Published in final edited form as:

Annu Rev Med. 2010 ; 61: 359–373. doi:10.1146/annurev.med.60.052907.094936.

Nanotechnology Applications in Surgical Oncology

Sunil Singhal¹, Shuming Nie², and May D. Wang³

¹Division of Thoracic Surgery, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; sunil.singhal@uphs.upenn.edu

²Departments of Biomedical Engineering and Chemistry, Emory University, Atlanta, Georgia 30322; snie@emory.edu

³Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, Georgia 30332; maywang@bme.gatech.edu

Abstract

Surgery is currently the most effective and widely used procedure in treating human cancers, and the single most important predictor of patient survival is a complete surgical resection. Major opportunities exist to develop new and innovative technologies that could help the surgeon to delineate tumor margins, to identify residual tumor cells and micrometastases, and to determine if the tumor has been completely removed. Here we discuss recent advances in nanotechnology and optical instrumentation, and how these advances can be integrated for applications in surgical oncology. A fundamental rationale is that nanometer-sized particles such as quantum dots and colloidal gold have functional and structural properties that are not available from either discrete molecules or bulk materials. When conjugated with targeting ligands such as monoclonal antibodies, peptides, or small molecules, these nanoparticles can be used to target malignant tumor cells and tumor microenvironments with high specificity and affinity. In the “mesoscopic” size range of 10–100 nm, nanoparticles also have large surface areas for conjugating to multiple diagnostic and therapeutic agents, opening new possibilities in integrated cancer imaging and therapy.

Keywords

quantum dots; Raman scattering; molecular probes; intraoperative imaging; cancer; instrumentation

Introduction

Most human cancers are treated by surgical resection, chemotherapy, and/or radiation. Surgery cures ~45% of all patients with cancer (1), whereas chemotherapy and radiation therapy together cure only 5%, and the remainder succumb to their diseases. To cure a cancer patient by surgery, the surgeon must remove the entire tumor at the time of surgery. A complete resection is the single most important predictor of patient survival for almost all cancers (2). This includes removal of the primary tumor, draining lymph nodes that may contain tumor cells, and small adjacent satellite nodules. In lung, breast, prostate, colon, and

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

pancreatic cancers, complete resection is associated with a three- to fivefold improvement in survival compared to a partial or incomplete resection (3–6).

Clearly, it is important to maximize the efficacy of surgery because it is the most important method that exists to cure people of cancer. Advances in the field of cancer surgery during the past 50 years include minimally invasive approaches, laparoscopy, preoperative imaging modalities, better anesthesia, and improved postoperative management strategies. But the cure rate from surgical intervention has changed little, and the tools that surgeons use in the operating room to determine if the tumor has been completely resected have remained largely the same. The surgeon uses cutting instruments, his or her eyes and hands, intuition, and experience. No intraoperative tools or devices have successfully improved the surgeon's ability to find and remove a tumor in over half a century.

Preoperative imaging with computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and their combinations (such as CT/PET) has greatly improved tumor detection, but these modalities do not provide much help to the cancer surgeon during surgery. The intraoperative challenges that a clinician must meet include the following: (a) accurate identification of the malignant lesion, (b) complete removal of the entire tumor with negative surgical margins, (c) preservation of normal uninvolved structures, (d) removal of lymph nodes that drain from the tumor, and (e) identification of small local residual tumor deposits. The long-term outcome of the patient depends on how well the individual surgeon manages these challenges, which in turn depends on that individual's skill and experience (7,8). These qualities are subjective and imprecise. For example, at the University of Miami, one experienced urologist performed 100 consecutive radical prostatectomies and recorded intraoperatively if he suspected the tumor margins were positive or negative based on his visual clues and palpation (9). Despite his intraoperative decision that the surgical margins were negative in all 100 cases, the true pathological margins were positive in 39% of the cases. The intraoperative assessment of the margin status had a high false-negative rate and a sensitivity of only 7%. The sensitivity of the intraoperative assessment of tumor location was 73%, and the positive predictive value was 65%. For breast cancer, Abraham and colleagues at the University of Pennsylvania reviewed 97 consecutive cases of breast cancer specimens in which no cancer appeared to be present, but 48% of the specimens contained invasive or in situ carcinoma (10).

Thus, there are urgent needs and major opportunities to develop new and innovative technologies that could help the surgeon to delineate tumor margins, to identify residual tumor cells and micrometastases, and to determine if the tumor has been completely removed. Such technologies would be applicable to many organ sites, such as lung, pancreatic, ovarian, brain, breast, and prostate cancers. In this article, we discuss recent advances in nanotechnology and optical instrumentation and how these advances can be integrated for applications in surgical oncology. A fundamental rationale is that nanometer-sized particles such as quantum dots, colloidal gold, and polymeric liposomes have functional and structural properties that are not available from either discrete molecules or bulk materials (11–13). When conjugated with targeting ligands such as monoclonal antibodies, peptides, or small molecules, these nanoparticles can be used to target malignant tumor cells and tumor microenvironments (such as tumor stroma and tumor vasculatures) with high specificity and affinity (14,15). In the “mesoscopic” size range of 10–100 nm diameter, nanoparticles also have large surface areas for conjugating to multiple diagnostic and therapeutic agents. This versatility opens new possibilities in integrated diagnostic imaging and therapy (called “theranostics”) for cancer (16,17).

