RECENT DEVELOPMENTS IN CANCER THERAPY BY THE USE OF NANOTECHNOLOGY

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Cancer is a leading cause of death worldwide. The biological application of nanoparticle 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 multiple simultaneous profiling of tumour biomarkers and for detection of multiple genes and matrix RNA with fluorescent in-situ hybridisation. A solid or hollow structure, with diameter in the 1 – 1,000 nanometre range nanoparticles have large surface areas and functional groups for conjugating to multiple diagnostic (e.g., optical, magnetic or radioisotopic,) and therapeutic (e.g., anticancer) agents. Bioaffinity of nanoparticle probes have led for molecular and cellular imaging, targeted nanoparticle drug for cancer therapy and integrated nanodevices for early cancer detection and screening. In this review, we give an overview of the use of bioconjugated nanoparticles for the delivery and targeting of anticancer drugs.

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1. Introduction

In modern scenario naanotechnology is used to provide more accurate and timely medical information for diagnosing disease, and miniature devices that can administer treatment automatically if required. It is used worldwide in the treatment of diabetes (1), respiratory diseases (2), Cancer (3) etc. like precarious diseases. Cancer is a complex disease occurring as a result of a progressive accumulation of genetic and epigenetic changes that enable escape from normal cellular and environmental control (4) . Cancer is a generic term for a group of more than 100 diseases that can affect any part of the body. Other terms used are malignant tumours and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells which grow beyond their usual boundaries, and which can invade adjoining

parts of the body and spreads to other organs, a process referred to as metastasis. Metastases are the major cause of death from cancer (5).

In this new era of world a new technology is emerging that have a diagnostic and therapeutic potential against this disease. In this review we are trying to focus on the role nanoscience in cancer diagnosis and therapy. It is expected that nanotechnology will be developed at several levels: materials, devices and systems. At present, the nanomaterials level is the most advanced in scientific knowledge as well as in commercial applications. A decade ago, nanoparticles were studied because of their size-dependent, physical and chemical properties (6). 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 (7, 8). Traditionally, the most common cancer treatments were limited to chemotherapy, radiation, and surgery. Limitations in cancer treatment are a result of current challenges seen in established cancer therapies, including lack of early disease detection, nonspecific systemic distribution, inadequate drug concentrations reaching the tumor, and inability to monitor therapeutic responses. Poor drug delivery and residence at the target site leads to significant complications, such as multi-drug resistance (9).

The field of nanotechnology was first predicated by Professor Richard P. Feynman in 1959 (Nobel laureate in physics, 1965) with his famous Cal Tech Lecturer entitled, “There’s plenty of Room at the Bottom” (10). Nanotechnology has achieved the status as one of the critical research endeavors of the early 21st century, as scientists harness the unique properties of atomic and molecular assemblages built at the nanometer scale.Ability to manipulate the physical, chemical, and biological properties of these particles affords researchers the capability to rationally design and use nanoparticles for drug delivery, as image contrast agents, and for diagnostic purposes (11). New technologies using metal and semiconductor nanoparticles are also under intense development for molecular profiling studies and multiplexed biological assays (12-16).

Recently functional nanoparticles have developed that are covalently linked to biological molecules such as peptides, proteins, nucleic acids, or small-molecule ligands (17-24). Medical applications have also appeared, such as the use of superparamagnetic iron oxide nanoparticles as a contrast agent for lymph node prostate cancer detection (25) and the use of polymeric nanoparticles for targeted gene delivery to tumor vasculatures (26).

2. Cancer Disease

Cancer is a leading cause of death worldwide. From a total of 58 million deaths worldwide in 2005, cancer accounts for 7.6 million (or 13%) of all deaths. More than 70% of all cancer deaths in 2005 occurred in low and middle-income countries. Deaths from cancer in the world are projected to continue rising, with an estimated 9 million people dying from cancer in 2015 and 11.4 million dying in 2030.The most frequent cancer types worldwide are (a) among men: lung, stomach, liver, colorectal, oesophagus and prostate; and (b) among women: breast, lung stomach, colorectal and cervical (27).

