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Quantum dots for diagnosis of cancers

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ABSTRACT

The most widely researched and investigated disease, both medically and scientifically, in the current era is the formidable disease cancer. The chances of successful treatment and hence the curability increases if it is diagnosed at an early stage. This can be done only by increasing awareness amongst people about its early diagnosis and screening tests. Cancer screening exams refers to the medical tests to identify people who have disease, often before symptoms of the illness occur. These tests help detecting cancer at its earliest stage when the chances for curing the disease are greatest. Advancements in nanotechnology have made the early screening of cancer possible. In this review, we have discussed the developments in nanotechnology that have encouraged the more recent innovative solutions for early diagnosis and treatment of cancer. Quantum dots, nanometer-sized semiconductors, are the new class of novel biosensors, now being exclusively employed as alternative fluorescent probes due to their unique properties, such as intense and stable fluorescence for a longer time, resistance to photobleaching, large molar extinction coefficients, and highly sensitive detection, due to their ability to absorb and emit light very efficiently. Their size approximates that of individual biomolecules, which offers unique possibilities for the ultrasensitive detection of cancer in persons' serum, tissues, and other body fluids, when tagged with specific antibodies against specific tumor markers. In this review, we have account briefly the applications of semiconductor QDs employed for the early screening and diagnosis of cancer biomarkers between the years 2009-2012. We believe that this review will enable workers in the field to devise new applications of these materials for the early detection of cancer, and ultimate reduction in incidence of the disease. Copyright © 2013 VBRI press.

Keywords: Biomarkers; cancer; core/shell; detection; diagnosis; imaging; quantum dots; targeting; therapy.

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1. Introduction

Cancer continues to be a major problem in the world for the past many years. Early diagnosis and complete treatment of cancer is the corner-stone of the cancer prevention and control strategy, while inappropriate diagnosis and irregular/incomplete treatment with anti-cancer therapy may lead to complications, disease spread and emergence of drug-resistant cancer. In order to ensure proper cancer diagnosis and address the problems of emergence and spread of drug-resistant cancer, it is essential to have complete information of cancer cases.

Cancer is defined as an abnormal and uncontrolled cell growth due to the accumulation of specific genetic and epigenetic defects, both environmental and hereditary in origin, where the cells forget how to die, unlike normal cells which multiply only when the body needs them and die when they are of no use. When cells divide in an unregulated fashion, there is formation of a tumor mass which later gets out of control. The formed tumor cells become resistant to apoptosis and other antigrowth defenses within the body, which ultimately spread to other body organs, and become difficult to be treated **[1]**. Cancer may develop in any part of the body, including any tissue or organ. Various common types of cancers have been reported, including lung, prostate, breast, ovarian, hematologic, skin, and colon cancer, etc. Environmental factors, such as tobacco smoke, certain chemicals, ionizing radiation, sunlight, alcohol, and genetic factors, such as inherited mutations in oncogenes, tumor suppresser genes and autoimmune dysfunction, are responsible for the development of cancer. Bacterial and viral infections also account strongly for stomach and cervical cancers, respectively. As per recent data, a total of 1,638,910 new cancer cases and 577,190 deaths from cancer are estimated to have occurred in the United States in the year 2012. There has been an overall decline of 0.6% per year in cancer incidence rates in men, with no change in women, whereas cancer death rates decreased by 1.8% per year in men, and by 1.6% per year in women, in the five years from 2004 to 2008 **[2]**. Due to the various new and advanced techniques developed for the early diagnosis of cancer, the American Cancer Society (ACS) assessed an increase in the average 5-year survival rate for all cancers for the years 1996–2004 to 66%, compared to 50% for the years 1975–1977, whereas the 5-year survival for certain cancers, such as liver, pancreatic, and lung still remains very low (6%–16%). Mainly the amendment in proto-oncogenes, tumor suppressor genes and DNA repair genes contributes to the alteration in normal cell functioning, along with the mismanagement of cell proliferation events, resulting in the development of cancer genotype and phenotype, which is resistant to the natural and inherent death mechanisms. Oncogene causes those cells to survive which are supposed to die, and activation of oncogenes occurs due to the mutation of normal genes, which further affects cell growth and their differentiation. This is responsible for the activation or excess levels of a normal gene product, causing gene rearrangements, point mutations, leading to disturbances in molecular pathways regulating cell growth, increase in the cell division, disturbances in cell growth mechanisms and their survival, metastasis, and, ultimately, tumor formation. This tumor mass turns into a more aggressive disease: cancer. Certain carcinogenic agents cause mutations in proto-oncogenes to become oncogenes. Although there is a significant role of genetic and genome based technologies in the diagnosis and prognosis of cancer, the emanating biosensor technologies hold promise, and have now been widely employed for early cancer diagnosis and therapy **[1, 3]**.

Tumor suppressor genes (TSGs) or anti-oncogenes are mainly the transcription factors which suppress process mitosis (a cell division process) and, hence, tumor formation, by blocking unscheduled cell growth. The commonly involved TSGs in cancer are retinoblastoma protein (Rb), BRCA1/2, and p53. Rb regulates cell division. Inactivation of the Rb gene leads to point mutations and deletions, and the major cause of many cancers is mutation of the Rb gene **[4-6]**. The functioning of normal cells is controlled by the apoptosis complex, triggered by the p53 tumor suppressor protein, which mainly regulates programmed cell death. Nearly half of all cancers involve altered p53 genes. Brain, breast, colon, lung, hepatocellular carcinomas and leukemia are found to be associated with the mutations in p53. A DNA repair enzyme, BRCA1 carries out the proofreading of newly replicated DNA for the presence of mutations and removes replication errors, if any, before the cell divides. About 50% of hereditary breast cancers and 80%–90% of hereditary breast and ovarian cancers are due to BCRA1 gene mutations **[7, 8]**. Thus, cancer results from cumulative mutations of proto-oncogenes and suppressor genes, which together cause the unregulated growth of cells. Therefore, we conclude that the mechanism of cancer involves simultaneous occurrence of two processes leading to cellular malfunctioning occurring at the same time: (i) permanent enabling of cellular replication due to genetic mutation or chromosomal translocation, and (ii) permanent disabling of the apoptosis complex, the suicide complex. The malfunctioning of these two cellular processes leads to uncontrolled cell division, resulting in a cluster of unspecialized cells committing to divide, which ultimately becomes larger and larger, and releases chemicals to promote the formation of malignant tumor, which starts damaging the surrounding tissue by withdrawing essential nutrients and displacing healthy cells **[9]**. For example, the human epidermal growth factor receptor HER-2 gene is responsible for making HER-2 proteins (receptors on breast cells) which control the development of the breast. However, in case of any abnormality in the HER-2 gene, too many copies of this gene are formed, called as amplification of the gene, which results in an overexpression of the HER-2 protein, causing the breast cells to grow aggressively, with the formation of tumor, leading to the development of breast cancer. A recombinant humanized monoclonal antibody, named trastuzumab, developed against HER-2, is nowadays being used as a standard treatment for breast cancer **[10]**.

