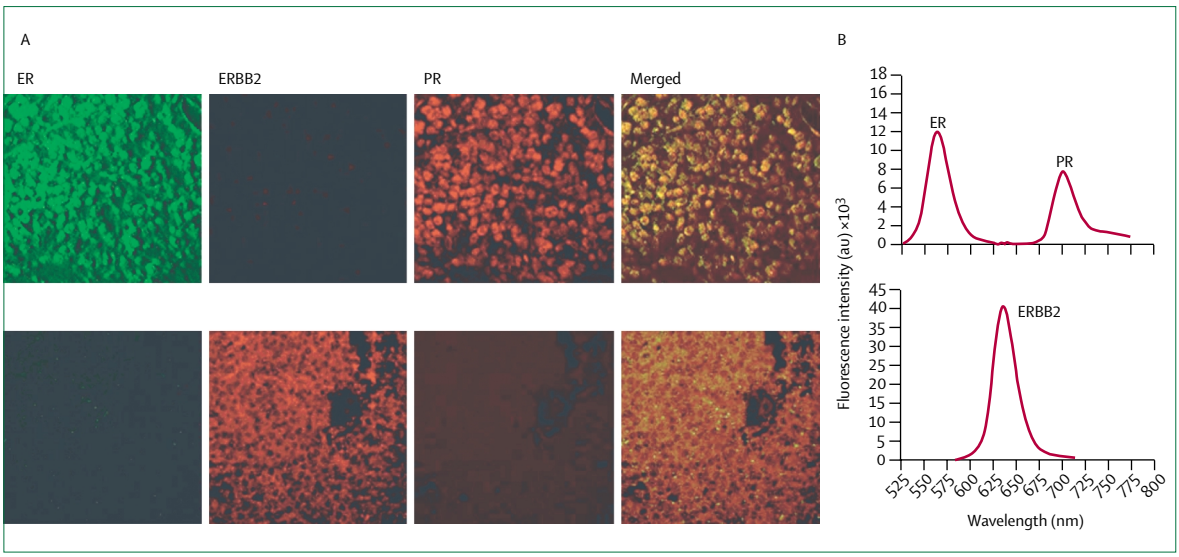

Figure 4: **Use of quantum dots to detect protein expression in tumour expressing oestrogen receptor and progesterone receptor (top) or ERBB2 (bottom)**
(A) Paraffin-embedded human breast tumours stained with human antibodies against oestrogen receptor (ER), ERBB2, and progesterone receptor (PR) conjugated with quantum dots (565 nm, 655 nm, and 605 nm, respectively). (B) Fluorescent intensity from quantum dots shows level of labelled biomarker expression in each tumour. au=arbitrary units.

visualisation of multiple subcellular structures by electron microscopy.[41]

Several groups[22,23] have assessed the best method of conjugating antibodies and peptides to nanoparticles such as quantum dots (figure 2). The most established method of bioconjugation involves by use of streptavidin and biotin as adapter molecules[20] and labelling of a sample with a primary and a biotinilated secondary antibody, followed by incubation with streptavidin-coated quantum dots (figure 2). With this approach, Wu and colleagues[20] showed specific ERBB2 labelling of fixed ERBB2-positive breast-cancer cells and human ERBB2-positive breast-cancer xenografts. Although this method is easy to use and highly effective for single staining of cell proteins, it is not optimum for multiplex protein detection. Direct conjugation of targeted antibodies to the surface of quantum dots, without use of secondary antibodies, might be the best approach to achieve multiplex detection of molecular targets. Direct conjugation results in the formation of covalent bonds between antibody fragments and the polymer on the surface of quantum dots in a molar ratio of four to one (figure 3). Direct quantum-dot bioconjugates preserve high affinity and cause minimum non-specific binding.[3]