Nanotechnology

Quantum Dots

Semiconductor quantum dots (QDs) are emerging as a new class of fluorescent labels for biology and medicine (11–14). In comparison with organic dyes and fluorescent proteins, these tiny light-emitting particles have unique optical and electronic properties, with size-tunable light emission, superior signal brightness, resistance to photobleaching, and broad absorption spectra for simultaneous excitation of multiple fluorescence colors. QDs also provide a versatile nanoscale scaffold for designing multifunctional nanoparticles with both imaging and therapeutic functions. For in vivo and intraoperative tumor imaging, QDs hold great promise mainly due to their intense fluorescent signals and multiplexing capabilities, which could allow a high degree of sensitivity and selectivity (see Figure 1). Toward this goal, Akerman et al. conjugated QDs to peptides with affinity for various tumor cells and their vasculatures (18). After intravenous injection of these probes into tumor-bearing mice, microscopic fluorescence imaging of tissue sections demonstrated that the QDs specifically homed to the tumor vasculature. In 2004, Gao et al. (19) demonstrated that tumor targeting with QDs could generate tumor contrast on the scale of whole-animal imaging. QDs were conjugated to an antibody against the prostate-specific membrane antigen (PSMA), and the conjugates were intravenously injected into mice bearing subcutaneous human prostate cancers. Tumor fluorescence was significantly greater with the actively targeted conjugates than with nonconjugated QDs. Using similar methods, Yu et al. (20) were able to actively target and image mouse models of human liver cancer with QDs conjugated to an antibody against alpha-fetoprotein, and Cai et al. (21) showed that labeling QDs with RGD peptide significantly increased their uptake in human glioblastoma tumors.

The development of clinically relevant QD contrast agents for in vivo imaging is certain to encounter many roadblocks in the near future, but QDs can currently be used as powerful imaging agents for the study of the complex anatomy and pathophysiology of cancer in animal models. Stroh et al. (22) demonstrated that QDs greatly enhance current intravital microscopy techniques for the imaging of tumor microenvironment. The authors used QDs as fluorescent contrast agents for blood vessels using two-photon excitation, and they simultaneously captured images of extracellular matrix from autofluorescent collagen and perivascular cell contrast from fluorescent protein expression. The use of QDs allowed stark contrast between the tumor constituents due to their intense brightness, tunable wavelengths, and reduced propensity to extravasate into the tumor, compared to organic dye conjugates. In this work, the authors also used QD-tagged beads with variable sizes to model the size-dependent distribution of various nanotherapeutics in tumors. As well, the authors demonstrated that bone marrow lineage-negative cells, which are thought to be progenitors for neovascular endothelium, were labeled ex vivo with QDs and imaged in vivo as they flowed and adhered to tumor blood vessels following intravenous administration. More recently, Tada et al. (23) used QDs to study the biological processes involved in active targeting of nanoparticles. The authors used QDs labeled with an antibody against human epidermal growth factor receptor 2 (HER2) to target human breast cancer in a mouse model. Through intravital fluorescence microscopy of the tumor following systemic QD administration, the authors could distinctly observe individual QDs as they circulated in the bloodstream, extravasated into the tumor, diffused in extracellular matrix, bound to their receptors on tumor cells, and then translocated into the perinuclear region of the cells. The combination of sensitive QD probes with powerful techniques like intravital microscopy and in vivo animal imaging could help to gain a better understanding of tumor biology, to improve early-detection schemes, and to guide new therapeutic designs.

SERS Nanoparticles

To overcome the potential toxicity concerns of QDs, Qian et al. (28) have developed a new class of nontoxic nanoparticles for in vivo tumor targeting and spectroscopic detection based on the use of pegylated colloidal gold and surface-enhanced Raman scattering (SERS). Colloidal gold has been safely used to treat rheumatoid arthritis for half a century (29,30), and recent work indicates that nanoparticles of pegylated gold—that is, colloidal gold coated with a protective layer of polyethylene glycol (PEG)—exhibit excellent in vivo biodistribution and pharmacokinetic properties upon systemic injection (31–33). In contrast to cadmium-containing QDs and other toxic or immunogenic nanoparticles, gold colloids have little or no long-term toxicity or other adverse effects in vivo (34,35). In addition, colloidal gold nanoparticles have unique optical properties and can amplify the Raman scattering efficiencies of adsorbed molecules by as much as 10^{14} – 10^{15} -fold, allowing spectroscopic detection and identification of single molecules at room temperature (36,37). Like QDs and other nanoparticles, SERS nanotags can be delivered to tumors by either a passive targeting mechanism or an active targeting mechanism (see Figure 2). In the passive mode, nanometer-sized particles are accumulated preferentially at tumor sites through an enhanced permeability and retention (EPR) effect (24–27). For active tumor targeting, ligand molecules such as antibodies and peptides are used to recognize specific tumor antigens. In comparison with QDs, the gold nanoparticles provide much richer spectroscopic information, and their emission peaks (full width at half maximum = 1–2 nm) are 20–30 times narrower than those of QDs (FWHM = 40–60 nm). Under identical experimental conditions, the pegylated gold particles are >200 times brighter (on a particle-to-particle basis) than near-infrared-emitting QDs in the spectral range of 650–750 nm (28).

For in vivo tumor targeting and spectroscopic detection, Qian et al. (28) injected small doses of SERS nanoparticles into subcutaneous and deep muscular sites in live animals. The results demonstrate that highly resolved SERS signals are obtained from subcutaneous as well as muscular injections. The in vivo SERS spectra are identical to those obtained in vitro (saline solution), although the absolute intensities are attenuated by 1–2 orders of magnitude. Based on the high signal-to-noise ratios, the achievable penetration depth is ~1–2 cm for in vivo SERS tumor detection. Similarly, intense SERS spectra can be from gold nanoparticles dispersed in whole blood at 785 nm excitation (but not at 633 nm excitation), owing to reduced light absorption and scattering in the near infrared. For in vivo tumor targeting and spectroscopy, the gold nanoparticles conjugated with the ScFv antibody are injected systemically (via tail veins) into nude mice bearing a human head and neck tumor. Figure 3 shows SERS spectra obtained 5 h after nanoparticle injection by focusing a near-infrared (785 nm) laser beam to the tumor site or to other anatomical locations (e.g., the liver or a leg) (28). Significant differences are observed between the targeted and nontargeted nanoparticles in the tumor signal intensities, whereas the SERS signals from nonspecific liver uptake are similar. This result indicates that the ScFv-conjugated gold nanoparticles are able to target EGFR-positive tumors in vivo. Time-dependent SERS data further indicate that nanoparticles are gradually accumulated in the tumor for 4–6 h and that most of the accumulated particles stay in the tumor for >24–48 h.