2. Cancer biomarkers

The National Cancer Institute defines a tumor marker as "A biological molecule found in blood, other body fluids or tissues that is a sign of a normal or abnormal process or of a condition or disease" **[11]**. Tumor markers could be a broad range of biochemical entities, basically the endogenous

proteins, lipids, sugars, nucleic acids or other cytogenetic parameters present in blood or other body fluids, tumor tissues whose changes in amount, any abnormality or modifications symbolizes the tumor state, its progress with time and response to various therapies. These tumor associated antigens are very helpful in cancer diagnosis and markers of how well the body responds to a treatment for a disease or condition **[3, 12]**. The most challenging task in cancer prognosis is to develop a connecting link between cancer biomarkers and the clinically developed therapies for non-invasive detection of tumors at an early stage. Effective tumor markers are in great demand since they have the potential to reduce cancer mortality rates by facilitating diagnosis of cancers at early stages. During the last decade, an improved understanding of carcinogenesis, tumor progression, and advancement in nanotechnology have revealed a large number of potential tumor markers. A partial list of tumor biomarkers is presented in **Table 1**.

Table1. Common biomarkers utilized for cancer detection **[13-15].**

Cancer Type	Biomarker
Breast	BRCAI, BRCA2, CA15-3, CA 125, CA 27.29, MUC1, NY-BR-I, ING-I, HER2/NEU, ER/PR
Colon	CEA, EGF, p53
Esophageal	SCC
Liver	AFP, CEA
Lung	CEA, CA19-9, SCC, NSE, NY-ESO-I, CYFRA21-1
Melanoma	Tyrosinase, NY-ESO-I
Ovarian	CA125, HCG, p53, CEA, CA 549, CASA, CA 19-9, CA 15-3, MCA, MOV-I, TAG72
Prostate	PSA, PAP

3. Quantum dots in early diagnosis of cancer

It becomes difficult to treat cancer when the cancer cells have metastasized into a certain sized tumor, which essentially proves the desirability of the early prognosis of cancer. The commonly employed methods for diagnosis of cancer include chemotherapy, immunotherapy, surgery medical imaging, enzyme linked immunosorbent assay (ELISA) and tissue biopsy, etc., but these are less sensitive, and are reliable only for early-stage cancer detection **[16]**. Colloidal quantum dots (QDs) are bright, photostable fluorophores, a few nanometers in diameter. Also, the nanometer scale is the scale of biological function, i.e. it is the same size range as that of enzymes, DNA and other cellular components **[17]**, which makes possible to employ water-soluble QD complexes to target and image tumor cells. QDs are now being used as alternative fluorescent probes in the biological world. Application of traditional fluorophores, e.g. organic dyes and fluorescent proteins, is limited due to their narrow absorption range, broad emission spectra and short fluorescent lifetimes, whereas QDs, which exhibit broad absorption and narrow emission spectra, are less susceptible to photobleaching than organic dye molecules, due to their inorganic composition. Their absorption and emission wavelengths are tunable by particle size. Their unique optical properties makes them strongly attractive as *in vivo* and *in vitro* fluorophores in various biological and medical applications, including multiplexed imaging of live tissues, detection and therapy of various diseases, including cancer **[18-24]**. The best materials for quantum dots are considered to be cadmium sulfide, and cadmium selenide, but these can be highly toxic due to the leaching of cadmium atoms in the biological system. Hence, various encapsulation techniques need to be employed in order to enhance biocompatibility and hence the bio-applicability of these nanoparticles (NPs) **[25]**.

Semiconductor QDs have proven their potential in biomedical fields due to their excellent optical properties. They exhibit size-dependent discrete energy levels. The energy gap increases with decrease in the size of the nanocrystal, thus yielding a size-dependent rainbow of colors **(Fig. 1)**. Light wavelengths from ultraviolet to infrared region can be achieved with variation of the size and composition of QDs, making them highly suited for the simultaneous examination of multiple events and molecules **[26]**.

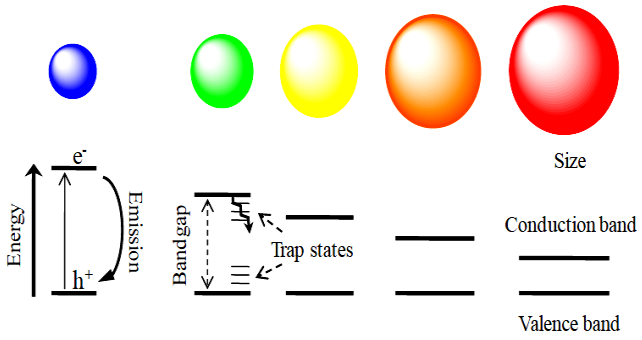

Fig. 1. Illustration of size-tunable QDs and creation of electron-hole pair on photoexcitation **[27]**.