Yezhelyev and colleagues[3,24,25] developed a quantum-dot-based assay that allows quantitative detection of oestrogen receptor, progesterone receptor, and ERBB2 in paraffin-embedded human breast-cancer cells. Breast-cancer cell lines known to have differential expression of oestrogen receptor, progesterone receptor, and ERBB2 (eg, MCF-7, BT474, MDA-231 cells) were stained simultaneously with multiple quantum dots, which were directly bioconjugated to targeting antibodies for these three proteins. Quantitative expression of the breast-cancer biomarkers, detected simultaneously on single samples of breast-cancer cell lines, by use of these conjugates and spectrometry, correlated with conventional immunohistochemical analysis and semiquantitative western blotting.[24,25] In addition, oestrogen receptor, progesterone receptor, and ERBB2 have been detected and quantified on paraffin-embedded human breast tumours (figure 4).[42] Quantum dots are available in multiple sizes and emission spectra, which allows multiple proteins to be detected simultaneously in small tumour samples. Al-Hajj and colleagues[42] have shown simultaneous multiplex detection of six breast-cancer proteins by use of direct conjugation of quantum dots to antibodies on fixed paraffin-embedded tumour samples.

Fluorescent in-situ hybridisation (FISH) is the standard method of determining gene amplification or matrix RNA distribution by use of fluorescent-labelled DNA or RNA probes. Use of organic fluorescent molecules as

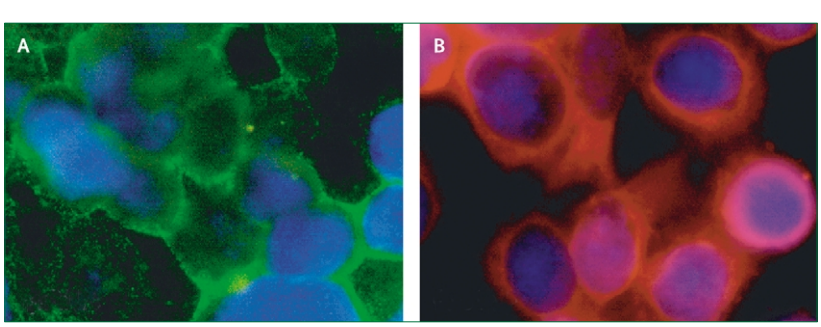

Figure 5: **FISH of E-cadherin mRNA (A) and protein (B) with quantum dots in androgen-repressed prostate-cancer cells**

tags for oligonucleotide probes has some limitations, which are similar to those seen with fluorescent immunostaining. Fairly weak signals and photobleaching problems, with complicated mechanisms for separating the emission signal of fluorophores from autofluorescence of tissues, makes detection and quantification of gene amplification technically difficult.[5,43,44]

Nanotechnology could overcome the limitations associated with FISH. Quantum dots used as fluorescent tags conjugated to oligonucleotide probes results in bright and stable fluorescent signals that are easy to detect and quantify (figure 5). Xiao and colleagues[44] noted that use of quantum dots as fluorescent tags was better than to standard FISH. Incubation of breast-cancer cells with biotinylated DNA probes for human *ERBB2* labelled with streptavidin-coated quantum dots for visualisation resulted in highly sensitive hybridisation that identified *ERBB2*, even at low levels of expression. These data suggest that use of quantum-dot-labelled oligonucleotides as a new FISH method of detecting gene amplification might offer advantages over standard FISH, particularly in the identification of genes expressed at low levels.

Moreover, oligonucleotides labelled with quantum dots are site specific. By use of linker molecules, quantum dots can be bioconjugated to either the 3′ or 5′ end of an oligo sequence. Xiao and Barker[43] have discussed the ability to control the number of attached oligonucleotides by use of a streptavidin-biotin quantum dot system. This technique allowed simultaneous detection of matrix RNA for dopamine D2 receptor, ε-sarcoglycan, tyrosine hydroxylase, and mouse vesicular monoamine transporter by the use of two different quantum-dot fluorophores and two different organic fluorophores within a single mouse midbrain neuron.[43] The same group has reported[44] combined quantum-dot-based FISH of mRNA and quantum-dot labelling of the protein on the same section of tissue. These results offer the possibility of correlating gene expression of genes at the matrix RNA level and the number of protein copies simultaneously in tumour cells.