3. Biomarkers of Cancer

Biomarkers or biomolecule markers include altered or mutant genes, RNAs, proteins, carbohydrates, lipids, and small metabolite molecules, and their altered expressions that are correlated with a biological behavior or a clinical outcome. Most cancer biomarkers are discovered by molecular profiling studies based on an association or correlation between a molecular signature and cancer behavior. In the cases of both breast and prostate cancer, a deadly step is the appearance of so-called lethal phenotypes, such as bone-metastatic, hormone-independent, and radiationand chemotherapy-resistant phenotypes. It has been hypothesized that each of these aggressive behaviors or phenotypes could be understood and predicted by a defining set of biomarkers (28). Biomarkers have tremendous therapeutic impact in clinical oncology, especially if the biomarker is detected before clinical symptoms or enable real-time monitoring of drug response. Protein signatures in cancer provide valuable information that may be an aid to more effective diagnosis, prognosis, and response to therapy. The recent progress of proteomics has opened new avenues for cancer-related biomarker discovery. Advances in proteomics are contributing to the understanding of patho-physiology of neoplasia, cancer diagnosis, and anticancer drug discovery. Continued refinement of techniques and methods to determine the abundance and status of proteins holds great promise for the future study of cancer and the development of cancer therapies (29, 30).

Early diagnosis of cancer is difficult because of the lack of specific symptoms in early disease and the limited understanding of etiology and oncogenesis. For example, blood tumor

markers for breast cancer such as cancer antigen (CA) 15-3 are useless for early detection because of low sensitivity (31). More than 98% of cervical cancer is related to human papilloma virus (HPV) infection. The identification and functional verification of host proteins associated with HPV E6 and E7 oncoproteins may provide useful information for the understanding of cervical carcinogenesis and the development of cervical cancer-specific markers (32). There is a critical need for expedited development of biomarkers and their use to improve diagnosis and treatment for cancer (33).

4. Nanotechnology in cancer therapy:

4.1 Quantum dots

Quantum dots are novel semiconductor nanocrystals with broad potential for use in various applications in the research, management, and treatment of cancer (34, 35). Quantum dots owe their fluorescence emission to electron excitation (36). To overcome the limitations of imaging in the visible spectra, such as autofluorescence from tissues like intestine and suboptimal tissue penetrance, some investigators have constructed quantum dots that fluoresce in the near infrared (NIR) spectra (700–1000 nm)(37). This property potentially makes NIR quantum dots attractive for in vivo imaging (38, 39, 40, 41-44). NIR quantum dots have been used for in vivo lymphatic mapping in several animal models (38, 41, 42). Because of their composition of heavy metals and previous reports of cytotoxicity, the potential use of quantum dots in humans may be limited (45). Uncoated or nonpolymer-protected quantum dots are unstable when exposed to ultraviolet (UV) radiation and have been shown to release toxic cadmium (45). Modification of quantum dots (i.e., PEGylation and micelle encapsulation) may limit the release of toxic metals in response to UV radiation (46).

4.2 Gold nanoparticles

Colloidal gold nanoparticles are another attractive platform for cancer diagnosis and therapy (47).These are attractive because gold has been approved and used for treatment of human disease. Gold nanoparticles have been used as contrast agents in vitro based on their ability to scatter visible light(48). Sokolov et al. successfully used gold nanoparticles conjugated to EGFR antibodies to label cervical biopsies for identification of precancerous lesions(48). Photoacoustic tomography has been used to image gold nanoparticles to a depth of 6 cm in experiments using gelatin phantoms(49). Based on this property, photoacoustic tomography may be useful for in vivo imaging of gold nanoparticles. Gold nanoparticles also have been

used as a platform for novel experimental cancer therapy. In a subcutaneous model of colon cancer, it was demonstrated that systemically delivered gold nanoparticles (size, approximately 33 nm) conjugated to tumor necrosis factor (TNF) accumulated in tumors(47).