Quantitative biodistribution studies using inductively coupled plasma–mass spectrometry (ICP-MS) reveal that the targeted gold nanoparticles are accumulated in the tumor 10 times more efficiently than the nontargeted particles (28). The ICP-MS data also confirm nonspecific particle uptake by the liver and the spleen, but little or no accumulation in the brain, muscle, or other major organs. Ultrastructural transmission electron microscopy (TEM) studies further reveal that the SERS nanoparticles are taken up by the EGFR-positive tumor cells and are localized in intracellular organelles such as endosomes and lysosomes. The in vivo endocytosed nanoparticles have crystalline and faceted structures. The pegylated gold particles are intact and stable in systemic circulation as well as after being taken up into

intracellular organelles. No toxicity or other physiological complications have been observed for the animals 2–3 months after gold particle injection.

Activatable Probes and Tumor Paints

Another class of contrast agents for tumor visualization is based on covalent conjugation of organic dyes with tumor targeting ligands. In particular, Hoffman and coworkers (38,39) have conjugated organic fluorophores with monoclonal antibodies for intraoperative visualization of colon and pancreatic tumors, achieving remarkable sensitivity and specificity. Kobayashi, Choyke, and their coworkers (40–42) have developed “activatable” fluorescent imaging probes with improved signal-to-background ratios for targeting and visualizing lung metastases in animal models. Several mechanisms have been demonstrated for probe activation, including photon-induced electron transfer (40,41), self-quenching (42), quencher-fluorophore interactions (43), and pH changes (40) (that is, when the probes are internalized into tumor cells, their fluorescence is activated by the acidic environment of endosomes and lysosomes). By attaching Cy5.5 to a tumor-targeting peptide called chlorotoxin, Olson and coworkers (44) have developed an imaging agent that effectively “paints” cancerous tissue. This class of “tumor paints” can be used to detect metastatic cancer foci as small as 200–2000 cancer cells, making it about 500 times more sensitive than MRI. In mice, the team demonstrated that they could light up brain tumors as small as 1 mm in diameter without lighting up the surrounding normal brain tissue. In a prostate cancer model, as few as 200 cancer cells traveling in a mouse lymph channel could be detected.

Instrumentation

Optical instrumentation provides unique advantages for intraoperative cancer detection that are not available from other imaging modalities. In the visible spectrum, optically labeled tumors are visible to the human eye (38–43) and can be seen and resected by the surgeon without any aid. In the near-infrared spectrum, standard fiber optics and silicon-based CCD (charge-coupled device) cameras can be used for tumor visualization at high sensitivity and low costs. Also, optical techniques provide both signal intensity and wavelength information, and they can be used to detect two or more cancer biomarkers simultaneously (45,46). This “wavelength-multiplexing” capability permits multicolor “ratiometric” cancer imaging as well as studies of in vivo tumor heterogeneity (variations from region to region in the same tumor or from one tumor to another). For multiplexed tumor targeting and detection, SERS nanoparticles with distinct spectroscopic signatures are particularly attractive because they are biocompatible and nontoxic, and can be prepared with the same particle size, surface coating, and in vivo pharmacokinetic properties. Similarly, semiconductor QDs are well suited for spectral multiplexing because of their broad absorption profiles and multicolor fluorescence emission (14,19).

One potential problem is that optical methods have limited penetration depths owing to tissue scattering and blood absorption. Even in the near-infrared “clear” window, it is difficult to achieve more than 1 cm tissue penetration (19,28). For intraoperative cancer detection, however, this is no longer a major limitation because the tumors are surgically exposed and are accessible to optical illumination and detection. One can even argue that a limited penetration depth could help the surgeon to more precisely determine the tumor location in a complex surgical field.

Another potential problem is that nanoparticles and macromolecules (such as monoclonal antibodies) are unable to deeply penetrate solid tumors (24–27). For detection of tumor margins during surgery, however, the nanoparticles are detected at the tumor periphery, and their deep tumor penetration is not required.

There are several prototypes for intraoperative optical imaging systems. These systems are necessary for visualizing particles that may not be immediately visible, such as near-infrared particles. QD technology has the advantage that it can be visualized by a simple UV light source (see Figure 1). Frangioni and colleagues (47) have developed an operational prototype designed specifically for use during large animal surgery. Such a system serves as a foundation for future clinical studies. Using their prototype and near-infrared fluorophore indocyanine green (ICG), they demonstrated vascular imaging in a large animal (a 35-kg pig). Cancer-specific applications of this imaging system included image-guided cancer resection with real-time assessment of surgical margins, image-guided sentinel lymph node mapping, intraoperative mapping of tumor and normal vasculature, image-guided avoidance of critical structures such as nerves, and intraoperative detection of occult metastases in the surgical field. A more advanced system, the FLARE system (fluorescence-assisted resection and exploration), has also been developed for large animal surgery and visualization of fluorescent particles (48). Two light sources, one for white light and one for near-infrared fluorescence excitation light, illuminate the surgical field. White light reflected from the surgical field is collected by a motorized zoom lens and directed to a color video camera. Normally, there is virtually no near-infrared fluorescence emission from the surgical field. However, when an exogenous near-infrared fluorophore is introduced, for example, as a lymphatic tracer, the invisible near-infrared fluorescent light emitted from the fluorophore is directed to a near-infrared camera. Both cameras acquire their images simultaneously, permitting “anatomy” (from the color video camera) and “function” (from the near-infrared camera) to be displayed separately or merged together.