SERS probes have potential application in ultrasensitive optical detection and spectroscopy. Raman scattering immunohistochemistry involves staining tissues with biomarker-specific antibodies linked to gold nanoparticles and fluorescent dyes (figure 6). Biomarker-specific antibodies and Raman reporter molecules are first adsorbed onto the gold nanoparticle surface. After the antibody binds to its target, the Raman scattering signal can be detected, and visualised by fluorescent microscopy. Once the probes are bound to their targets, silver ionic solution and a reducing agent are applied to form a silver shell around the gold nanoparticles (ie, silver enhancement). The resulting complex shows strong Raman scattering signals when excited by a monochromatic light source (figure 6). Silica-shell-coated SERS probes have opened new possibilities in use of SERS for spectroscopic labelling of multiple biomarkers in tissue samples.[27] With optimised gold cores and silica shells, the core-shell nanoparticles are stable in both aqueous electrolytes and organic solvents. SERS signals do not originate from the target molecules but from the reporter (ie, organic dye with isothiocyanate group) that is embedded in the core-shell structure (figure 6). By comparison with other biolabels, such as fluorescent dyes and quantum dots, SERS-active particles have a built-in mechanism for signal enhancement and give a rich spectroscopic information in ambient conditions.[27]

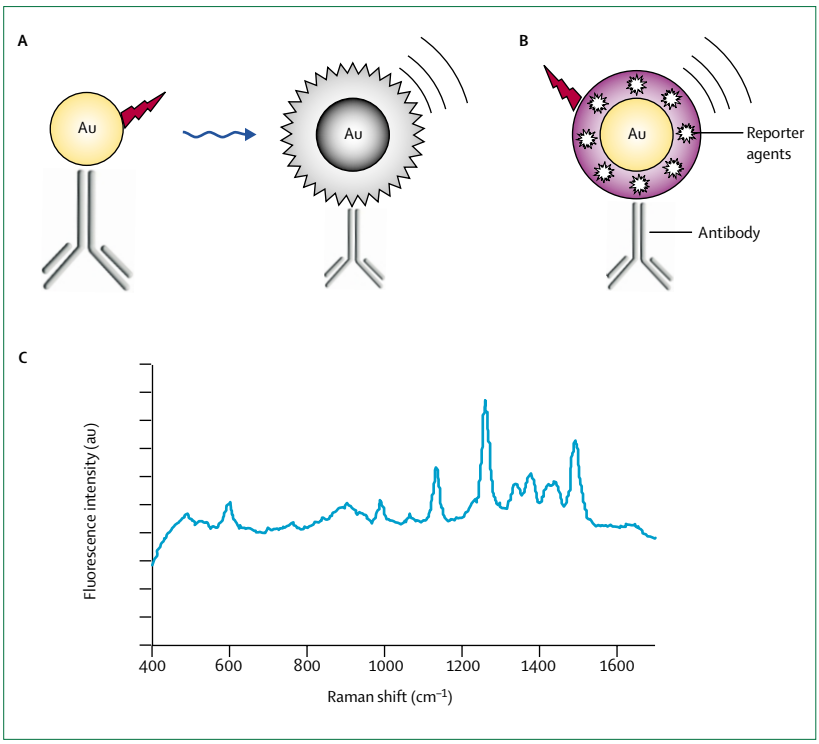

Figure 6: **Raman scattering immunocytochemistry**
Gold (Au) nanoparticle is incubated with nanoparticle–antibody conjugates (A), and silver ionic solution and reducing agent are then applied to form silver shell (B), which shows strong Raman scattering signals when excited by monochromatic light source (C, D)

In summary, use of quantum-dot conjugates and Raman probes offer the possibility of quantifying multiple proteins simultaneously on single tumour sections or small cancer samples; treatment decisions can then be made on the basis of these results. Obviously, several issues need to be addressed before conjugated nanoparticles can be used in routine surgical pathology practice. Although profiling of breast tumours with direct quantum-dot–antibody conjugation showed good antigen-binding affinity, this method still needs to be optimised, and a method of bioconjugation of antibody to quantum dots in a ratio of one to one needs to be developed. In addition, the use of quantum-dot conjugates and Raman probes, and in particular the spectral microscopes needed for accurate quantification of labelled molecular markers, is costly, which could restrict widespread applicability.

