

***The Current and Prospective Uses of
Nanotechnology in Enhancing Clinical Oncology.***

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PASS WITH MERIT

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Abstract:

The size of a human cell ranges from 4-135 μm . And each of these cells are molecular machines: assortments of different chemicals in different places, to form life as we know it. This chemical balance is fragile, and can malfunction. We call this malfunction disease, and one such malfunction is uncontrolled growth- or cancer. But what if we could get as small as these cells to bring forward a whole new method of treatment? And what if we could get smaller? This research paper aims to illustrate several of the uses of nanotechnology in cancer treatment at present and prospective uses in the future, in short how nanotechnology will be the best way to develop oncology.

Introduction:

The Problem:

Cancer is the second leading cause of death worldwide^[1]. Its current, most common treatment methods are:

- **Surgery:** commonly used as both a method of identification by taking a biopsy and partial treatment of cancer by removing the cancerous tissue from the body as well as a small clear margin (normal tissue around the cancer), causing local trauma and tissue damage.
- **Radiotherapy:** the use of ionizing radiation to control malignant cells. It is applied to the tumour and often the surrounding lymph nodes to kill the cells. This also requires a clear margin, and causes damage to non-cancerous cells within the beam of radiation.
- **Chemotherapy:** the treatment of cancer using drugs which will eventually cause the death of the cancerous cells, also causing damage to non-cancerous cells.

Each of these treatment methods produces serious side effects, often causing more discomfort than the tumour itself. With more research into cancer and its causes, we would be able to increase the effectiveness of the therapy, as well as change the therapy itself. Nanotechnology promises this development.

Introduction to Nanotechnology:

Richard Feynman first introduced us to nanotechnology in 1959, in a talk aimed at the America Physical Society: "There's Plenty of Room at the Bottom". 52 years later, we are at a point where this is now possible, and being developed readily.

A nanometre is a billionth of a metre, and the study of manipulating materials at an atomic and molecular scale is called nanotechnology, dealing with structures between 1 and 100 nanometres.

Nanoparticles:

Nanoparticles are “particles with one or more dimension of 100nm or less” [2], but a nanoparticle will also show “different properties which differentiate nanoparticles from the bulk material” [ibid]. These differences in properties are resultant of the fact that at nanoscale, atoms and molecules are affected differently by physical laws because of their size. It is the exploitation of these new characteristics which makes nanotechnology so diverse.

A fullerene is a nanoparticle made entirely of carbon, in the form of a sphere, ellipsoid or tube. The Buckminsterfullerene (C_{60}) was the first Fullerene to be discovered, and is composed of 60 Carbon atoms in a spherical shape (See figure 1), called a Buckyball. Since the discovery of the C_{60} buckyball, it has been found that fullerenes are obtainable from varying numbers of Carbon atoms, the smallest being C_{20} . Buckyballs of C_{60} , C_{70} , C_{76} and C_{84} have been found in nature, in minerals, in soot formed by lightning discharges in the atmosphere, and have also been found in outer space.

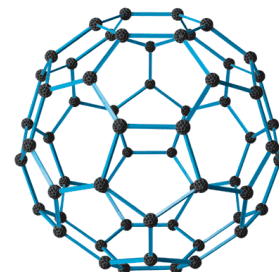


Figure 1:
Buckminsterfullerene

Following the buckyball, carbon nanotubes have also been made (See figure 2). These have a diameter of a few nanometres, but can be a thousand times as long. You can also get layered fullerenes, in the forms of nano-onions (carbon layers around a buckyball core) and Multiple Walled Nanotubes.

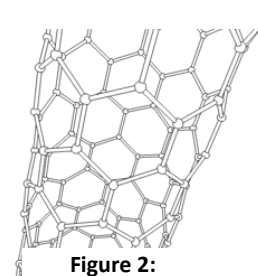


Figure 2:
Carbon Nanotube

Nanoparticles are not just allotropes of carbon; they can also be made out of metallic and molecular compounds. A good example is gold, which shows many different characteristics when in a nanostructure, including solubility, reactivity, and use as a catalyst. [3, 4]

Drug Delivery Systems:

A drug delivery system is simply the way in which a drug is administered. By improving these systems using nanotechnology, we can increase the efficiency of a drug, as well as reducing deterioration of the drug, and damage to normal tissue (like chemotherapy).

Many drugs are currently administered into the body orally. If these are protein or peptides, the bioavailability (amount of the drug that reaches the intended target) is greatly reduced due to the high pH in the stomach and protease enzymes in the digestive system, causing protein degradation. If they are macromolecular drugs, they are often unable to pass through the wall of the intestinal cells, also reducing the bioavailability. This also affects nucleic acid drugs, used as parts of a vaccine or in gene therapy. One proposed method to increase bioavailability of medicines is to form the drug or gene into a nanoparticle [5], some of which are already commercially available as delivery systems eg Nab-Paclitaxel. However researchers are still looking into delivery systems employing different molecules, dependent on the purpose of the drug and the intended location.