Nowadays QDs are widely employed for targeted anticancer therapies due to an easy manipulation of their surface chemistry by either conjugating them with antibodies, ligands or other biomolecules, directly or indirectly linking them by strategies such as streptavidin-biotin interaction, and by altering their optical and magnetic properties **[28]**. A single QD can be conjugated to various molecules due to its large surface area to volume ratio, and hence QDs can be designed into more complex multifunctional nanostructures. The antibodies conjugated QDs allow specific recognition and tracking of surface antigens. QDs have been linked covalently to various biomolecules, such as nucleic acids, antibodies, peptides and other ligands for *in vivo* imaging, where the encoding of genes, proteins and various other biomolecules is done by multiple colors and changes in intensities of the QDs **[29, 30]**. Several reports by researchers prove QDs to be ideal candidates for identifying various types of biomarkers, such as proteins, specific DNA or mRNA sequences, tumor cells, and hence a more effective diagnosis of cancer can be achieved.

QDs possess an important property of easily transferring energy. Thus, the energy of the light falling on a quantum dot passes along to a nearby molecule, which uses this energy to show fluorescence. Self-illuminating QD

conjugates luminesce by bioluminescence resonance energy transfer (BRET) in the absence of external excitation. In this process, the energy from a light-emitting donor molecule is transferred to a nearby acceptor fluorescent molecule nonradiatively, leading to an enhanced sensitivity in bioimaging. As discussed above, one of the greatest advantage of QDs which makes them ideal for *in vivo* imaging is that their emission wavelengths can be tuned throughout the near-infrared (NIR) spectrum with the manipulation in their size, resulting in photostable fluorophores which are highly stable at biological pH values. A deep tissue optical imaging is best in the near-infrared spectrum. Also, hemoglobin and water have local minima in absorption in this spectrum **[31, 32]**.

The applicability of potential QDs to identify live breast cancer cells by employing QDs linked to immunoglobulin G (IgG) and streptavidin to label the HER-2 cancer marker present on the surface of live breast cancer cells was proved **[33]**. Simultaneous labeling of HER-2 on the cell surface as well as in the nucleus was achieved. Two cellular targets were simultaneously detected with a single excitation wavelength, which proves that different sized and, hence differently colored, QDs could be used together to distinguish different parts of a single cell, leading to multiplex target detection **[33]**. Antibody-conjugated QDs have made possible the real-time imaging and tracking of single receptor molecules on the surface of living cells with improved sensitivity and resolution **[34]**.

Therefore, compared to other assays, which are time consuming, expensive, labor intensive and have no multiplexing capability, QDs based technology is rapid, easy and economical, and allows quick detection of cancer markers. However, the clinical outcome of any assay developed for cancer strongly depends on the stage at which the malignancy is detected, especially for breast cancer in women and prostate cancer in men **[35]**. This makes early screening and prognosis of cancer highly important.

4. Surface-modification of quantum dots

Quantum dots are used as bare core only or as core/shell structures. In core/shell structures, the core of a semiconductor is surrounded by a shell (generally a wider bandgap semiconductor material), which allows better passivation of surface defects and enhances the photostability and improves its optical properties. For example, in the absence of a protective shell, CdSe exhibits low quantum yield, but when it is passivated with a higher band gap semiconductor material, ZnS, the CdSe QD's luminescence is improved. Therefore, core/shell structures are better for biological applications than the core-only structures. Alloyed quantum dots, which offer continuous tuning of quantum confinement by variation in the size of the quantum dots or modulation of their chemical composition, have also been synthesized. QDs (whether single or core/shell structures) do not exhibit aqueous solubility as they are generally synthesized in organic solution and are surface-stabilized with hydrophobic organic ligands. Thus, they are necessarily made water soluble by surface modifying them with various bifunctional surface ligands or caps to promote aqueous solubility and enhancing bio-compatibility **[36-41]**. Quantum dots interact with biomolecules through different types of interactions mechanisms; importantly, peptide linkages or S-S disulfide bonds, electrostatic interactions, hydrophobic and van der Waals interactions. There are various methods for surface modifications of QDs such as (i) silanization, which is done by introducing silica shell covering onto the QDs, (ii) by exchanging the hydrophobic surfactant molecules with bifunctional molecules, i.e. molecules having a hydrophilic end on one side and a hydrophobic end on the other, the most commonly used bifunctional molecules being cysteine, mercaptosuccinic acid, glutathione and mercaptoacetic acid, or (iii) by coating the hydrophobic surface of the QD with a cross-linked amphiphilic polymer, the hydrophilic component of which provides water solubility and the hydrophobic part interacts with the hydrophobic surface of the QD **[40]**. Other coating techniques include electrostatic interaction, micelle encapsulation, and hydroxylation, as shown in **Fig. 2**.

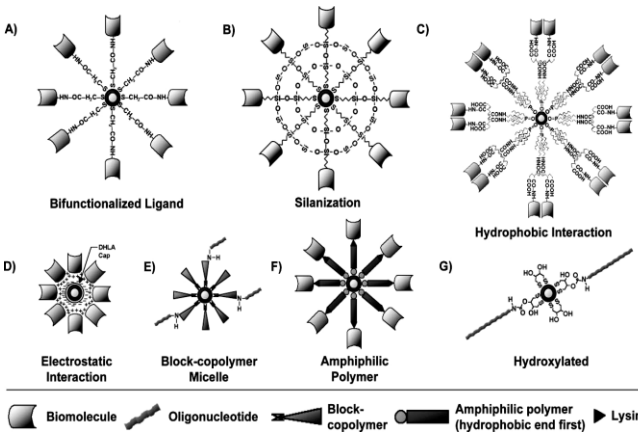

Fig. 2. Surface coatings that permit QDs to interface with biological systems and biorecognition molecules **[42]**.

5. Techniques employed for cancer detection

Various techniques are employed in the biomedical field for the detection of various cancers, out of which the two most widely employed are sentinel lymph node biopsy and photodynamic therapy. These techniques initially used to employ fluorescent dyes, but due to certain advantages of QDs over conventional dyes, QDs have replaced conventional dyes in these techniques. Here, we are giving a brief description of these two techniques.