Tumour imaging in vivo

At present, magnetic nanoparticles are attracting attention because of their potential use as contrast agents for MRI.[45–48] Key advantages of the magnetic nanoparticles are low toxic effects, biocompatibility, and high level of accumulation in the target tissue.[49] Although metal particles with cobalt and nickel have been proposed, magnetic nanoparticles containing a ferric-oxide core have been used more commonly. Supermagnetic nanoparticles (3–10 nm) have been developed as MRI contrast agents and used in clinical diagnosis as a negative contrast for their effects on signal reduction on T2-weighted images.[47] Bismuth-based nanoparticles have improved over the contrast agents used for CT, which despite a good absorption, have non-specific distribution and rapid pharmacokinetics. Coating with an outer polymer protects particles from degradation and therefore prevents the cytotoxic effects of bismuth.[45] These nanoparticles showed excellent stability at high concentrations, high x-ray absorption, long circulation time in vivo (ie, >2 h), and a ratio of efficacy to safety that is better than that for iodinated imaging agents.[45]

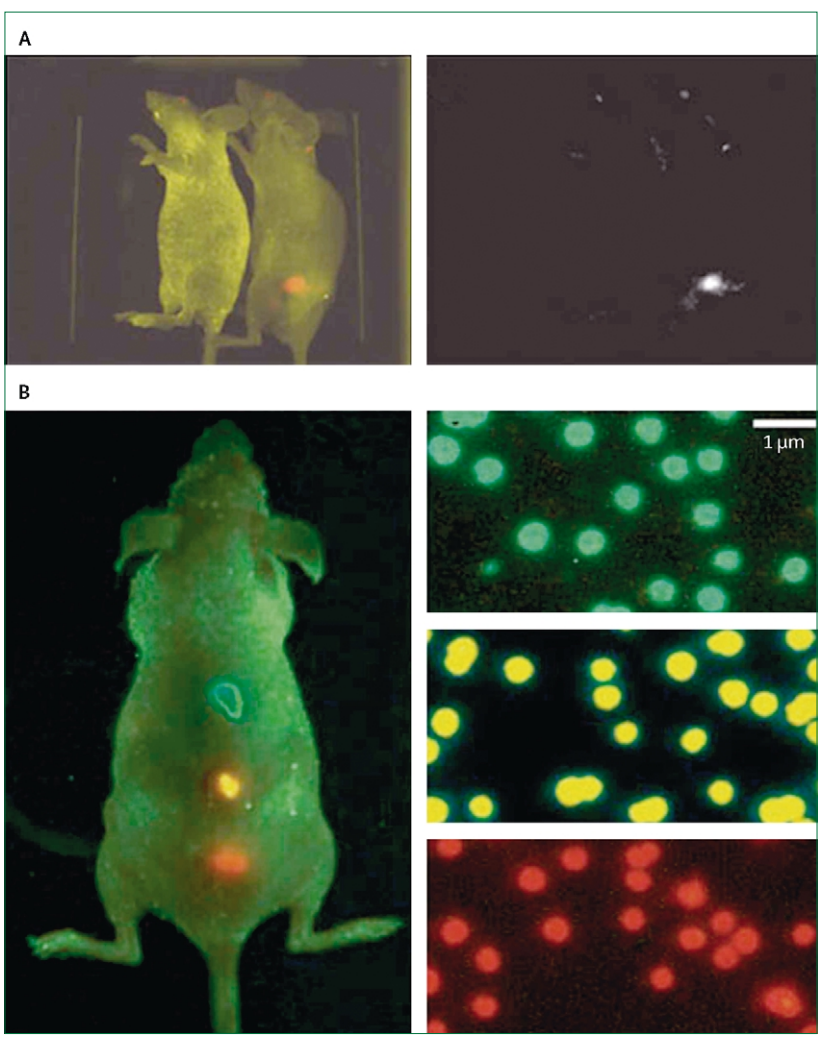

Figure 7: **Quantum dots for tumour imaging in mice**
(A) Orange-red fluorescence signals show prostate tumour in live mouse on superimposed image (left) and unmixed quantum-dot image (right). (B) About 1–2 million beads in green, yellow, or red light (right) were injected subcutaneously at three adjacent locations in mouse and visualised with tungsten or mercury-lamp excitation (left). Reproduced with permission from ref 7.

