

Nanotechnology Applications in the Field of Medicine

The Potential of Quantum Dots in the Early Diagnosis and Treatment of Cancer



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Abstract

Early diagnosis and treatment of cancer are arguably modern society's most pressing challenges. There is active research in investigating the efficacy of a number of new technologies in improving current methods for treating cancer. One such is the Quantum Dot (QD) technology. The promise of QDs lies chiefly in their fluorescent properties and ability to bind to biomolecules - they can serve as fluorescent markers, enabling doctors to identify cancer cells, and can play a role in subsequent treatment by delivering targeted therapy. This dissertation explores the potential of this emerging technology as well as the number of challenges that remain.

Introduction

In 2008 cancer claimed the lives of 156,723 people in the UK. In 2008-09, £6.3bn of NHS costs were cancer-related, a significant proportion of an already stretched budget. While practical issues are the concern of the health providers, the human cost of cancer in terms of the distress and emotional strain it causes to patients and their loved ones cannot be underestimated - it is clear that reliable methods of early diagnosis and treatment are essential.

Before proceeding further it will be helpful to understand the underlying nature of the disease and the dynamics of how it spreads. In a healthy cell, genes present in the nuclei dictate processes including cell division, DNA repair and **apoptosis**¹. However, gene mutations caused by genetic or environmental factors create cells which divide uncontrollably and do not die. This abnormal growth of cells is known as a tumour². Cancer cells are capable of invading adjacent tissues and of metastasis - the spreading of cancer from the primary tumour to remote locations in the body. The metastasising cancer cells break away from the primary tumour and travel via the circulatory and lymphatic systems, to establish metastatic tumours elsewhere.

The fight against cancer has been aided by the development of a variety of increasingly sophisticated methods for its detection and treatment. However, these methods are, in many cases, unable to offer a definitive solution and can be time-consuming, labour-intensive and costly.

The use of imaging techniques such as Computer Tomography (CT) and Magnetic Resonance Imaging (MRI) gives doctors a view of the inside of the

¹ Terms highlighted in bold font are explained in the glossary

² The term 'tumour' is often incorrectly used synonymously with the term 'cancer' - only malignant tumours should be referred to as cancerous

body which aids in diagnosis. Although valuable, they are insufficiently sensitive to detect small primary or metastatic tumours, and incapable of detecting tumour antigens on the surface of tumour cells. Both of these are important in the early diagnosis of cancer and can serve as targets for treatment.

The underlying principle of QDs is the excitation of nanoparticles through exposure to light of a particular wavelength, causing the emission of light on return to the **ground state**. QDs can bind to tumour cells, acting as fluorescent markers and labelling them for attention, or delivering them targeted therapy. The brightness, sensitivity and specificity of QDs increases the chances of even small tumours being detected and of targeted treatment being successful, with minimal collateral damage.

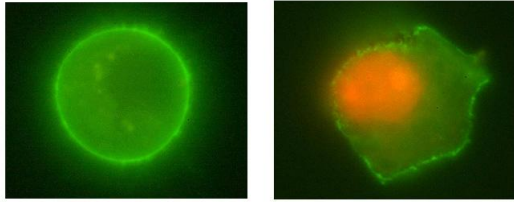
In this dissertation I will first provide a background to the QD technology and then go on to discuss its application in cancer detection and treatment. I will then elaborate on the challenges that will need to be tackled if QDs are to prove viable. I present my conclusions in the final section.

QD Technology - Background

A QD is a semiconductor nanocrystal with a diameter between 2 and 10nm. The majority of QDs are binary semiconductors - their core consists of two atoms, one of which occurs in Group 2 of the Periodic Table, and the other in Group 6 (alternatively a Group 3 element can be used with a Group 5 element) . A common combination is cadmium with selenium in the form of a cadmium selenide core with a zinc sulphide shell and a monolayer of **surfactant**. To make the QD water-soluble, it is encapsulated in another layer of molecules - either **amphiphilic** or **hydrophilic** which are capable of interacting with the water molecules.