Surgical Applications

Tumor Localization

Gao et al. (19) reported the first simultaneous in vivo targeting and imaging of tumors in live animals using QDs tagged to antibodies. Both active prostate-specific antigen targeting and passive enhanced permeability and retention allowed nanoparticle uptake at tumor sites in murine models. A whole-body macroillumination system was also integrated with wavelength-resolved spectral imaging for efficient background removal and precise delineation of weak spectral signatures. This work demonstrated QDs' capacity for the tumor localization that is critically important to oncological surgery. The fundamental first step in any intraoperative improvement to cancer surgery is the ability to deliver sufficient QDs to a tumor in order to make the neoplasm visible, either to the naked eye or with the assistance of imaging devices.

Tumor Margins

In breast cancer, as many as 20%–55% of patients undergoing partial mastectomy require a second surgical procedure owing to positive margins (49).

Breast cancer patients often receive preoperative radiation, which results in a fibroplastic scar and destroys normal tissue planes. Preoperative chemotherapy can also cause a local inflammatory reaction that makes it difficult to distinguish normal tissue planes, inflammatory adhesions, and residual cancer deposits. Because of the inability to discern which tissues are normal and which are cancerous, surgeons may perform incomplete resections. Pathological review of resected tumor specimens often demonstrates residual tumor at the edge (margin) of the removed specimen. This implies that tumor deposits are left behind in the patient at the time of surgery. Without complete removal of the tumor, long-term prognosis is dramatically reduced.

Weissleder, Josephson, and coworkers have developed a nanoparticle, CLIO-Cy5.5, which is both an MRI contrast agent and a near-infrared fluorescent optical probe (50). CLIO-

Cy5.5 is composed of a superparamagnetic iron oxide core coated with crosslinked dextran to which the fluorochrome Cy5.5 is covalently attached. This group examined the accuracy of tumor margin delineation of orthotopic tumors implanted in hosts. MRI was performed on brain-tumor-bearing rats after the administration of CLIO-Cy5.5. Hypointense tumor uptake relative to the surrounding T2-weighted images indicated nanoparticle accumulation. Rats then underwent craniotomy and were exposed to various light spectra to delineate the tumor margin. Histological examination was performed on the resected tumor using the fluorescence to guide margins.

Identification of Important Adjacent Structures

Although surgically removing the entire cancer is the single most important factor in determining long-term survival in patients with local cancers, the surgeon must be cautious about removing “too much” tissue. This is typically termed the morbidity of the operation. For example, a local tumor may abut adjacent vessels. In lung cancer, the aorta and superior vena cava can be close to a large lung cancer. Inadvertent incision into these structures can lead to intraoperative exsanguination and death. Posterior-based lung cancers can invade critical neural structures such as the vertebral column, and excessive dissection around the spinal canal can result in paralysis. In prostate cancer, excessive removal of surrounding neural tissue around the male organ results in erectile dysfunction. In pancreatic cancer, aggressive margin control can result in injuries to the portal vein or gastroduodenal artery. Resection of excessive tissue can also cause aesthetic complications. In breast cancer, for example, minimizing the amount of breast tissue removed in a partial mastectomy procedure is the main factor in preserving cosmetic appearance.

The main problem is the inability to intraoperatively distinguish the tumor margin from vessels and nerves. If the tumor margin could be better assessed, a precise decision could be made regarding the resection margins. This would dramatically reduce postoperative morbidity.

Optical methods, including optical spectroscopy and the preoperative injection of fluorescent dyes, have been investigated to aid in surgical resection. However, these techniques are still limited in their application because of difficulties with tissue discrimination, specificity of tumor uptake, and photobleaching of fluorescent dyes. Toms and colleagues at the Cleveland Clinic utilized the fact that a variety of nanoparticles are phagocytized by macrophages in vivo (51). This feature may allow optical nanoparticles, such as QDs, to colocalize with brain tumors and serve as an optical aid in the surgical resection or biopsy of brain tumors. In particular, the authors found that macrophages and microglia colocalized with the glioma cells, carrying the QD and thereby optically outlining the tumor. Excitation with blue or UV wavelengths stimulated the QDs, which gave off a deep red fluorescence detectable with CCD cameras, optical spectroscopy units, and dark-field fluorescence microscopy. The optical signal was detected, allowing improved identification and visualization of tumors.

Nanotechnology also holds great promise in identifying normal arterial and neural structures intraoperatively. For example, during left colon surgery, one of the most worrisome complications is injury to the left ureter (a tubular structure that connects the kidney to the bladder). A fluorescent optical probe would be very useful in highlighting this structure. In one example, a near-infrared fluorophore dye was injected intravenously in rats and pigs, and renal clearance kinetics and imaging performance were quantified (47). The clinically available near-infrared fluorophore indocyanine green was also used via retrograde injection into the ureter, and imaging of the ureters was achieved under the conditions of steady state, intraluminal foreign bodies, and injury. In rat models, the highest signal-to-background ratio for visualization occurred after intravenous injection at 10–30 min, and in pig models, the

fluorophores clearly visualized the normal ureter and intraluminal foreign bodies as small as 2.5 mm in diameter. Retrograde injection of 10 μ M indocyanine green also permitted the detection of normal ureter and pinpointed urine leakage caused by injury. This successful use of clinically approved fluorophores for ureter visualization suggests that nanoparticles could be utilized for the same purpose.