5. Alkylating Agents

Alkylating agents are able to target tumor cells in various and multiple phases of the cell cycle and are better suited for the treatment of slow growing cancers. Alkylating agents stunt tumor growth by cross-linking guanine nucleobases resulting in abnormal base pairing or DNA strand breaks. Tumor DNA is unable to uncoil and separate which prevents the cell from dividing. Typically, alkylating agents act nonspecifically requiring conversion into active substances *in vivo* (50). Cisplatin is one of the most widely used antineoplastic alkylating agents for the treatment of certain cancers such as testicular, ovarian carcinomas, and carcinomas of the head and neck (51). The aqua cisplatin-DPPG micelles were converted into liposomes 100-160 nm in diameter by mixing with vesicle forming lipids followed by dialysis and extrusion through membranes, entrapping and encapsulating cisplatin with a very high yield. Therapeutic efficacy was determined utilizing a human breast carcinoma MCF-7 bearing murine model. Significant MCF-7 tumor regression due to apoptosis was seen after intravenous injections of the liposome encapsulated cisplatin(52).

6. Lipid/Polymer

Positively charged lipid-based nanoparticles are known to trigger strong immune responses when injected into the body. This can be problematic when attempting to use this type of nanoparticle as a drug delivery vehicle. Lipid-based cationic nanoparticles (53) are a new promising option for tumor therapy, because they display enhanced binding and uptake at the neo-angiogenic endothelial cells, which a tumor needs for its nutrition and growth. By loading suitable cytotoxic compounds to the cationic carrier, the tumor endothelial and consequently also the tumor itself can be destroyed. For the development of such novel anti-tumor agents, the control of drug loading and drug release from the carrier matrix is essential. Screening of different matrices for a given drug may be useful for fast and efficient optimization of drug/lipid combinations in pharmaceutical development.

In a new therapeutic approach, targeted drug delivery is performed not to the tumor itself, but to the neo-angiogenic blood vessels that the tumor stimulates to grow for its nutrition. This procedure is based on the observation that cationic liposomes show enhanced

binding and uptake at tumor endothelial cells. In this context Munich Biotech AG has developed a series of cationic, lipid based, nanoparticulate agents for tumor therapy and diagnosis. Hassan *et al*. proposed the utilization of nanoparticles for intravascular injections for cancer treatment and/or diagnosis and extravascular injections to provide controlled release of the drug at the site of injection for prolonged drug effects with minimized multiple dosing.

7. Dendrimers

Dendrimers (54, 55) are synthetic, nanometer-sized macromolecules that can be modified to suit a specific application. Several types of dendrimers are commercially available, among which Polyamidoamine (PAMAM) dendrimers are the most extensively studied for biological applications (56,57). They have a unique architecture based on *â*-alanine subunits with primary amine groups on the surface that are available for the attachment of several types of biological material (58) . Their aqueous solubility and biocompatibility are well suited to carry ligands, fluorochromes, and drugs for targeting, imaging (59), and drug delivery (60-63). Some of the issues associated with immunoconjugates, such as decreased solubility and reduced binding efficiency, can be addressed using dendrimers as carrier molecules attached to antibodies (64). Several groups have studied the conjugation of dendrimers to antibodies for targeting applications (65, 66). Antibody-dendrimer conjugates have been used for radiolabeling (67) with minimal loss of immunoreactivity (65). Some research shows that the anti-PSMA antibody J591 when conjugated to a dendrimer containing a fluorochrome, can be used for targeting prostate cancer and has potential as an efficient delivery system for therapeutics and imaging agents (68).

8. Conclusions

Nanotechnology is definitely a medical boon for diagnosis, treatment and prevention of cancer disease. It will radically change the way we diagnose, treat and prevent cancer to help meet the goal of eliminating suffering and death from cancer. The integration of nanotechnology into cancer diagnostics and therapeutics is a rapidly advancing field, and there is a need for wide understanding of these emerging concepts. The development of new nanoscale platforms offers great potential for improvements in the care of cancer patients in the near future. Areas of greatest clinical impact likely include novel, targeted drug-delivery vehicles, molecularly targeted contrast agents for cancer imaging, targeted thermal tumor

ablation, and magnetic field targeting of tumors. Because nanotechnology is a rapidly progressing field, future advances in nanotechnology research and development likely will be associated with the further development of novel, high-impact approaches to cancer diagnosis and treatment.

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References

1. Awadhesh Kumar Arya, Lalit Kumar, Deepa Pokharia, Kamlakar Tripathi. Applications of nanotechnology in diabetes: Digest Journal of Nanomaterials and Biostructures **3**, 221 – 225 (2008).