QDs when exposed to light undergo excitation with the electrons jumping energy levels (from the valence band to the conduction band), leaving a hole and creating an electron-hole pair known as an exciton. QD atoms release energy in the form of photons to return to the ground state, the process which gives them their fluorescent properties. As the size of the QD core gets smaller, the gap between conduction and valence bands gets larger and the return to ground state releases more energy. Therefore, during fluorescence, QDs with smaller cores emit light of higher energy and frequency and with wavelengths closer to the blue and ultraviolet end of the spectrum. Conversely, QDs with larger cores emit in the red and infrared regions.

The traditional method of fluorescent labelling in biological experiments was through the use of organic **fluorophores**. However, a complication posed by



Human cancer cells labelled with green-emitting QDs

this technique is that the particles have a tendency for photobleaching - their fluorescent properties degenerate rapidly through light exposure necessary to produce emissions. This problem can be overcome through use of inorganic QDs which have a high resistance to photobleaching enabling the structures to which they become attached to be studied

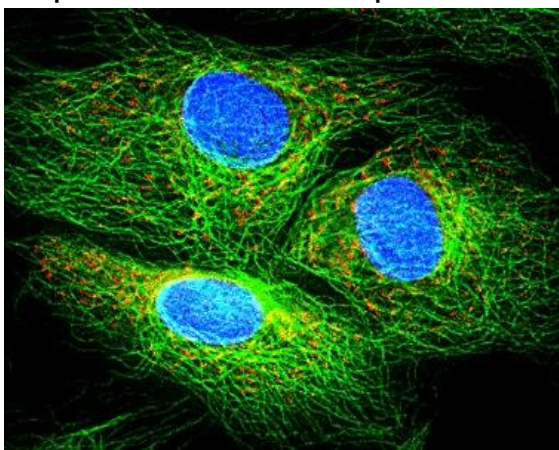
over relatively longer periods without their fluorescence fading.

The other desirable property of QD technology is its ability to conjugate to biomolecules. Encapsulator molecules which coat the QD bear functional groups, while biomolecules can be modified to possess functional groups. The functional groups on the encapsulator molecules can then bind covalently to the functional groups on the biomolecules, a process facilitated by the use of **cross-linkers**. Two or several biomolecules can generally be attached to each QD. This binding ability of QDs is exploited in the diagnosis and treatment of cancer which is explored further in this dissertation.

QDs in Cancer Diagnosis

The binding and fluorescent properties of QDs offer the potential for the early detection of certain types of cancer.

One feature of cancer cells that sets them apart from normal cells is the over-expression of certain proteins on their surfaces, and this can be exploited for their detection. These proteins are termed as tumour antigens or cancer cell-specific antigens. For example, cancer antigen 125 (CA-125) is a protein highly expressed on the surface of many ovarian cancer cells.



QDs bound to NIH 3T3 mouse fibroblasts. Green-emitting and red-emitting QDs shown. Nuclei are stained blue.

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As mentioned earlier, QDs can be conjugated to appropriate biomolecules and injected into the body. This can act as an antibody to the antigen which is over-expressed by the cancer cells (the antigen is recognised specifically and will bind to it.) Therefore, majority of the QDs will localise at the site of a primary or metastatic tumour, and by tracking their

progress, doctors can be alerted to the presence of suspicious growths. In addition, the surgeon can be confident that the fluorescent tumour has been removed during the operation.

Experiments to test the feasibility of this concept have been performed on small animals. One test involved the conjugation of cadmium selenide QDs to PSMA-specific antibodies. These antibodies are specific to the prostate-specific membrane antigen, a glycoprotein that is over-expressed in cancerous prostate cells. The conjugated QDs were injected into the veins of laboratory mice which possessed transplanted cancerous prostate cells, bearing the PSMA. After only 2 hours of circulation the QDs had localised at the site of the tumour. During a control experiment, QDs conjugated to another antigen were injected at a concentration 15-times higher than in the initial experiment. These were found to take 24 hours before they localised at the tumour, presumably by means of diffusion given that tumours have ineffective **lymphatic drainage systems**.