Several groups[7,49–54] have shown the potential of using quantum dots (especially with emission wavelength in the near infrared region) and magnetic nanoparticles as optical and contrast probes for non-invasive tumour imaging in vivo. Gao and colleagues[7] modified the surface of quantum dots for tumour labelling in vivo. These modified quantum dots contain an amphiphilic triblock copolymer (hydrophilic polymethacrylic segments, and two hydrophobic polybutylacrylate and polyethylacrylate segments) that prevents degradation and have multiple polyethylene glycol molecules to improve biocapability and intravascular circulation. Conjugation with a targeting antibody against prostate-specific membrane antigen allowed specific biomarker labelling of human prostate cancer xenografts with reduced accumulation of quantum dots in the liver and bone marrow (figure 7).[7] In addition, three 0·5-µm polymer beads, all with green, yellow, or red quantum dots, were visualised simultaneously in three different locations in vivo (figure 7).

Several other groups[39,50,51] have reported on the possible advantages of using quantum dots for tumour imaging. Stroh and colleagues[50] showed the use of quantum dots for labelling tumour blood vessels in vivo. The use of quantum dots allowed differentiation of tumour vasculature from perivascular cells and tumour matrix. Unlike dextran conjugates coupled with organic dyes, which are commonly infused to highlight tumour vessels, quantum dots showed clear demarcation of the vessel wall in a transgenic mouse with perivascular cells expressing green fluorescent protein.[50] Akerman and colleagues[49] showed simultaneous differential targeting of several tumour structures in a breast-cancer xenograft. Whereas quantum dots linked to an endotheliocyte-sensitive agent were localised in the tumour vasculature, quantum dots targeted against tumour tissue and lymphatic vessels were distributed within the tumour area.

Use of quantum dots that emit in the near-infrared spectrum is an alternative approach for the imaging of tumour structures in vivo. Fluorescent emission peaks of these nanoparticles are in the 800–1000 nm range, distant from the typical spectrum of tissue autofluorescence (400–600 nm). This unique feature of near-infrared quantum dots makes probes easily recognisable under near-infrared light, even in the tissues with high fluorescent background. Intraoperative detection of sentinel lymph nodes is routine for staging melanomas and breast cancers, and is associated with less morbidity than standard dissection of lymph nodes.[52–55]

Current methods of identifying sentinel lymph nodes include the use of blue dye or injection of radioisotope.[55,56]

Quantum dots offer a new method of optically tracing these nodes by use of intraoperative near-infrared fluorescence imaging without the use of radioactive tracer or blue dye.[52] After injection of near-infrared quantum dots into the skin of a tumour-bearing animal, lymphatic flow could be followed to the sentinel lymph node and its location could be quickly identified.[52] With an optimum size of 18·8 nm, near-infrared quantum dots do not flow past the sentinel lymph node and therefore allow precise localisation, which could simplify this surgical procedure in the management of breast cancer and melanoma. The widespread use of near-infrared quantum dots for the identification of sentinel lymph nodes is limited by their toxic effects.[26] However, nanoparticles with reduced toxic effects are in development, which could allow the use of this nanotechnological approach in surgical oncology in the near future. In summary, the use of nanoparticles offers exciting possibilities in imaging cancers, both in staging and ultimately in early detection.

Concurrent imaging and therapeutic targeting

As outlined above, nanoparticles can be bioconjugated to different affinity ligands and used as contrast agents, which allow imaging technologies at a subcellular level in vivo. Nanoparticles conjugated to a targeting antibody enable simultaneous cancer diagnosis and anticancer treatment. Preliminary studies in vitro and in vivo have shown the potential of this approach.[8,47,48]

One approach for bioconjugation of targeting ligands to nanoparticles involves the use of biotin and streptavidin linkers. This technique has been used to conjugate an antiERBB2 to a modified metal nanoparticle to form nanoshells.[57] The construct comprises of a spherical dielectric core nanoparticle, made of silica, surrounded by a thin gold shell. These near-infrared-emitting nanoshells convert light into thermal energy, and have been used to produce thermal tumour ablation. Thermal induction after near-infrared exposure with these nanoparticles is more than one million times more efficient than with comparable dye molecules.[57] After bioconjugation with an antiERBB2, Ito and colleagues[47] showed specific labelling of these magnetite nanoparticles to ERBB2-positive SK-BR-3 breast-cancer cells. When exposed to near-infrared light, these conjugated nanoshells induced hyperthermia, with an average temperature far above the threshold necessary to induce irreversible tissue damage, resulting in tumour-cell death. Thus, targeted nanoshells can be used to achieve localised, irreversible photothermal ablation of breast tumours in vivo.