Sentinel Lymph Nodes

One of the most promising applications of nanotechnology is to detect and visualize sentinel lymph nodes by intraoperative near-infrared fluorescence imaging. Sentinel lymph node mapping has been tested in animal models for a variety of organs and tissues including skin, lung, pleural space, germ cell tumors, mammary tissue, esophagus, stomach, small intestines, and colon (52–57). In both melanoma and breast cancer, the clinician relies on the status of the sentinel lymph node to guide therapy. Frangioni and colleagues studied the use of QDs in a spontaneous melanoma model in six small pigs (48). QDs were injected in four quadrants around melanoma tumors in the animals. Injections, lymphatic tracking, and sentinel lymph node resection were imaged in real time, and actual pulsations could be identified. Sentinel lymph nodes were identified in all the injections. After tissue resection, nodal tissue was resected and confirmed histologically. QD technology has a dual role in these procedures. Pathologists can use the fluorescence to guide their dissection as well as perform high-resolution sectioning at the sinus entry point.

Detection of Residual Tumor Cells

Despite apparently adequate resection of tumors, histological examination of normal-appearing tissue surrounding a cancer may reveal small adjacent tumor deposits that are not visible to the human eye or palpable with the fingertips of the surgeon. For example, in breast cancer, half of all patients undergoing surgery can have residual carcinoma outside the palpable tumor when the tissue specimens are reviewed under the microscope (58). In lung cancer, as many as 15% of patients who have complete resection of their tumors can have residual tumor deposits on the bronchial resection stump that are as far away as 2.5 cm from the primary tumor and that are too small to be seen by the human eye (59). The best opportunity to remove these residual cells is at the initial surgery. Although chemotherapy and radiation therapy are often used to “clean up” small tumor deposits that may have escaped the surgeon, this approach is not as successful as mechanically removing all disease from the patient. For example, in early-stage lung cancer, residual tumor deposits after a curative resection are associated with up to a 35% reduction in survival regardless of adjuvant chemotherapy or radiotherapy (59).

The limiting factor in detecting microscopic tumors at the time of surgery is the sensitivity of the human eye. The human eye cannot detect microscopic tumors or cell clusters in a background of normal tissue. The sensitivity of the human eye depends on the intensity of the signal, background noise, excitation lighting, and adjacent contrast. With increasing light intensity, the ability of the eye to distinguish detail increases (60). The use of nanoparticles such as QDs and SERS nanoparticles can enhance the sensitivity of the human eye. If residual tumor cells could be detected in the operating room, surgical resection could be more accurate. In principle, a complete resection of tumor is the most important prognostic indicator for most cancers and use of this technology should result in better cure rates following surgery.

Future Directions

This article has highlighted important opportunities to develop nanoparticle contrast agents and optical instrumentation that could help the surgeon to delineate tumor margins and to

detect residual tumor cells or micrometastases. By reducing cancer recurrence and saving patients from second or third surgeries, nanotechnology could ultimately make a broad impact on how cancer patients are treated. Looking into the future, we note four research directions that are particularly promising but require concerted effort for success.

Theranostics

There is a need to develop multifunctional nanoparticles. For cancer and other medical applications, important functions include imaging, therapy, and targeting. With each added function, nanoparticles could be designed to have novel properties and applications. For example, nanoparticles with two functions could be developed for molecular imaging, targeted therapy, or for simultaneous imaging and therapy. Nanoparticles with three functions could be designed for simultaneous imaging and therapy with targeting, targeted dual-modality imaging, or targeted dual-drug therapy.

Minimizing RES Uptake

There is an urgent need to develop biocompatible nanoparticles that can target tumors by active molecular binding while escaping nonspecific uptake in reticuloendothelial (RES) organs. This in vivo biodistribution barrier might be mitigated by systematically optimizing the size, shape, and surface chemistry of imaging and therapeutic nanoparticles. For in vivo and intraoperative cancer imaging, it is highly desirable to have the injected nanoparticles cleared from nonspecific organs and tissue, but accumulated and retained in tumors. To achieve this goal, it will be important to develop biodegradable or self-assembled nanoparticle probes that can be cleared by renal filtration or hepatobiliary excretion.

Nanotoxicology

Great concern has been raised over the potential toxicity of QDs in living cells and animals. Presently, the most commonly used QDs contain divalent cadmium, a nephrotoxin in its ionic form. Although this element is incorporated into a nanocrystalline core, surrounded by biologically inert zinc sulfide, and encapsulated within a stable polymer, it is still unclear if these toxic ions will impact the use of QDs as clinical contrast agents. It may be of greater concern that QDs, and many other types of nanoparticles, have been found to aggregate, bind nonspecifically to cellular membranes and intracellular proteins, and induce the formation of reactive oxygen species. Pegylated colloidal gold raises less concern because it is not usually dissolved or released under in vivo conditions, but its long-term retention in the liver and spleen could still pose a problem. Detailed toxicology studies of various nanoparticles are an essential step toward their clinical application.

Integration with Robotics, High-Speed Computing, and 3D Graphics

The nanoparticle agents and miniaturized instrumentation can also be used for robotic and minimally invasive surgery, such as endoscopic and laparoscopic procedures to detect and resect solid tumors. With high-speed computing and 3D graphics, microscopic metastases and residual tumor cells could be detected and visualized with unprecedented sensitivity and spatial resolution, enabling the surgeon to completely remove tumors with minimal invasiveness or tissue damage (61). The convergence of nanotechnology with molecular biomarkers and biocomputing holds great promise for individualized cancer treatment in which surgical decisions are based on real-time molecular and cellular information (62).