Conventional diagnostic methods, including medical imaging and biopsy of tissue samples cannot detect a cancer until it has reached a certain size. At this point the tumour will consist of millions of cells and may already have metastasised, reducing the chances of treatment being successful. Tumours must have a minimum diameter of 2-5mm before they will show up on a routine CT scan, In contrast, QD technology allows detection of tumours with a diameter as small as 10nm.

The specificity of the antibodies conjugated to the QDs means there is less concern of their binding to molecules other than the antigens they were intended to target. The sensitivity of the antibodies in recognising their complementary antigen means that even small cancers can be detected enabling early diagnosis. QD-based detection is also quick, relatively easy in practice, economical and less invasive than some alternative methods.

According to 2010 statistics, only 6% of pancreatic cancer patients survive for more than five years after diagnosis. This grim survival rate is driven by limitations in early detection methods - the cancer is generally diagnosed only in its advanced stages, often after metastasis has occurred. QDs could significantly improve the chances of pancreatic cancer patients by facilitating early detection - QDs conjugated to the monoclonal antibody anti-Claudin-4 have been demonstrated to target the receptor antigen Claudin-4 which is over-expressed on the surface of pancreatic cancer cells. However, another problem is posed given the lack of specific symptoms for pancreatic cancer, which may mean that patients remain unaware of the developing cancer and do not come forward for tests, defeating the purpose of early diagnosis. I would propose the establishment of a system of regular check-ups (not unlike

the smear test in the case of cervical cancer), particularly for people with a family history of pancreatic cancer.

QDs in Cancer Treatment

I considered two areas of cancer treatment where QDs appear to have the potential to make a difference. In the mapping of **sentinel lymph nodes** prior to their removal they could be used in prognosis and to determine the most appropriate method of treatment. Although QDs appear to possess some advantages over conventional methods in the case of certain cancers (for example thoracic oesophageal cancer), existing techniques are generally successful. Such techniques include the injection of radioactive substances or Isosulfan Blue dye. For this reason, I have decided to focus on the use of QDs in drug therapy where they could be used to facilitate targeted administration of medicine to cancer cells.

Once cancer cells have been detected in the body, efforts must turn to treatment. Techniques involving QDs exploit their conjugation property that was also key to cancer detection - the over-expression of certain proteins on the surface of cancer cells.

An example of one such protein is transmembrane-receptor tyrosine kinase. These proteins are present on the surface of cancerous cells. Since tyrosine kinase is key to the development and spread of cancer, its identification as target for treatment could be an effective method of curing the cancer. QDs can be conjugated to both antibodies and **enzyme inhibitors** of the receptor tyrosine kinase. Conjugation to antibodies enables QDs to bind specifically to cancer cells which over-express tyrosine kinase - the inhibitors can then suppress the enzyme and by so doing treat the cancer.

Targeted delivery of drugs makes it possible for doctors to use smaller, safer doses - treatment administered to the body as a whole often entails toxic side effects. The specificity of the biomolecules to which the QDs are bound also minimises the risk of drug treatment being delivered to healthy cells.

However, this method of treatment can only be successful if the tyrosine kinase on the surface of the cells is expressed to a certain extent which is not the case for all cancer cells. It would be ineffective and possibly dangerous to inject QDs conjugated to antibodies for the kinase if it is not expressed to a sufficient degree for the QDs to recognise and bind to the tumour cells. For example, Human Epidermal growth factor Receptor 2 (HER2) is a receptor tyrosine kinase which is only expressed highly in approximately 20-30% of breast cancer patients.

A further complication is posed by the fact that the level of expression of HER2 can change even within the same patient at different stages of cancer development, particularly after metastasis has occurred. In order to determine the likelihood of success using QDs, a surgery would have to be performed and the degree of expression assessed. While the surgical procedure is relatively simple in the case of assessing primary tumours, difficulties arise when metastatic tumours are involved, given that it is not feasible to attempt to take biopsy samples of tumour cells which have spread throughout the body. Also, certain healthy cells can sometimes express the protein being targeted.