Treatment of breast cancer

Tumour-selective delivery of anticancer agents is desirable to increase the cell-kill effect, while protecting the healthy tissue from exposure to a cytotoxic agent, thereby reducing systemic toxic effects, and nanoparticles could be used for this purpose. Much preclinical research has been done on the use of nanoparticles as a means of targeted therapy in oncology. Some of these ideas have already been brought into the clinic. We will focus on the use of nanoparticle formulations in the treatment of breast cancer.

Liposomal anthracyclines

Anthracyclines are some of the most active agents in the treatment of breast cancer,[34] and are widely used in all stages of disease. However, the use of anthracyclines is limited by cardiac toxic effects, which occurs with high cumulative doses of these agents. Trastuzumab, a monoclonal antibody that targets ERBB2, has improved treatment of this aggressive form of breast cancer;[58,59] however, its use is limited by a risk of cardiac toxic effects, which occur almost exclusively in patients previously treated with anthracyclines.[58]

Liposomal anthracycline formulations were developed to improve the therapeutic index of conventional anthracyclines, while maintaining their widespread antitumour activity. Three liposomal anthracyclines, all of which are nanoparticles measuring about 100 nm, are being assessed in human cancers: liposomal daunorubicin, approved in the USA for the treatment of Kaposi's sarcoma; liposomal doxorubicin, which, in combination with cyclophosphamide, is approved for the treatment of metastatic breast cancer in Europe; and pegylated liposomal doxorubicin, approved for both Kaposi's sarcoma and refractory ovarian cancer in the USA.

Both liposomal doxorubicin and pegylated liposomal doxorubicin have been compared with conventional doxorubicin in first-line treatment of patients with metastatic breast cancer.[9,10] 297 patients with metastatic breast cancer, who had received no previous chemotherapy, were randomly assigned to 60 mg/m² liposomal doxorubicin or 60 mg/m² conventional doxorubicin, both in combination with 600 mg/m² cyclophosphamide every 3 weeks, until disease progression or unacceptable toxic effects. Efficacy did not differ significantly between the two groups (response rate 43% *vs* 43%, median time to progression 5·1 *vs* 5·5 months, and median survival 19 *vs* 16 months).[9] However, significantly fewer patients allocated to liposomal doxorubicin developed cardiac toxic effects compared with treated with conventional doxorubicin (6% *vs* 21%, respectively, p=0·0001).[9] Overall, patients assigned liposomal doxorubicin were 80% less likely to develop cardiac toxic effects than were those assigned conventional doxorubicin. Liposomal doxorubicin was also associated with less neutropenia than was conventional doxorubicin.

Pegylated liposomal doxorubicin was compared with conventional doxorubicin in patients with previously untreated metastatic breast cancer. 509 patients were randomly assigned to single-agent pegylated liposomal

doxorubicin (50 mg/m² every 4 weeks) or doxorubicin (60 mg/m² every 3 weeks). Both agents had similar efficacy, with response rates of 33% and 38%, and progression-free survival of 6·9 and 7·8 months, respectively.[10] The risk of cardiac toxic effects was significantly higher in patients assigned doxorubicin than in those assigned pegylated liposomal doxorubicin (hazard ratio 3·16, p<0·001). Neutropenia and gastrointestinal toxic effects were reported more commonly with doxorubicin, whereas palmar-plantar erythrodysaesthesia was more common with pegylated liposomal doxorubicin.

Liposomal doxorubicin has been investigated in combination with trastuzumab in a phase I/II trial in patients with metastatic breast cancer. A response rate of 59% was noted, even though patients could have received trastuzumab previously. Cardiac toxic effects were reported in two patients, both of whom had previously received conventional doxorubicin.[60] Anthracyclines are highly effective in ERBB2-positive breast cancer,[58] so the combination of liposomal formulations and trastuzumab warrant further study.