Summary Points

1. Surgery is currently the most effective procedure in treating human cancers, and the single most important predictor of patient survival for almost all cancers is a complete surgical resection.
2. Nanometer-sized particles such as quantum dots and colloidal gold have novel size-tunable properties that neither discrete molecules nor bulk materials can provide.
3. Nanotechnology's applications in surgical oncology include tumor localization, tumor margin detection, identification of important adjacent structures, mapping of sentinel lymph nodes, and detection of residual tumor cells or micrometastases.
4. Nanoparticle contrast agents can be accumulated in solid tumors through both passive and active targeting mechanisms.
5. Intraoperative imaging overcomes the tissue penetration problem of optical methods.
6. Surface-enhanced Raman scattering (SERS) nanoparticles are approximately 200 times brighter than near-infrared quantum dots and $2-4 \times 10^3$ -fold more intense than organic dyes.
7. A major remaining challenge is to design and develop nanoparticle agents that are accumulated in tumors but are cleared from other organs and tissues.
8. For clinical use, the long-term fate and toxicity of nanoparticles need to be further studied.

Acknowledgments

We are grateful to Drs. Aaron Mohs, Ximei Qian, Andrew Smith, Michael Mancini, Brian Leyland-Jones, Lily Yang, and James Provenzale for insightful discussions. This work was supported by grants from the US National Cancer Institute Centers of Cancer Nanotechnology Excellence (CCNE) Program (U54CA119338) and the Bioengineering Research Partnerships Program (BRP) (R01CA108468). M.D.W. and S.N. are distinguished scholars of the Georgia Cancer Coalition (GCC).

Literature Cited

1. De Grand AM, Frangioni JV. An operational near-infrared fluorescence imaging system prototype for large animal surgery. *Technol Cancer Res Treat* 2003;2:553–62. [PubMed: 14640766]
2. Gebitekin C, Gupta NK, Satur CM, et al. Fate of patients with residual tumour at the bronchial resection margin. *Eur J Cardiothorac Surg* 1994;8:339–42. discussion 342–34. [PubMed: 7946410]
3. Meric F, Mirza NQ, Vlastos G, et al. Positive surgical margins and ipsilateral breast tumor recurrence predict disease-specific survival after breast-conserving therapy. *Cancer* 2003;97:926–33. [PubMed: 12569592]
4. Sienel W, Stremmel C, Kirschbaum A, et al. Frequency of local recurrence following segmentectomy of stage IA nonsmall cell lung cancer is influenced by segment localisation and width of resection margins—implications for patient selection for segmentectomy. *Eur J Cardiothorac Surg* 2007;31:522–27. discussion 527–28. [PubMed: 17229574]
5. Karni T, Pappo I, Sandbank J, et al. A device for real-time, intraoperative margin assessment in breast-conservation surgery. *Am J Surg* 2007;194:467–73. [PubMed: 17826057]

6. Neoptolemos JP, Stocken DD, Dunn JA, et al. Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. *Ann Surg* 2001;234:758–68. [PubMed: 11729382]
7. Sosa JA, Bowman HM, Tielsch JM, et al. The importance of surgeon experience for clinical and economic outcomes from thyroidectomy. *Ann Surg* 1998;228:320–30. [PubMed: 9742915]
8. Dimick JB, Pronovost PJ, Cowan JA, et al. Surgical volume and quality of care for esophageal resection: Do high-volume hospitals have fewer complications? *Ann Thorac Surg* 2003;75:337–41. [PubMed: 12607635]
9. Vaidya A, Hawke C, Tiguert R, et al. Intraoperative T staging in radical retropubic prostatectomy. *Urology* 2001;57:949–54. [PubMed: 11337301]
10. Abraham SC, Fox K, Fraker D, et al. Sampling of grossly benign breast reexcisions: a multidisciplinary approach to assessing adequacy. *Am J Surg Pathol* 1999;23:316–22. [PubMed: 10078923]
11. Alivisatos P. The use of nanocrystals in biological detection. *Nat Biotechnol* 2004;22:47–52. [PubMed: 14704706]
12. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer* 2005;5:161–71. [PubMed: 15738981]
13. Nie SM, Xing Y, Kim GJ, et al. Nanotechnology applications in cancer. *Annu Rev Biomed Eng* 2007;9:257–88. [PubMed: 17439359]
14. Gao XH, Yang L, Petros JA, et al. In-vivo molecular and cellular imaging with quantum dots. *Curr Opin Biotechnol* 2005;16:63–72. [PubMed: 15722017]
15. Liu Z, Cai W, He L, et al. In vivo biodistribution and highly efficient tumor targeting of carbon nanotubes in mice. *Nat Nanotechnol* 2007;2:47–52. [PubMed: 18654207]
16. Heath JR, Davis ME. Nanotechnology and cancer. *Annu Rev Med* 2008;59:251–65. [PubMed: 17937588]
17. Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat Rev Drug Disc* 2008;7:771–82.
18. Akerman ME, Chan WCW, Laakkonen P, et al. Nanocrystal targeting in vivo. *Proc Natl Acad Sci USA* 2002;99:12617–21. [PubMed: 12235356]
19. Gao XH, Cui YY, Levenson RM, et al. In vivo cancer targeting and imaging with semiconductor quantum dots. *Nat Biotechnol* 2004;22:969–76. [PubMed: 15258594]
20. Yu XF, Chen LD, Li KY, et al. Immunofluorescence detection with quantum dot bioconjugates for hepatoma in vivo. *J Biomed Opt* 2007;12:014008. [PubMed: 17343483]
21. Cai WB, Shin DW, Chen K, et al. Peptide-labeled near-infrared quantum dots for imaging tumor vasculature in living subjects. *Nano Lett* 2006;6:669–76. [PubMed: 16608262]
22. Stroh M, Zimmer JP, Duda DG, et al. Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo. *Nat Med* 2005;11:678–82. [PubMed: 15880117]
23. Tada H, Higuchi H, Wanatabe TM, et al. In vivo real-time tracking of single quantum dots conjugated with monoclonal anti-HER2 antibody in tumors of mice. *Cancer Res* 2007;67:1138–44. [PubMed: 17283148]
24. Jain RK. Delivery of molecular medicine to solid tumors: lessons from in vivo imaging of gene expression and function. *J Control Release* 2001;74:7–25. [PubMed: 11489479]
25. Jain RK. Transport of molecules, particles, and cells in solid tumors. *Annu Rev Biomed Eng* 1999;1:241–63. [PubMed: 11701489]
26. Maeda H, Wu J, Sawa T, et al. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release* 2000;65:271–84. [PubMed: 10699287]
27. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer-chemotherapy —mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. *Cancer Res* 1986;46:6387–92. [PubMed: 2946403]
28. Qian XM, Peng XH, Ansari DO, et al. In vivo tumor targeting and spectroscopic detection with surface-enhanced Raman nanoparticle tags. *Nat Biotechnol* 2008;26:83–90. [PubMed: 18157119]
29. Merchant B. Gold, the noble metal and the paradoxes of its toxicology. *Biologicals* 1998;26:49–59. [PubMed: 9637749]