Alternative therapeutic targets to tyrosine kinase can be used in the treatment of cancer (still involving the same principle of an antibody being conjugated to a QD and binding to an antigen on a tumour cell) - integrins are non-kinase cell-surface receptors which play a fundamental role in tumour growth and metastasis. Targeting of the integrins could be another effective approach to treating the cancer. The integrin inhibitor is also conjugated to the QD and can treat the cancer by employing one or both of the following methods - by inhibiting the formation of new blood vessels (angiogenesis) to the tumour, leading to its 'starvation' thus preventing further growth and metastasis, or by acting on the tumour itself and stopping it from receiving signals vital for it to survive, develop and metastasise.

An example of a cancer drug which could be delivered by this method is doxorubicin, as it causes undesirable side effects when administered to the whole body at doses needed to for it to be effective. However, using this method of targeted delivery, whereby the drug is incorporated into silicon QDs and is released as the latter degrades, would reduce the dosage level and not lead to other tissues being damaged.

Challenges of Quantum Dots

Many of the treatments discussed so far have only been tested *in vitro* or in animal models. Granted QDs potentially offer substantial benefits for cancer treatment, but it is equally important that any possible adverse human health effects as well as environmental impacts are investigated, analysed and understood.

Studies carried out at Rutgers on the side effects of the technology concluded that the presence of QDs does not affect cell growth, although some controversy does exist. Experiments by the University of Minnesota Cancer Centre have tested the effect of QDs on metabolism and behaviour by injecting them into pigs and mice, and no adverse impact was observed. However, it is worth noting that the QDs were only present in the bodies of the test subjects for brief periods of time, and the results in animals may not

scale as favourably to humans. In my opinion, the jury is still out on the safety and human side-effects of QD technology, and is the subject of active research.

The effect of conjugating QDs to biomolecules on the latter's ability to carry out regular functions must also be examined. Conjugation does not affect the binding ability of some biomolecules to their specific receptors - studies on QD conjugation to **transferrin** have shown that the biomolecule's ability to bind to iron is not impeded. However, while many functions look likely to proceed uninterrupted, certain issues may arise. For example, QD conjugation to the serotonin transporter protein may impair the protein's ability to bind to and transport serotonin, a **neurotransmitter**. Such issues must be better understood and resolved before we can gain confidence in QDs.

Another challenge that has been highlighted relates to the potential toxicity of binary QDs owing to the presence of heavy metals such as cadmium in their cores. If a situation were to arise involving the release of cadmium ions into the body (possibly accompanied by cancer drugs previously incorporated into the nanoparticles), damage could be sustained by surrounding tissues. Although the presence of a shell around the nanoparticle might lead it to be deemed stable enough to prevent the release of cadmium ions, the effects of possible shell disintegration must be thoroughly understood before QDs can be deployed with confidence.

It must also be determined as to whether injected QDs will be excreted by the body or whether they will linger in the long-term. Recent studies suggest that the non-biodegradable nature of cadmium selenide QDs leads to them accumulating in certain organs after they have served their purpose. While non-conjugated QDs appear to be excreted effectively through the kidney and QDs conjugated to certain proteins travel to the liver and leave the body in faeces, QDs conjugated to larger proteins are retained in the liver, raising questions about potential toxicity.

However, progress is being made in overcoming this hurdle. Research at the University of California, San Diego has resulted in the development of QDs which remain in the body long enough to serve their purpose and subsequently break down into benign by-products. These QDs do not contain heavy metals but instead consist of silicon wafers. An experiment carried out on mice showed that the injected QDs gradually degraded to silicic acid, a chemical which naturally occurs in the body and no toxicity was detected. Although this is significant progress, it must be recognised that the newly developed QDs are not entirely harmless, and safe levels have to be determined before they can proceed to clinical trials.

Environmental implications must also be considered - possible disintegration of QD shells and the release of toxic chemicals, as well as being a danger to the human body could have consequences for the environment.

The disposal of QDs is a further issue which has to be carefully considered. More research is needed urgently to understand the impact of QDs on the environment and their impact on pollution to soil and water - as they might then be absorbed into the ecosystem through plants and enter the food cycle.