2. Ulrich Pison, Tobias Welte, Michael Giersig, David A. Groneberg Nanomedicine for respiratory diseases: European Journal of Pharmacology, **533,** 341-350 (2006).

3. Priya Pathak , V. K. Katiyar , Multi-Functional Nanoparticles and Their Role in Cancer Drug Delivery – A Review: 10.2240/azojono0114, **3**, 3-17 (2007).

4. Weinberg, R.A. ,How cancer arises: Sci. Am. ,**275,** 62-70,(2006)

5. Pan American Health Organisation, Regional Office of the World Health Organisation (WHO),Cancer(WHO Fact Sheet No. 297), 24/Oct/2006.

6. C. B. Murray, C. R. Kagan, M. G. Bawendi, Annu Rev Mater Sci **30**, 545-610 (2000).

7. Jain K. ,Nanotechnology in clinical laboratory diagnostics: Clin Chim Act **358,**37–54 (2005).

8. Ferrari M., Cancer nanotechnology: opportunities and challenges: Nat Rev Cancer, **5**, 161–71 (2005)

9. B. Ehdaie, Int. J. Biol. Sci., **3**, 108-110 (2007).

10. James R Baker , Jr., Antonia Quintana, Lars Pehlerel, Mark Banazak- Holl al, Donold Tomalia, Ewa Raczka. The synthesis and testing of anti- acancer therapeutic nanodevices: Biomedical microdevices, **3:1**, 61-69 (2001).

11. Scott E. Mc Neil , Nanotechnology for Biologist: Journal of Leukocyte Biology, **78,** 585-591 (2005).

12.Walt DR., Imaging optical sensor arrays: Curr. Opin. Chem. Biol. **6**, 689–95 (2002).
13. Nicewarner-Pena SR, Freeman RG, Reiss BD, He L, Pena DJ, et al., Submicrometer metallic barcodes : Science, **294**, 137–141(2001).
14 . Cunin F, Schmedake TA, Link JR, Li YY, Koh J, et al., Biomolecular screening with encoded porous-silicon photonic crystals: Nat. Mater , **1**,39–41(2002).
15. Dejneka MJ, Streltsov A, Pal S, Frutos AG, Powell CL, et al., Rare earthdoped glass microbarcodes: Proc. Natl. Acad. Sci. USA, **100** ,389–393 (2003).
16. Cao YWC, Jin RC, Mirkin CA. , Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection: Science , **297**,1536–1540 (2002).
17. Alivisatos P. ,The use of nanocrystals in biological detection: Nat. Biotechnol, **22**, 47–52 (2004).
18. Alivisatos AP., Semiconductor clusters, nanocrystals, and quantum dots: Science, **271**, 933–937 (1996).
19 . Alivisatos AP, Gu WW, Larabell C., Quantum dots as cellular probes: Annu. Rev. Biomed. Eng., **7**, 55–76 (2005).
20. Pinaud F, Michalet X, Bentolila LA, Tsay JM, Doose S, et al., Advances in fluorescence imaging with quantum dot bio-probes: Biomaterials, **27**, 1679–1687 (2006).
21. Michalet X, Pinaud FF, Bentolila LA,Tsay JM, Doose S, et al., Quantum dots for live cells, in vivo imaging, and diagnostics: Science **307**, 538–544 (2005).
22 . Gao XH, Yang LL, Petros JA, Marshal FF, Simons JW, Nie SM**.,** In vivo molecular and cellular imaging with quantum dots: Curr. Opin. Biotechnol**, 16**, 63–72 (2005).
23.Smith AM, Gao X, Nie S., Quantum dot nanocrystals for in vivo molecular and cellular imaging: Photochem. Photobiol,. **80**, 377–385 (2004).
24. Chan WCW, Maxwell DJ, Gao XH, Bailey RE, Han MY, Nie SM., Luminescent quantum dots for multiplexed biological detection and imaging: Curr. Opin. Biotechnol, **13**, 40–46 (2002).
25.Action of clinically occult lymph-node metastases in prostate cancer*:* N. Engl. J. Med, **348**, 2491–2499 (2003).
26. Hood JD, Bednarski M, Frausto R, Guccione S, Reisfeld RA, et al., Tumor regression by targeted gene delivery to the neovasculature: Science **296**, 2404–2407 (2002).
27. Pan American Health Organisation , Regional Office of the World Health Organisation (WHO),Cancer(WHO Fact Sheet No. 297), 24/Oct/2006.