30. Root SW, Andrews GA, Kniseley RM, et al. The distribution and radiation effects of intravenously administered colloidal gold-198 in man. *Cancer* 1954;7:856–66. [PubMed: 13199762]
31. Paciotti GF, Kingston DGI, Tamarkin L. Colloidal gold nanoparticles: a novel nanoparticle platform for developing multifunctional tumor-targeted drug delivery vectors. *Drug Dev Res* 2006;67:47–54.
32. Paciotti GF, Myer L, Weinreich D, et al. Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. *Drug Deliv* 2004;11:169–83. [PubMed: 15204636]
33. James WD, Hirsch LR, West JL, et al. Application of INAA to the build-up and clearance of gold nanoshells in clinical studies in mice. *J Radioanal Nucl Chem* 2007;271:455–59.
34. Connor EE, Mwamuka J, Gole A, et al. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 2005;1:325–27. [PubMed: 17193451]
35. Shukla R, Bansal V, Chaudhary M, et al. Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: a microscopic overview. *Langmuir* 2005;21:10644–54. [PubMed: 16262332]
36. Nie SM, Emory SR. Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. *Science* 1997;275:1102–6. [PubMed: 9027306]
37. Kneipp K, Wang Y, Kneipp H, et al. Single molecule detection using surface enhanced Raman scattering. *Phys Rev Lett* 1997;78:1667–70.
38. Kaushal S, McElroy MK, Luiken GA, et al. Fluorophore-conjugated anti-CEA antibody for the intraoperative imaging of pancreatic and colorectal cancer. *J Gastrointest Surg* 2008;12:1938–50. [PubMed: 18665430]
39. McElroy M, Kaushal S, Luiken GA, et al. Imaging of primary and metastatic pancreatic cancer using a fluorophore-conjugated anti-CA19-9 antibody for surgical navigation. *World J Surg* 2008;32:1057–66. [PubMed: 18264829]
40. Urano Y, Asanuma D, Hama Y, et al. Selective molecular imaging of viable cancer cells with pH-activatable fluorescence probes. *Nat Med* 2009;15:104–9. [PubMed: 19029979]
41. Kamiya M, Kobayashi H, Hama Y, et al. An enzymatically activated fluorescence probe for targeted tumor imaging. *J Am Chem Soc* 2007;129:3918–29. [PubMed: 17352471]
42. Hama Y, Urano Y, Koyama Y, et al. A target cell-specific activatable fluorescence probe for in vivo molecular imaging of cancer based on a self-quenched avidin-rhodamine conjugate. *Cancer Res* 2007;67:2791–99. [PubMed: 17363601]
43. Ogawa M, Kosaka N, Longmire MR, et al. Fluorophore-quencher based activatable targeted optical probes for detecting in vivo cancer metastases. *Mol Pharm* 2009;6:386–95. [PubMed: 19718793]
44. Veisheh M, Gabikian P, Bahrami SB, et al. Tumor paint: a chlorotoxin: Cy5.5 bioconjugate for intraoperative visualization of cancer foci. *Cancer Res* 2007;67:6882–88. [PubMed: 17638899]
45. Kosaka N, Ogawa M, Longmire MR, et al. Multi-targeted multi-color in vivo optical imaging in a model of disseminated peritoneal ovarian cancer. *J Biomed Optics* 2009;14:014023.
46. Longmire M, Kosaka N, Ogawa M, et al. Multicolor in vivo targeted imaging to guide real-time surgery of HER2-positive micrometastases in a two-tumor coincident model of ovarian cancer. *Cancer Sci* 2009;100:1099–104. [PubMed: 19302283]
47. Ohnishi S, Lomnes SJ, Laurence RG, et al. Organic alternatives to quantum dots for intraoperative near-infrared fluorescent sentinel lymph node mapping. *Mol Imaging* 2005;4:172–81. [PubMed: 16194449]
48. Tanaka E, Choi HS, Fujii H, et al. Image-guided oncologic surgery using invisible light: completed preclinical development for sentinel lymph node mapping. *Ann Surg Oncol* 2006;13:1671–81. [PubMed: 17009138]
49. Walls J, Knox F, Baildam AD, et al. Can preoperative factors predict for residual malignancy after breast biopsy for invasive cancer? *Ann R Coll Surg Engl* 1995;77:248–51. [PubMed: 7574313]
50. Kircher MF, Mahmood U, King RS, et al. A multimodal nanoparticle for preoperative magnetic resonance imaging and intraoperative optical brain tumor delineation. *Cancer Res* 2003;63:8122–25. [PubMed: 14678964]