5.1. Sentinel lymph node biopsy (SLNB)

A sentinel lymph node is the first lymph node to which cancer cells are most prone to metastasize from a primary tumor, and sentinel lymph node biopsy is a surgery that takes out the lymph node tissue to look for cancer in order to determine if a known cancer has spread from the original cancer site. This technique is a means of ultra-staging cancer metastasis and is now the standard in breast cancer surgery **(Fig. 3)**.

It is based on targeting the first draining lymph node at the cancer site to determine the extent of disease spread. A negative SLNB result suggests that cancer has not

developed the ability to spread to nearby lymph nodes or any other organ, and a positive SLNB is an indication of the presence of cancer in other nearby lymph nodes and possibly to other organs as well. Current tracers for SLNB are blue dye and radioisotopes, but they have certain limitations, which can be overcome by the use of QDs that emit in the NIR range (700 nm - 900 nm), as light within this range has maximum depth of tissue penetration and least tissue autofluorescence interference (emission between 400 nm and 600 nm). Thus, the use of QDs overcomes the problem of background tissue autofluorescence, the main problem associated with live animal imaging **[43]**.

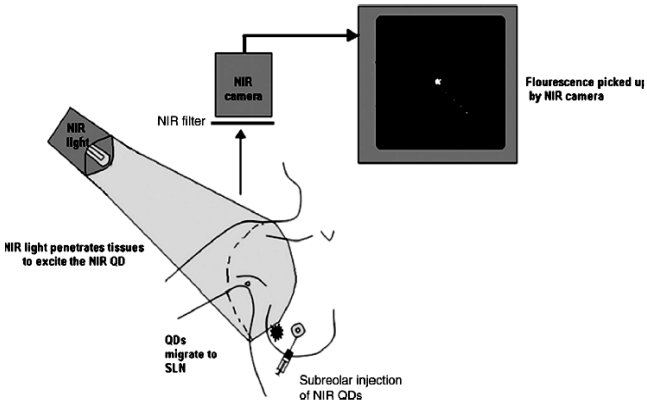

Fig. 3. Near infrared imaging system for SLNB in breast cancer surgery **[44]**.

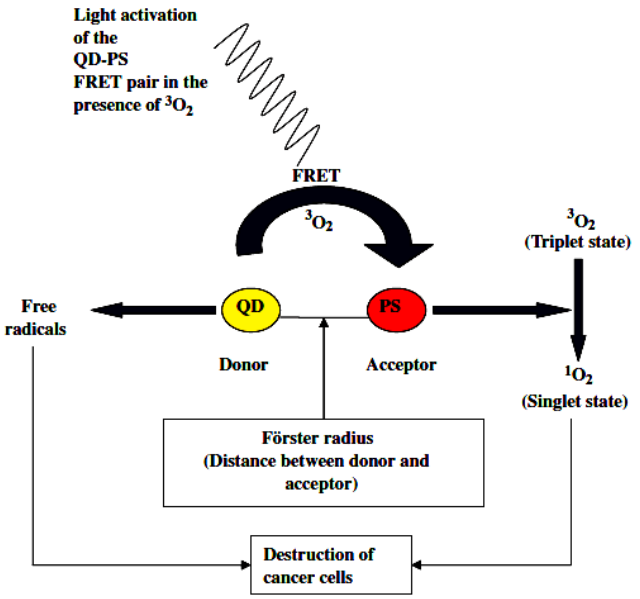

Fig. 4. Mechanism of PDT using quantum dots. Activation of a QD-PS FRET pair by light of a particular wavelength and generation of singlet oxygen which is toxic to cancerous cells and destroys tumor mass **[44]**.

5.2. Photodynamic therapy (PDT)

PDT is considered to be one of the major advances in least invasive therapies for cancer treatment and is now being widely employed instead of surgery in treating various superficial malignancies, including basal cell skin carcinoma, oral, esophageal and lung cancers **[44, 45]**. This is based on the destruction of diseased tissues via oxidation and degradation of cellular components using the cytotoxic singlet oxygen ($^1O_2$) generated from a non-toxic photosensitiser (PS) activated by light of a specific wavelength in the presence of molecular oxygen ($^3O_2$). Singlet oxygen leads to cellular necrosis and apoptosis of target cells **(Fig. 4)**.

Higher quantum efficiency, greater photostability, high molar extinction coefficients and tunable emission spectra in the near infra red region make QDs ideal donors for the fluorescence resonance energy transfer (FRET) process in order to know the exact cancer site for specific targeted action **[46]**.

6. Cancer detection using quantum dots

Earlier studies open new avenues for application of QDs as bioimaging tools and their applicability in multiplexed imaging by various researchers. Their photostability makes them ideal candidates for multicolor imaging and for studying various events in living cells. **Fig. 5** shows the biological applications of quantum dots in imaging and in cellular tracking.

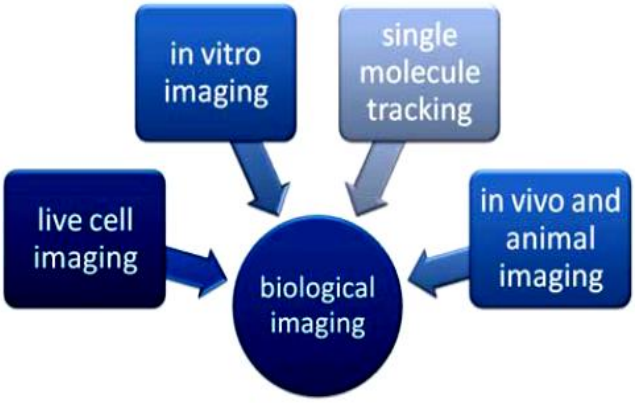

Fig. 5. Biomedical applications of quantum dots **[47]**.