NAB paclitaxel

The taxanes paclitaxel and docetaxel are some of the most important agents in the treatment of solid tumours, and are widely used in all stages of breast cancer. Both drugs are highly hydrophobic, and have to be delivered in synthetic vehicles (polyethylated castor oil for paclitaxel and polysorbate-ethanol for docetaxel). The toxic effects associated with both taxanes are increasingly recognised to be cause by these synthetic vehicles, and not the agents themselves.[61,62] Several new formulations of these agents have been developed in an attempt to decrease the toxic effects associated with the taxanes. NAB paclitaxel—a nanoparticle with a core containing paclitaxel surrounded by albumin, the naturally occurring vehicle for hydrophobic molecules—has shown efficacy in breast cancer (figure 2). Preclinical studies[63] showed that NAB paclitaxel resulted in improved tumour penetration compared with conventional paclitaxel. In addition, it resulted in a higher plasma clearance and larger volume of distribution than did paclitaxel, consistent with a lack of sequestration by castor-oil micelles.[63]

After phase I trials, a phase II trial[12] in 63 patients with metastatic breast cancer showed a response of 48% to NAB paclitaxel at a dose of 300 mg/m² every 3 weeks. In a phase III trial[10] comparing NAB paclitaxel with conventional castor-oil-based paclitaxel, 460 patients with taxane-naive metastatic breast cancer were randomly assigned to castor-oil-based paclitaxel or NAB paclitaxel on a 3-weekly schedule until evidence of disease progression. Overall response was significantly higher in patients allocated NAB compared with those allocated the conventional formulation, irrespective of line of therapy (overall response in all patients 33% [95% CI 27·09–39·29] *vs* 19% [13·58–23·76], p=0·001; in patients receiving first-line treatment, 42% [32·44–52·10] *vs* 27% [17·76–36·19], p=0·029, for NAB paclitaxel versus conventional paclitaxel, respectively). Time to progression was significantly longer for those allocated NAB paclitaxel than for those allocated to conventional paclitaxel (23 weeks *vs* 17 weeks; p=0·006).[10] Although overall survival was not significantly different in the patients as a whole (p=0·374), patients in the second-line setting had a significantly higher survival with NAB paclitaxel at 56 weeks compared with conventional paclitaxel at 47 weeks (p=0·024). Most importantly, tolerability improved with NAB compared with conventional paclitaxel. Although patients allocated NAB paclitaxel did not receive any drugs before the trial, no hypersensitivity reactions were noted. In addition, grade IV neutropenia was significantly lower and incidence of grade 3 neuropathy signifcantly higher in patients allocated to NAB paclitaxel compared with those allocated to the conventional formulation (p<0·001 for both comparisons). (p<0·001). However, the NAB paclitaxel has been assessed on a weekly schedule in patients with heavily pretreated metastatic breast cancer.[64] Responses were noted in patients who had given paclitaxel or docetaxel, or both, previously, and preliminary data suggest that neuropathy is lessened with this weekly schedule. In summary, this nanoparticle formulation of paclitaxel offers advantages over castor-oil-based paclitaxel, with an overall decrease in toxic effects, an absence of need for pretreatment, and enhanced efficacy.

Targeted delivery of tamoxifen

About two-thirds of breast cancers express hormone receptors, of which about 50% benefit from endocrine therapy. Tamoxifen remains widely used in all stages of breast cancer, in both premenopausal and postmenopausal women. It undergoes substantial metabolism, and an inability to get active drug into breast tumours might hinder its effectiveness. Shenoy and Amiji[65] have developed a tamoxifen-loaded, polymeric nanoparticle to increase tumour penetration. By use of a human breast-cancer xenograft model, they showed a significant increase in the level of tumour accumulation of tamoxifen in mice given the loaded nanoparticles, compared with those given an intravenous formulation. The use of drug-loaded nanoparticles offers the promise of improved tumour penetration, with selective tumour targeting, and a subsequent decrease in toxic effects.

Gene therapy

Major strategies in breast-cancer gene therapy include transfer of tumour-suppressor genes, enhancement of immunological response, transfer of suicide genes, and bone-marrow protection by use of drug-resistance genes.[66] Breast-cancer genome abnormalities for which

gene therapy could be potentially useful include amplification or mutation of multiple genes, including *ERBB2*, *P53*, *MYC*, and cyclin D1.[67] However, human gene-therapy techniques have been hampered by the fact that oligonucleotide-containing substances undergo rapid enzymatic degradation in human plasma. Therefore, research is ongoing to identify the best delivery vehicle for gene therapy.