51. Jackson H, Muhammad O, Daneshvar H, et al. Quantum dots are phagocytized by macrophages and colocalize with experimental gliomas. *Neurosurgery* 2007;60:524–29. discussion 529–30. [PubMed: 17327798]
52. Ballou B, Ernst LA, Andreko S, et al. Sentinel lymph node imaging using quantum dots in mouse tumor models. *Bioconjug Chem* 2007;18:389–96. [PubMed: 17263568]
53. Parungo CP, Colson YL, Kim SW, et al. Sentinel lymph node mapping of the pleural space. *Chest* 2005;127:1799–804. [PubMed: 15888861]
54. Parungo CP, Ohnishi S, Kim SW, et al. Intraoperative identification of esophageal sentinel lymph nodes with near-infrared fluorescence imaging. *J Thorac Cardiovasc Surg* 2005;129:844–50. [PubMed: 15821653]
55. Soltesz EG, Kim S, Kim SW, et al. Sentinel lymph node mapping of the gastrointestinal tract by using invisible light. *Ann Surg Oncol* 2006;13:386–96. [PubMed: 16485157]
56. Kim S, Lim YT, Soltesz EG, et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat Biotechnol* 2004;22:93–97. [PubMed: 14661026]
57. Soltesz EG, Kim S, Laurence RG, et al. Intraoperative sentinel lymph node mapping of the lung using near-infrared fluorescent quantum dots. *Ann Thorac Surg* 2005;79:269–77. discussion 269–77. [PubMed: 15620956]
58. Connolly JL, Harris JR, Schnitt SJ. Understanding the distribution of cancer within the breast is important for optimizing breast-conserving treatment. *Cancer* 1995;76:1–3. [PubMed: 8630858]
59. Wind J, Smit EJ, Senan S, et al. Residual disease at the bronchial stump after curative resection for lung cancer. *Eur J Cardiothorac Surg* 2007;32:29–34. [PubMed: 17466532]
60. Pirenne MH, Denton EJ. Accuracy and sensitivity of the human eye. *Nature* 1952;170:1039–42. [PubMed: 13013307]
61. Wang MD, Simons JW, Nie SM. Biomedical nanotechnology with bioinformatics—the promise and current progress. *Proc IEEE* 2007;95:1386–89.
62. Phan JH, Moffitt RA, Stokes TH, et al. Convergence of biomarkers, bioinformatics and nanotechnology for individualized cancer treatment. *Trends Biotechnol* 27:350–58. [PubMed: 19409634]

Glossary

Laparoscopy or laparoscopic surgery	a type of minimally invasive surgery in which operations are performed through small incisions (usually 0.5–1.5 cm) instead of the larger incisions needed in traditional surgical procedures. Minimally invasive surgery performed on the thoracic or chest cavity is called thoracoscopic surgery. Laparoscopic and thoracoscopic surgery belong to the broader field of endoscopy
Computed tomography (CT)	a medical imaging modality that uses x-ray scanning and computed image reconstruction to obtain spatially resolved information on relatively dense or “opaque” structures, such as bone, inside the body
Magnetic resonance imaging (MRI)	a medical imaging modality that measures proton spin relaxations (T1 and T2) in primarily water molecules for three-dimensional reconstruction of soft tissue, such as the brain
Positron emission tomography (PET)	a molecular imaging modality that uses isotopes (such as fluorine-18) for high-sensitivity detection of tumor and other diseases at medium-to-low spatial resolutions (~1 mm)
Quantum dots (QDs)	particles on the nanometer scale with quantum-confinement properties such as size-tunable light emission, often made of semiconductors such as CdSe

Surface-enhanced
Raman scattering
(SERS)

a phenomenon in which the efficiency of inelastic light scattering is dramatically amplified by the use of metal nanostructures such as colloidal gold

Figure 1. Use of quantum dots (QDs) and related nanoparticles for direct visualization of tumors during surgery. Shown here are two color images of a pig lung obtained with (a) bright-field visible illumination and (b) near-UV illumination. Note that the fluorescence image shows a bright spot (*orange*) that corresponds to injected QDs.

Figure 2.

Schematic diagram showing nanoparticle accumulation in solid tumors through both active and passive targeting mechanisms. In the passive mode, nanometer-sized particles such as SERS nanoparticles and quantum dots accumulate at tumor sites through an enhanced permeability and retention (EPR) effect (24–27). For active tumor targeting, nanoparticles are conjugated to molecular ligands, such as antibodies and peptides, to recognize protein targets that are overexpressed on the surface of tumor cells, such as the epidermal growth factor receptor (EGFR), the transferrin receptor, or the folate receptor. (Courtesy of X. Qian and S. Nie, Emory University, Atlanta, GA; adapted for publication by Annual Reviews.)

a Targeted **b Nontargeted** **Figure 3.**

In vivo cancer targeting and surface-enhanced Raman scattering (SERS) detection by using ScFv-antibody conjugated gold nanoparticles that recognize the tumor biomarker EGFR. Photographs show a laser beam focusing on (a) the tumor site or (b) the anatomical location of liver. SERS spectra were obtained from the tumor and liver locations by using (a) targeted and (b) nontargeted nanoparticles. Two nude mice bearing human head and neck squamous cell carcinoma (Tu686) xenograft tumor (3 mm diameter) received 90 μ L of ScFv EGFR-conjugated SERS tags or pegylated SERS tags. Adapted with permission from Reference 28.