Nanosensors:

These may be biological, chemical or surgical sensors to show information about nanoparticles. The most common nanosensors are the natural receptors for taste and smell. These sense nanosized molecules. Nanosensors can respond to changes in volume, concentration and electrical forces. So they can be used to test the effectiveness of nerve cells, and abnormal changes in volume in different parts of the body.

Future predictions for nanosensors are that they will be able to detect specific DNA and recognize specific defects. Nanorobotics will also affect nanosensors, with scientists aiming to produce sensors which are able to communicate with other nanoparticles in the body.

Nanopores:

A nanopore is simply a nanosized hole in a membrane, but due to commercialisation of the technology, it is now understood as the use of a protein to make a hole in a membrane, through which molecules are able to pass. E.g. naturally, an α -hemolysin protein forms a hole in a membrane. This protein has a hole through it which is only a few nanometres thick, making it a nanopore. In industry, this protein can be placed into a lipid membrane with high electronic resistance. An electrical potential is then transmitted through the protein, causing a current through the hole in the nanopore. As molecules enter the pore, the current is distorted, and this change can be measured.

Aim:

The examples of nanotechnology mentioned beforehand can all be used to aid the development of cancer treatment and research. This research paper aims to illustrate the uses of different examples of nanotechnology in Oncology- highlighting its prospective uses in the research and treatment of cancer.

Discussion:

A cancer is caused by a series of mutations of genes within the DNA of a cell, causing uncontrolled cell division. Some cancers are brought upon by hereditary genes, some by age, and others by environmental factors affecting cells. So by looking into the genome of cancerous cells, we are able to determine exactly which oncogenes have malfunctioned to result in the uncontrolled division, and to identify the exact molecular cause of the malfunction.

Sequencing Techniques:

The most common way of sequencing DNA is the Sanger method (or chain-termination method), which involves the use of modified nucleotides to end a DNA chain. It also requires a single strand of

DNA, a DNA primer (method of labelling) and a DNA bond forming enzyme (a DNA polymerase). This involves adding the modified nucleotides to the existing strand of DNA, then using slab gel electrophoresis (separation technique) separating the four different bases. Originally, the primer would produce a radioactive label which required radiography to view, or fluorescent labels, requiring ultraviolet light. This has been adapted to use a dye primer, meaning it can be viewed optically, and the results originally had to be recorded manually.^[6]

Nanotechnology Alternative:

Now, nanopore technology offers a primer free way of sequencing individual molecules, removing several of the labour intensive procedures involved in the Sanger Method, including the manual reading of the displayed results.

In “Molecular Diagnostics for personal medicine using a nanopore” (2010)^[7], Mirsaidov et al discuss how it is possible to use a “nanopore to detect base-pair mutations in dsDNA by measuring the binding of a restriction enzyme”.

Exonuclease sequencing (as shown in figure 3) involves an α -hemolysin nanopore and, a processive enzyme—an exonuclease (shown in green) and a restriction enzyme (shown in orange), so can and is used to sequence DNA. The exonuclease breaks individual bases off DNA or RNA, which then go through the nanopore and the change in current produced by the molecule is noted. Different bases produce different levels of distortion in the current (shown in the membrane).^[8]



Figure 3: Exonuclease sequencing using a nanopore

Using the DNA sequence:

To differentiate between normal DNA and a mutation the genome of healthy cells and cancerous cells will need to be sequenced and compared. Some cells have mutations which do not lead to uncontrollable growth, so several cells will have to be tested to find that person’s correct genome. The genome of the cancerous cells can then be compared to this correct genome and the exact location of the oncogene (cancer causing gene) leading to the abnormal growth can be discovered.

The location and nature of the mutation can be compared with the mutation in other tumours, from different patients and different parts of the body to try and locate similarities between the cancers, other than the oncogene. Hence by knowing the genome of cancers, we will be able to develop a broader research base than that which we currently have. This will allow more weaknesses in the cancer cell to be discovered e.g. the HER2 gene and receptor which will be discussed later in the paper.

The development of nanopore technology has led to the sequence time of a whole genome to be simplified and reduced, with some estimates showing an entire genome could be sequenced within a few hours^[9]. And as our knowledge of human genetics increases – with the aid of nanopore sequencing

– so will “a substantial advancement in our ability to study, predict and cure diseases from the perspective of the genetic makeup of each individual.” [Ibid]

Ethical Issues:

There are currently several ethical issues regarding genomics. This is mainly due to the lack of restrictions and guidelines as a result of it being a relatively new field of research.