28. Shuming Nie, Yun Xing, Gloria J. Kim, and JonathanW. Simons, Nanotechnology Applications in Cancer: Annu. Rev. Biomed. Eng.,**9**, 257-288 (2007).

29. W. C. Cho, Sci. J. **56**, 14-17 (2004).

30. W. C. Cho. Research progress in SELDI-TOF MS and its clinical applications. Sheng Wu Gong Cheng Xue Bao **22**, 871-876 (2006).

31 W. C. S. Cho, Molecular Cancer **6**, 25 (2007)

32. E. K. Yim, J. S. Park, Expert Rev Proteomics **3**, 21-36 (2006).

33. Alok Kumar Singha, Anjita Pandeyb, Rajani Raib, Mallika Tewarib, H.P. Pandeya, H.S. Shukla, Nanomaterials emerging tool in cancer diagnosis and treatment: Digest Journal of Nanomaterials and Biostructures , **3**, 135 – 140 (2008).

34. Bruchez M Jr., Moronne M, Gin P, Weiss S, Alivisatos AP., Semiconductor nanocrystals as fluorescent biological labels: Science, **281**, 2013–2016 (1998) .

35. Seydel C. Quantum dots get wet. Science, **300**, 80–81.(2003).

36. Alper J. Shining a light on cancer research. NCI Alliance for Nanotechnology in Cancer USA, (2005).

37. Frangioni JV., In vivo near-infrared fluorescence imaging:Curr Opin Chem Biol. **7**, 626–634.(2003).

38. Parungo CP, Ohnishi S, De Grand AM, et al., In vivo optical imaging of pleural space drainage to lymph nodes of prognostic significance: Ann Surg Oncol, **11**, 1085–1092 (2004) .

39. Gao X, Cui Y, Levenson RM, Chung LW, Nie S., In vivo cancer targeting and imaging with semiconductor quantum dots: Nat Biotechnol, **22**, 69–976 (2004).

40. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A., In vivo imaging of quantum dots encapsulated in phospholipid micelles: Science. **298,**1759–1762. (2002).

41. Parungo CP, Colson YL, Kim SW, et al., Sentinel lymph node mapping of the pleural space: Chest, **127**, 1799– 1804 (2005) .

42. Parungo CP, Ohnishi S, Kim SW, et al., Intraoperative identification of esophageal sentinel lymph nodes with nearinfrared fluorescence imaging: J Thorac Cardiovasc Surg., **129**, 844–850 (2005).

43. Stroh M, Zimmer JP, Duda DG, et al., Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo: Nat Med. **11**, 678–682(2005).

44. Morgan NY, English S, Chen W, et al., Real time in vivo non-invasive optical imaging using near-infrared fluorescent quantum dots: Acad Radiol, **12**, 313–323 (2005) .

45. Derfus AM, Chan WC, Bhatia SN., Probing the cytotoxicity of semiconductor quantum dots: Nano Lett. **4**, 11–18 (2004).
46. Gao X, Yang L, Petros JA, Marshall FF, Simons JW, Nie S. ,In vivo molecular and cellular imaging with quantum dots: Curr Opin Biotechnol, **16**, 63–72 (2005).
47. Paciotti GF, Myer L, Weinreich D, et al., Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery: Drug Deliv. **11**,169–183 (2004) .
48. Sokolov K, Follen M, Aaron J, et al., Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles:Cancer Res. **63**, 1999–2004 (2003).
49. Copland JA, Eghtedari M, Popov VL, et al., Bioconjugated gold nanoparticles as a molecular based contrast agent: implications for imaging of deep tumors using optoacoustic Tomography,. Mol Imaging Biol., **6**, 341–349 (2004).