For the early and accurate cancer cells detection, use was made of dendrimer/QD nanocrystals (NCs) as an ECL signal probe for cancer cells **[48]**. This study is advantageous, since large numbers of CdSe/ZnS QDs were assembled onto the dendrimer NCs due to the many functional amine groups of NCs, which greatly amplifies QDs ECL signals. Targeting of blood vessels and cancer cells was done using QDs, wherein the surfaces were conjugated with specific peptides **[49]**. Earlier in 2004, QDs encapsulated with an ABC triblock copolymer proved the multiplexed fluorescence imaging of human prostate cancer biomarkers developing in mice due to the successful binding of the QD-antibody to tumor specific antigens **[50]**. QDs spontaneously endocytosed by HeLa cancer cells retained their bright fluorescence when mercaptoacetic acid-coated CdSe/ZnS QDs were covalently conjugated to the transferrin protein, indicating that QDs could be used as intracellular labels **[51]**.

Folic acid (FA), an oxidized form of folate, shows a high binding affinity for folate receptors (K_d ~10^{-10} M) **[52]**. An over-expression of the membrane associated folic acid receptor (FR) makes FA as a potential marker for a variety

of tumors, such as ovarian, prostate and breast cancers, since FR generally does not expressed in normal tissues but is over-expressed in these tumor cells **[53]**. The diagnosis of cancers in which FR is over-expressed was carried out **[54]** and the role of bovine serum albumin (BSA) as a bridge molecule to form FA-BSA-CdTe/ZnS QD conjugates for cancer diagnosis was elucidated. The greater selectivity of FA-BSA-QDs compared to only BSA coated QDs was concluded from this study.

Mucin 1 (MUC1), a glycoprotein, expressed on most epithelial cell surfaces, is considered to be a useful biomarker for the diagnosis of early cancers. Cheng *et al* designed A three-component DNA system (quencher, QD-labeled reporter and the MUC1 aptamer stem) was designed **[55]**, where the role of the fluorescent QDs is to selectively detect the MUC1 peptide. A strong fluorescence was observed in the absence of the MUC1 peptide, but the fluorescence intensity decreased in its presence. This allowed the detection of MUC1 in the nanomolar range. Burkitt's lymphoma, an acute blood cell cancer, is the most common cancer in children in equatorial Africa **[56]**, and the Ramos cell is a human Burkitt's lymphoma cell. An assay utilizing CdTe QDs for the sensitive qualitative and quantitative analysis of Ramos cells out of a mixture of various cancerous cells has been developed **[57]**. High fluorescence intensity was observed due to loading of large numbers of CdTe QDs tags on the surface of the Ramos cells and the formation of a DNA-CdTe QDs sheath.

6.1. Detection of lung cancer

Lung cancer has been ranked the No. 1 killer of all cancers. Only 15% of lung cancers are detected when they are localized, and the majority is diagnosed in the advanced stages of the disease, since there are few or no symptoms in the early stages of the disease. Hence, the early detection of lung cancer is highly desirable for improving survival from this disease. A new concept of detecting multiple cancer markers in a single sample was proposed by combining QD labels with enzyme labels for the simultaneous detection of three cancer markers in human serum with equal detection limits up to ng mL^{-1} level for the three markers. A multiplexed detection of the three lung cancer markers neuron-specific enolase (NSE), carcinoembryonic antigen (CEA) and cytokeratin fragment (Cyfra21-1) was achieved by coupling one QD label with two enzyme labels. This is considered to be a sensitive and selective method of detection of multiple targets, since no cross-reaction between the three cancer markers occurs when they are simultaneously detected **[58]**.

A comparative study of the ability of quantum dots immunofluorescence histochemistry (QDs-IHC) and conventional immunohistochemistry (IHC) for the detection of caveolin-1 and PCNA in the lung cancer tissue microarray **[59]** concluded that both methods could precisely detect the expression of caveolin-1 and PCNA markers, but a higher sensitivity is obtained with QDs-IHC than with conventional IHC. In another work **[60]**, the simultaneous detection of two lung cancer biomarkers (CEA and NSE) based on the use of dual-color QDs was carried out by employing two antibodies, two antigens and two detection antibodies, which on mixing formed sandwiched complexes in homogeneous solution. This was followed by the addition of streptavidin coated polystyrene beads into the resultant system. Dual-color QDs with emission maxima at 525 and 655 nm were added which reacted with the detection antibodies. The results showed that CEA and NSE could be sensitively determined with equal detection limits upto the 1.0 ng mL^{-1} level. This method is advantageous, as the multiplex fluorescence could be achieved simultaneously for CEA and NSE, and the homogeneous antibody-antigen reaction made the whole detection simpler and efficient. Also, no cross-reaction is observed during the simultaneous detection using the above-mentioned QDs **[60]**.

International standards divide lung cancer into two types: small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC accounts for approximately 85% of all cases of lung cancer and is a type of epithelial cancer **[61]**. Cytokeratins are intermediate filaments of the cytoskeleton that are specifically expressed in epithelial cells, considered to be protein markers for the detection of epithelial tumor cells **[62]**. Human lung-specific gene Lunx and surfactant protein-A (SP-A) have been employed as diagnostic markers for the detection of micrometastases in NSCLC patients **[63,64]**. A method for detecting lung cancer micrometastases in peripheral blood has been proposed **[61]** by synthesizing magnetic NPs over-coated with pan-cytokeratin (pan-ck) antibody and QDs over-coated with Lunx and SP-A antibodies. These doubly-labeled QDs were then utilized for the detection of lung cancer micrometastases in NSCLC patients.