Nanoparticle-based DNA and RNA delivery systems offer several potential advantages for gene delivery to various human tumours, including breast cancer. A DNA plasmid can be coupled with cationic and neutral lipids to form lipid–nucleic-acid nanoparticles.[68] DNA molecules are encapsulated into the nanoparticle and are thus protected from degradation. In addition, conjugation of a polyethylene glycol molecule to the surface of the nanoparticle with targeted antibody increases gene delivery into tumour cells. Hayes and colleagues[68] have used this method to allow gene delivery to human ERBB2-positive breast-cancer cells using a ERBB2-directed antibody conjugated to a nanoparticle. Another study[69] has shown successful transfer of *E1A* complexed with cationic liposome to human breast and ovarian cancers. Preclinical studies[70] have shown that adenovirus type 5 *E1A* is associated with antitumour activities by transcriptional repression of ERBB2. Patients with breast or ovarian cancer (ERBB2-positive or low ERBB2 expressing) were treated in a phase I trial with this cationic liposome-mediated *E1A* gene-transfer system, given by injection either into the thoracic or peritoneal cavity. *E1A* gene expression in tumour cells was detected by immunohistochemical analysis and reverse-transcriptase PCR, suggesting successful gene transfer. In addition, *E1A* expression was accompanied by ERBB2 downregulation, an increase in apoptosis, and a reduction in proliferation.[69] Prahba and Labhasetwar[71] showed antiproliferative activity of wild-type *P53*-loaded nanoparticles in a breast-cancer cell line. Nanoparticles containing plasmid DNA were formulated by a multiple-emulsion-solvent evaporation technique using a biocompatible polymer, poly(D,L-lactide-co-glycolide). Cells transfected with wildtype *P53* DNA-loaded nanoparticles showed significantly greater antiproliferative effect than did those with naked wildtype *P53* DNA, resulting in antiproliferative activity, which could be therapeutically beneficial in breast-cancer treatment.[71]

Transfection of tumour cells with small-interfering RNA (siRNA) is a rapidly growing gene-silencing technology with great potential for clinical application. Inhibition of breast-cancer oncogenes results in induction of apoptosis and an increase of chemotherapy sensitivity in breast-cancer cells.[72,73] Stability and cellular uptake of siRNA can be greatly improved by adsorption to polyalkylcyanoacrylate nanoparticles.[74] Nanoparticle–siRNA complexes directed to *Ras* matrix RNA selectively inhibited the proliferation of breast-cancer cells and markedly inhibited *Ha-ras*-dependent tumour growth in nude mice after injection under the skin. In addition, injection of a non-covalent siRNA-polyethylenimine targeting ERBB2 complex into the peritoneal cavity resulted in significant ERBB2 receptor downregulation in an animal, with a resultant reduction in tumour growth.[75] Despite this early stage of development, nanoparticle-based delivery systems have already shown significant benefits for targeted gene delivery, and indicate great potential for clinical use in breast-cancer therapy.

Search strategy and selection criteria

References were obtained by searches of PubMed using the MeSH search terms "nanotechnology", "nanoparticles", "breast cancer", "diagnostics", "quantum dots", "Raman probes", "dendrimers", "magnetic nanoparticles", "liposomes", "carbon nanotubes", "abraxane", "treatment", with additional search terms "biomarkers", "profiling", "in-vivo imaging", "targeting", and "small interfering RNA" required for specific aspects of the review. Only papers published between January, 1980, and December, 2005, in English were included.

Conclusion

The use of nanotechnology in oncology offers exciting possibilities, and is regarded an area of major importance by the US National Cancer Institute, which has recently awarded several Center of Cancer Nanotechnology Excellence grants. The use of nanoparticles conjugated to antibodies allows the possibility of simultaneously detecting multiple molecular targets in small tumour samples, on which treatment decisions can be made. Protein and gene expression in an individual tumour can be correlated using nanoparticle tags. The use of nanoparticles in imaging in vivo is rapidly evolving, and could allow simultaneous detection and targeting of cancer-related antigens. Nanoparticles offer a new method of tumour targeting, already available in clinical practice, which can concomitantly improve the efficacy and decrease the toxicity of existing or novel anticancer agents. In the near future, the use of nanotechnology could revolutionise not only oncology, but also the entire discipline of medicine.

Conflicts of interest

We declare no conflicts of interest.

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