50. What is chemotherapy: types of chemotherapy. May 30, 2006. www.chemocare.com/whatis/types_of_chemotherapy.asp
51. Kostova, I.,Platinum complexes as anticancer agents: Recent Pat Anti-Can Drug Discov 1: 1-22 (2006).
52.Boulikas, T.: US2003185879A1 (2003).
53. L. P. Cavalcanti, O. Konovalov, I. L. Torriani, H. Haas, Drug loading to lipid-based cationic nanoparticles: Nucl. Instr. and Meth. in Phys. Res., **238** , 290–293 (2005).
54. Newkome, G. R., Moorefield, C. N., and Vogtle, F., Dendrimers and Dendrons: Concepts, Syntheses, Applications:Wiley-VCH, Weinheim, Germany (2001).
55. Fre´chet, J. M. J., and Tomalia, D. A., Dendrimers and other dendritic polymers: Wiley, Chichester, UK. (2002).
56. Patri, A. K., Majoros, I., and Baker, J. R., Jr., Dendritic polymer macromolecular carriers for drug delivery: Curr. Opin. Chem. Biol. **6,** 466-471 (2002).
57. Cloninger, M. J. Biological applications of dendrimers : Curr. Opin. Chem. Biol. ***6***, 742-748 (2002).
58. Singh, P. ,Terminal groups in Starburst dendrimers: activation and reactions with proteins: Bioconjugate Chem**. 9**, 54-63(1998) .
59. Wu, C., Brechbiel, M. W., Kozak, R. W., and Gansow, O. A., Metal-chelate-dendrimer-antibody constructs for use in radioimmunotherapy and imaging: Bioorg. Med. Chem. Lett**. 4**, 449-454 (1994).

60. Kojima, C., Kono, K., Maruyama, K., and Takagishi, T., Synthesis of polyamidoamine dendrimers having poly- (ethylene glycol) grafts and their ability to encapsulate anticancer drugs: Bioconjugate Chem. **11,** 910-917 (2000).
61. Esfand, R., and Tomalia, D. A., Poly(amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications: Drug Discovery Today, **6**, 427-436 (2001).
62. Quintana, A., Raczka, E., Piehler, L., Lee, I., Myc, A., Majoros, I., Patri, A. K., Thomas, T., Mule, J., and Baker, J. R., Jr. Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor: Pharm. Res**. 19**, 1310-1316 (2002).
63. Baker, J. R., Quintana, A., Piehler, L. T., Banazak-Holl, M., Tomalia, D., and Raczka, E. The synthesis and testing of anti-cancer therapeutic nanodevices: Biomed. Microdevices, ***3***, 61-69 (2001).
64. Ong, K. K., Jenkins, A. L., Cheng, R., Tomalia, D. A., Durst, H. D., Jensen, J. L., Emanuel, P. A., Swim, C. R., and Yin, R. Dendrimer enhanced immunosensors for biological detection: Anal. Chim. Acta, **444**, 143-148 (2001).
65. Kobayashi, H., Sato, N., Saga, T., Nakamoto, Y., Ishimori, T., Toyama, S., Togashi, K., Konishi, J., Brechbiel, M. W., Monoclonal antibody-dendrimer conjugates enable radiolabeling of antibody with markedly high specific activity with minimal loss of immunoreactivity: Eur. J. Nucl. Med. ***27***, 1334-1339 (2000).

66. Wu, G., Barth, R. F., Yang, W., Chatterjee, M., Tjarks, W., Ciesielski, M. J., and Fenstermaker, R. A. Site-specific conjugation of boron-containing dendrimers to anti-EGF receptor monoclonal antibody cetuximab (IMC-C225) and its evaluation as a potential delivery agent for neutron capture therapy: Bioconjugate Chem**. 15**, 185-194 (2004) .
67. Roberts, J. C., Adams, Y. E., Tomalia, D. A., Mercer-Smith, J. A., and Lavallee, D. K., Using Starburst Dendrimers as Linker Molecules to Radiolabel Antibodies: Bioconjugate Chem. **1,** 305-308 (1990).
68. Patri, A. K., Thomas, T., Baker, J. R., Jr., Bander, N. H., Antibody-dendrimer conjugates for targeted prostate cancer therapy: Polym. Mater. Sci. Eng. **86**, 130 (2002).