6.2. Detection of breast cancer

Breast cancer (BC) is the second most common type of cancer and the fifth most common cause of cancer deaths in the US and the world. About 20%-30% breast cancer patients show over-expression of HER-2 (human epidermal growth factor receptor 2) in tumor cells **[65,66].** Hereditary breast cancer is commonly due to an inherited mutation in the BRCA1 and BRCA2 genes. In normal cells, these genes help preventing cancer by making proteins that keep the cells away from growing abnormally. An inherited mutated copy of either gene from a parent raises the risk of developing breast cancer during the lifetime of a child. Hence, development of therapeutic techniques for breast cancer biomarkers is highly important for the treatment of breast cancer. Since anti-HER-2 antibodies inhibit the growth of HER-2-overexpressing breast cancer cells, it is considered to be the most effective therapy in HER-2-positive breast cancer patients, and nowadays it is being widely employed by generating various recombinant monoclonal antibodies like trastuzumab **[10,67-69]**. Immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) used to be considered the most widely employed techniques for detecting HER-2 in breast cancer patients **[70]**, but, due to certain limitations of these two techniques, HER-2 detection using QDs based fluorescent probes has attracted much attention **[71-81]** in recent times. The applicability of QDs for detecting breast cancer biomarker HER-2 on the surface of breast cancer SK-BR-3 cells utilizing QD-535 and QD-630 has been proved **[71]**. The clinical application of QD-based technology for the quantitative determination of HER-2 expression in breast cancer tissues has been subsequently developed **[82]**. Later,

various research groups have worked upon and are still working on staining HER-2 overexpressing breast cancer cells using anti-HER-2 antibody conjugated QDs. A method for the early stage cancer diagnosis and imaging based on folate-decorated NPs of biodegradable polymers for QDs modification has been developed **[52]**. The *in vitro* cellular uptakes of these surface-modified QDs, as investigated by confocal laser scanning microscopy, proved that MCF-7 breast cancer cells are due to overexpression of folate receptors than to the cellular uptake by NIH 3T3 fibroblast cells which are of low expression of folate receptors **[52]**. Another QDs based breast cancer sensing **[83]** involved three kinds of antibodies: the first one, the capture antibody anti-Her2/neu, was used to capture the SK-BR3 breast cancer cells; the second antibody, the labeling antibody, anti-EpCAM, was used for labeling the captured SK-BR3 cells; and the third antibody, against anti-EpCAM, which was conjugated to QDs, was used for imaging the captured cells. These antibodies conjugated QDs proved to be promising candidates for the sensitive and specific imaging of cancer cells. The potentiality of QD-based immunofluorescent nanotechnology for the simultaneous imaging of HER-2 and estrogen receptor (ER) was explored by making use of CdSe/ZnS QDs conjugated with streptavidin (QD-SA) probes with an emission wavelength of 605 nm (605-QD-SA) and 545 nm (545-QD-SA), hence providing new insights into BC heterogeneity **[84]**. It was found that QD-IHC displays BC heterogeneity more sensitively than conventional IHC.

Virus-based NPs are also becoming popular nowadays in diagnostic techniques for the detection of tumors in the early stage. Work in this direction was initiated **[85]** by attaching QDs to HER-2 specific M13 bacteriophage antibodies for the detection of cancer lesions and cellular imaging, and by conjugating these HER-2 specific antibodies with end coat proteins of the phage to create HER-2 specific monoclonal antibodies. CdTe QDs attached to the phage displayed specific HER-2 antibodies to form a stable complex QD-Ab. The studies illustrated the value of HER-2 phage-QD complex as a useful cancer detection tool. An algorithm to assess the HER-2 status using QDs-based nanotechnology and spectral analysis by introducing a new parameter "total HER-2 load" **[86]** could be helpful in formulating a better targeted therapy for BC therapy.

6.3. Detection of prostate cancer

The most commonly diagnosed cancer and the second leading cause of cancer related deaths in American men is prostate cancer **[87],** which is now becoming an increasingly common cancer in some Asian and Eastern European countries as well, because of the adoption of the western lifestyle. In India, the prevalence is relatively low, but is increasing by 1% every year. The goal of early screening of prostate cancer is the hope that it can be treated more effectively.

Prostate stem cell antigen (PSCA), a cell surface antigen, predominantly expresses prostate specificity. Prostate-specific antigen (PSA) is made by both normal and cancerous cells in the prostate gland. It is mostly found in semen, but a small amount is also found in blood. The chances of developing prostate cancer increases as the PSA level raises. An extensive research has been carried out and still continues in order to develop therapies for curing prostate cancer. Use of cys-diabodies, which are small, bivalent tumor targeting antibody fragments, has made possible the simultaneous detection of two tumor antigens on LNCaP/PSCA prostate cancer cells (which express PSCA and HER2) in culture using two immunoQdots (iQdots), anti-HER2 iQdot 655 and anti-PSCA iQdot 800, and by conjugating cys-diabodies specific for HER-2 as well as prostate stem cell antigen with amino polyethylene glycol (PEG) Qdot 800 **[88]**. Correlation and comparison of the results for the detection of PSCA obtained via QDs based immunolabeling and those obtained via conventional IHC concluded that QDs exhibit superior sensitivity for higher PSCA expression in prostate tissue than conventional IHC, along with greater long-term photostability, proving QDs to be better candidates for *in-vivo* imaging **[89]**. However, in an earlier study **[90],** the results of QD based immunolabeling were compared with those from the conventional immunohistochemical staining for detecting PSCA in bladder tumor tissues, and it was concluded that both methods show similar sensitivity in the PSCA expression correlated with tumor stage **[90]**. Biosensing technology called surface plasmon-coupled emission (SPCE) is based on surface plasmon resonance (an optical detection process based on the absorption of light by a thin metal film when a polarized light hits a prism covered by the thin metal layer). The ZnS-capped CdSe QDs enhanced SPCE technique was used for the detection of prostate specific antigen (PSA) by conjugating QDs with PSA antibodies and by using a 405 nm wavelength laser in order to excite emission of QDs-labeled PSA antibodies. The limit of detection of PSA achieved by this technique was reported as 10 fg mL^{-1} **[91]**.