The size of the particles means that they persevere in the atmosphere which could lead to their inhalation by humans and animals - the effects on health of their long-term presence in the atmosphere need to be assessed. Given that it would be a very expensive operation to remove QDs from the atmosphere if they did prove harmful, scientists must have a thorough understanding of their effects before they are deployed as part of a clinical solution for early detection and treatment of cancer.

Conclusion

Following the first report discussing QD applications in biological sciences published in 1998, notable progress has been made in evolving the concept and developing its possible applications to yield practical benefit. QD technology should not be dismissed as 'Star Trek' science - significant amounts of research and time continue to be invested in its advancement. I believe this to be a promising technology with certain desirable properties such as sensitivity and specificity, and its resistance to photobleaching, which offer the potential to overcome many problems which confront conventional methods, and currently hinder timely diagnosis and effective treatment.

In my opinion the greatest benefit that QD technology can offer lies in the field of cancer diagnosis - QDs' capacity for detecting even small tumours which would be missed by less sensitive detection systems could mean earlier detection followed by an increased scope for successful treatment.

Another area where I feel QDs offer promise is in targeted drug delivery. QDs could provide a solution to the long-standing problem of administering drugs exclusively to cancerous cells without affecting the healthy ones.

Despite this promise, I must acknowledge that in common with any emerging technology, there are still a number of unanswered questions. These should not deter us from further exploring the technology's potential, but more targeted research has to be undertaken to ensure that there are no show-stoppers for deployment in a clinical setting.

Researching this topic has led me to speculate on whether the benefits of QD technology could extend into other areas of medicine. I propose that QDs be conjugated to antibodies complementary to an antigen, whose over-expression is indicative of the presence of a disease other than cancer. The conjugated QDs would bind to the antigens and allow the progression of the disease to be monitored.

For example, GP-123 is a protein expressed on the capsid of a Human Immunodeficiency Virus (HIV). The conjugation of QDs to these proteins could enable doctors to count the number of viral particles circulating and could provide an indication of whether the disease is present, and to what extent. If a patient is undergoing therapy, the levels of viral particles in the body could be monitored, allowing doctors to determine how successful treatment is proving and decide if a different approach is required.

QD technology could be applied to another disease called Systemic Lupus Erythematosus (SLE), a condition which involves the immune system attacking body cells. Anti-nuclear antigen is the protein over-expressed in an SLE sufferer. Post-treatment targeting of this protein to follow disease activity could provide some indication of how well the patient is responding and provide early warning of any relapse.

At present this is just my conjecture and without conducting experiments it is, of course, difficult for me to draw any conclusions regarding the viability of such a proposal - however, I would like to see this idea being explored.

The role of QD in cancer treatment is at a tantalising stage - whether its potential will be realised in full measure or whether it will be yet another false dawn remains to be seen. However, I remain optimistic that there will be some role for QD technology in early cancer diagnosis and treatment.

Glossary of Scientific Terms

Amphiphilic: A molecule which possesses a part which has an affinity for water, and another part which has affinity for fat.

Apoptosis: Programmed cell death which plays a crucial role in maintaining health by the elimination of old, unnecessary and unhealthy cells.

Cross-linker: A reagent which links one polymer chain to another.

Enzyme inhibitors: A molecule that binds to enzymes and decreases their activity.

Fluorophore: A molecule or functional group which is capable of fluorescence.

Ground state: The lowest energy state of an atom or other particle - all of the particle's electrons are in their lowest available energy levels.

Hydrophilic: A molecule which has a strong affinity for water.

Lymphatic drainage system: A system of vessels that drains fluid from the body and returns it to a central location - potentially harmful substances can be filtered from the fluid and destroyed.

Neurotransmitter: A neurochemical that transmits nerve impulses across a synapse.

Phagocyte: A white blood cell capable of engulfing and absorbing bacteria and other small cells and particles.

Sentinel lymph nodes: The first lymph nodes reached by metastasising cancer cells. A biopsy of the SLNs is regularly carried out to check for the presence of cancer cells.

Surfactant: A chemical agent capable of reducing the surface tension of the liquid in which it is dissolved.

Transferrin: A protein which binds and transports iron in blood serum.

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