E-cadherin, considered to be a principal mediator of cell-cell adhesion in epithelial tissues, has been extensively studied to determine its role in cancer metastasis. The loss of E-cadherin expression or function is linked to increased invasive potential **[92],** metastatic potential **[93]** and poorer disease diagnosis **[94, 95]**. A comparative study of the adhesion mechanisms in both healthy and cancerous epithelial cells was carried out **[96]** by utilizing the scanning near-field optical microscopy technique in conjunction with quantum dot labeling and the differences were studied both morphologically and phenotypically in healthy and cancerous cells. It was found that E-cadherin is predominantly located around the cell periphery and within filopodial extensions in healthy prostate epithelial cells (PNT2), whereas no E-cadherin labeling was found around the periphery of the cells in metastatic prostate adenocarcinoma cells (PC-3).

6.4. Detection of colorectal cancer

Colorectal cancer (CRC) is a term used to represent cancer that develops in the colon or the rectum, but, depending on where it starts, it is referred to separately as colon cancer or rectal cancer. CRC is the second most common cause of cancer related deaths worldwide **[97]** and the metastases derived from CRC are responsible for such cancer-related deaths **[98]**. In order to detect circulating colorectal cancer cells, an immunoassay developed **[99]** by making use of magnetic beads coupled with epithelial cell adhesion molecule (EpCAM) antibody and monoclonal cytokeratin

19 (CK19) antibody could separate the circulating tumor cells from body fluids. The formed complexes were then tagged with streptavidin-conjugated QDs and the fluorescent signal of QDs confirmed the detection of circulating tumor cells surface antigens.

6.5. Targeting and imaging melanoma

Melanoma, a type of skin cancer is the third and most deadly skin cancer which begins in melanocytes, but can also begin in other pigmented tissues like in the eye or in the intestines **[100]** and accounts for about 75% of skin cancer deaths worldwide **[101, 102]**. Although, compared to other skin cancers, melanoma is less common; it is much more dangerous and causes the majority of deaths related to skin cancer. Therefore, a method of melanoma targeting and imaging is very important for its early prognosis and effective therapy. Melanoma can often be detected early when it is most likely to be cured. In the direction of early detection of melanoma, extensive research has been done on the applicability of QDs, and this has been worked upon **[103]** with the utilization of dendrimers functionalized nonmaterial. Dendrimers are repetitively branched polymers and highly ordered structures which, when coated over the NP surfaces, result in an alteration in the charge, functionality, stability and reactivity of NPs. Biocompatibility and cellular uptake of NPs are exclusively enhanced when modified with dendrimers **[104-106]**. CdSe QDs surface modified with polyamidoamine dendrimers **[107]** were conjugated with arginine-glycine-aspartic acid peptides. These modified QDs were water-soluble with high quantum yield and good biocompatibility specifically used to target human umbilical vein endothelial cells (HUVEC) and A375 melanoma cells and exhibited great potential in tumor prognosis and therapy. Human melanoma cell adhesion molecule CD146 is overexpressed on the surface of melanoma cells. Highly fluorescent PEG-capped CdSe/ZnS QDs **[108]** were synthesized, and melanoma detection was demonstrated by conjugating these QDs with streptavidin (QD-SA) and linking QD-SA with biotinylated goat anti-mouse IgG and mouse anti-human CD146 to label CD146 overexpressed on live and fixed cells. Labeled cells were highly bright and with high photostability, which made possible their easy detection. In order to mimic the *in vivo* tumor environment for evaluating the target specificity of polymer coated QDs, a coculture system consisting of cancer cells mixed with normal cells was developed **[109]**, and the specificity of melanoma antibody-conjugated QDs for melanoma cells rather than melanocytes in the coculture model was proved.
A surface antigen ganglioside (GLS) is known in melanoma cells **[110]** which is not expressed in normal cells but can be expressed in melanoma cells and the extent of GLS expression in melanoma cells depends on the extent of metastasis **[111]**. Thus, in order to develop an early diagnostic probe of melanoma, the human malignant melanoma ganglioside single-chain antibody (ScFv) was chosen and an anti-human melanoma ganglioside single chain antibody-CdTe QD nanoprobe was developed **[112]**, and the specificity of the nanoprobe for only the melanoma cells was proved by comparing its affinity for human stomach cancer cells and melanoma cells.

Cancer stem cells (CSCs) possess a high potential of differentiation and are more resistant to chemotherapy than non-stem cells. The effect of QDs on the expression of two plasma membrane associated glycoproteins CD44 and CD133, which are considered to be common markers of CSCs **[113]** in the expression of four different cancerous cells glioblastoma, melanoma, pancreatic and prostate adenocarcinoma, were studied **[114]** and a positive response for both the markers was found in case of melanoma cells only, while all others were positive only for CD44.

6.6. Imaging pancreatic cancer

The fourth leading cause of cancer related deaths in the United States is considered to be pancreatic cancer with the mean survival rate estimated to be 6 months and less than 5% of all patients diagnosed with pancreatic cancer survive beyond five years **[115, 116]**, mainly due to lack of specific symptoms, as a result of which the disease is diagnosed after reaching an advanced stage **[117]**. Thus, the only way to increase the survival rate of this disease is by developing novel bioimaging probes which would specifically diagnose pancreatic cancer *in vivo* at the earliest stage without producing any systemic toxicity. For expressing pancreatic cancer cells such as Mia-PaCa, cadmium free InP QDs which possess greater optical stability and no leakage of the toxic ions in biological systems were chosen **[118]** and mercaptosuccinic-coated InP/ZnS QDs conjugated with antibodies such as anticlaudin 4 and anti-PSCA whose corresponding antigen receptors are known to be overexpressed in both primary and metastatic pancreatic cancer **[119-121]** were constructed. A method of synthesizing lysine-coated Mn doped CdTeSe/CdS QDs conjugated with monoclonal and polyclonal antibodies with excellent quantum yield has been reported **[122]**. The Mn species induces magnetism in these QDs. hown The successful labeling of QD bioconjugates in pancreatic cancer cells such as Panc-1 and MiaPaCa has been shown, which is further helpful in the development of nanoprobes for *in vivo* imaging and therapeutic applications **[122]**.

The applicability of tetraiodothyroacetic acid (tetrac), a thyroid hormone antagonist and anti-proliferative agent, in the treatment of human pancreatic cancer by conjugating PEG-QDs to tetrac has been discussed **[123]**. A higher level of cellular entry of PEG-QDs (tetrac-PEG-QDs) into pancreatic cancer (PANC-1) cells than the unconjugated PEG-QDs was found.

6.7. Detection of cancer marker type IV collagenase

Type IV collagenase, an extracellular neutral metalloprotease, is involved in tumor invasion and metastasis **[124-126]**. Alongwith the degradation of type IV collagen (major component of cell membranes), it can degrade type 3, 5 collagen and gelatin. This compelled researchers to develop a rapid, selective and sensitive method for the determination of type IV collagenase for early diagnosis of type IV collagenase-relevant diseases. Conventionally applied methods like gelatin zymography and enzyme linked immunosorbent assay (ELISA) are not often employed for monitoring the activity of type IV collagenase due to their limitations as a multiplexed and a

high-throughput process **[127]**. A QDs-based FRET biosensor was successfully applied for the detection of the cancer marker type IV collagenase **[128]** in order to resolve the abovesaid limitations, and it was found to be a much more sensitive and selective method too. This was done by linking peptide between the donors luminescent QDs and small sized acceptor-gold nanoparticles (SAuNPs). After the addition of type IV collagenase to the system, the SAuNPs could detach from QDs because the enzyme cleaves the peptide leading to the disappearance of FRET which allowed the fluorescence of the QDs to return. The enzymatic activity of type IV collagenase was related to the PL change of QDs-based FRET probes and the concentration of type IV collagenase was determined with a detection limit of 18 ng mL^{-1} **[128]**.

6.8. Detection of thyroid carcinoma antigen

The specificity of the JT-95 antibody, which belongs to the class of immunoglobulin M (IgM) group, for detecting thyroid carcinomas antigen has been reported **[129,130]**, and a new detection system has been built by the combination QDs and JT-95 antibody in microscopic analysis, western blotting analysis and ELISA-like system. The possibility of even IgM antibodies to be applicable to the detection system with QDs, which are usually neglected as detection tools due to their lower affinity (dissociation constant > 10^{-5} M), has also been proved. An effective application of luminescent surface modified CdSe QDs conjugated with the IgM antibody to recognize the associated thyroid carcinoma antigen has been reported **[131]**, and it was possible to quantify the antigen in the range of 1.56-100 µg mL^{-1}. Thus, the feasibility of labeling of JT95 and other IgM class antibodies with QDs further proves the applicability of IgM antibodies in the diagnosis of cancers **[131]**.

7. Conclusion

Various researches carried out using quantum dots prove the applicability of QDs as promising diagnostic tools for the early and accurate detection of tumor cells, multiplexed tissue, intracellular imaging, and immunohistochemistry. Due to their advantages, such as single source excitation, narrow emission, high quantum yield, long fluorescence time and high photostability, bioconjugated-QDs have now replaced the conventionally used organic fluorescent dyes in various tumor targeting and imaging applications. The major problem associated with QDs is their toxicity and hydrophobicity, which hinders their applicability in biological systems and the biomedical field. These problems are overcome by using non-cadmium based QDs such as InP QDs and carrying out surface passivation. Recent developments in the field of QDs therefore generate a hope for the early detection of cancer cells. This is due to their encapsulation using polymers, conjugation with various biomolecules and antibodies, thereby making them suitable for targeting specific cancerous cells. Therefore, the high fluorescence of QDs in comparison to other detection methods and their unique size-dependent optical properties can be explored in the design and development of highly sensitive molecular imaging tools for *in vivo* imaging and therapeutic applications.

8. Future perspectives

Our ultimate goal is to convert QD technology back into clinical diagnostic purposes. QDs have proved to be excellent fluorescent bio-probes in biological and biomedical research. Their properties render them to be used for *in vivo* and *in vitro* molecular and cellular imaging and lead to major advances in cancer detection and diagnosis, and image-guided drug delivery of chemotherapeutic agents. QDs have replaced the current tracers in SLNB, and are employed as photosensitizes in PDT. Although QD technology is still not in much use due to their hydrophobicity, toxicity and many issues need to be solved in order to apply them safely in clinical medicine and assays for targeting, imaging and drug delivery. However, there is still a hope for further improvements in enabling their applications as more sensitive, qualitative and quantitative tools for measurements of population of cancerous cells, in targeting and localizing metastasis, improving signal intensity, and tracking drug delivery in live tissues. QD technology can prove to be a simple, rapid and successful platform for the early and sensitive prognosis of cancer biomarkers with great precision and accuracy, and hence anti-cancer therapies in future in order to completely eradicate cancer.

Abbreviations

BC Breast cancer, **BRET** Bioluminescence resonance energy transfer, **BSA** Bovine serum albumin, **CRC** Colorectal cancer, **CSCs** Cancer stem cells, **ELISA** Enzyme linked immunosorbent assay, **ER** Estrogen receptor, **FA** Folic acid, **FISH** Fluorescence *in situ* hybridization, **FR** Folic acid receptor, **FRET** Fluorescence resonance energy transfer, **GLS** Ganglioside, **IgG** Immunoglobulin G, **IgM** Immunoglobulin M, **IHC** Immunohistochemistry, **iQdots** ImmunoQdots, **MUC1** Mucin 1, **NPs** Nanoparticles, **NSCLC** Non-small cell lung cancer, **PDT** Photodynamic therapy, **PEG** Polyethylene glycol, **PS** Photosensitiser, **QDs** Quantum dots, **QDs-IHC** Quantum dots Immunofluorescence histochemistry, **SLNB** Sentinel lymph node biopsy, **SPCE** Surface plasmon-coupled emission, **SAuNPs** Small sized acceptor-gold nanoparticles, **TSGs** Tumor suppressor genes.

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