One such issue is the concern over the storage of genetic information and possible misuse by organisations and individuals looking to exploit the knowledge of people’s genomes. This could be in the form of insurance companies increasing the costs of their product for people with certain genes making them less healthy than others, or even by employers discriminating against certain employees because of their genes.

Another issue is the situation presented when someone will develop a genetic disease but shows no current symptoms- for example certain cases of breast cancer, and how you deal with such a situation. By telling someone, you potentially change the rest of their life, as they will be living with the fact that they will develop a disease. This is not as life changing in cancer, because often, the earlier you treat the tumour the greater the likelihood it will be killed, however with diseases like Huntingtons, no cure is known, and treatment is insufficient to stop the disease. By sequencing a human genome, it is not just the cancer causing genes that may be found.

Next is the issue raised in people with genetic diseases having children: One of the best ways to get rid of a genetic disease is to stop the disease being passed on to the next generation by not having children; however this raises several issues, and the question of ‘Is parenthood a right or privilege?’ Many people with these diseases still want to raise children, and many have already had children before being diagnosed with the disease.

Lastly is the development from sequencing DNA into altering it, and then creating whole new genotypes. This issue is concern over the ethics of germline and somatic gene therapy, which although not fully possible yet, may become reality soon enough, especially with nanopore technology making it much easier to sequence DNA. Is it morally right to tailor genes to have desired characteristics? Most people would agree the use of such techniques to remove genetic diseases and defects is acceptable, whereas creating a genotype purely based on ‘desirable’ characteristics isn’t acceptable. But the problem lies in where one draws the line between a genetic defect and undesirable gene, and whether prospective parents should be able to design their own baby. The major concern is with the possible production of a super race, with geneticists acting God-like with their creation. Another view is that removal of certain genes is tantamount to genocide.

Nanotechnology in the treatment of cancer:

Anti-Cancer Drugs using a Nanoparticle Drug Delivery System:

Currently, there are already some anti-cancer nanodrugs commercially available. Protein-bound (nab-) Paclitaxel –for treating metastatic breast cancer – is one such example. This is a nanoparticle

alternative to conventional Paclitaxel which is dissolved in Cremophor EL and ethanol as the delivery system, the nanoparticle however is bound to albumin- the most common protein in human blood plasma. This means Nab-paclitaxel does not contain any toxic solvents and is much safer than Paclitaxel with a solvent delivery system. This allows administration of 50% more chemotherapy in one cycle with much safer results than the original ^[10]. Regardless of this, the protein bound drug showed a response rate almost doubling that of the solvent based drug.

The albumin removes the need for Cremophor which is used to overcome poor water solubility of certain drugs. Albumin is soluble, and is already naturally present in the blood plasma. The use of the protein allows a higher bioavailability of the drug in the tumour cells. It delivers the drug to the tumour cells by utilising a previously unknown “tumour-activated biological pathway with a nanoshell” ^[11]. This is said to activate “an albumin-specific (Gp60) receptor-mediated transcytosis path through the cell wall of proliferating tumour cells” ^[11]. Once in the stromal (connective tissue) cells, the tumour secretes an albumin-specific binding protein, which causes the breakdown of the albumen. This then leaves the drug to surround the tumour cells and once inside the cells, it interferes with the normal breakdown of the microtubules during mitosis- stopping the division from being able to take place. Nab-Paclitaxel is usually used in combination with other drugs which are cytotoxic and kill the cancerous cells.

This is one example of how further understanding of cancer cells has led to a discovery in pathway for albumin into a tumour, and then nanotechnology being employed as a delivery and treatment system as a result. So hopefully this new breakthrough in the effectiveness of Nab-Paclitaxel will encourage other drug manufacturers to replace toxic solvents with nanoparticles, decreasing the side-effects induced by the toxicity of the delivery system and increasing the response rate and bioavailability of chemotherapy drugs.

Anti-Cancer Treatments involving Nanoparticles:

Herceptin is an example of a Monoclonal Antibody (mAbs) treatment and is used in conjunction with Paclitaxel to form a synergy. Herceptin targets the HER2 receptors of the HER2 oncogene (cancer causing gene) which surround cancer cells and bind to them specifically. There are HER2 receptors and genes in normal cells, however in cancerous cells, there are many more HER2 receptors, and these are not picked up by the immune system. Once specifically bound to the cancer, the mAbs in herceptin can act as a tumour marker, triggering an immune response which stimulates Natural Killer cells to attack the cancerous cells.

This is the precise type of treatment Jie Gao et al¹ discuss, stating “The development of targeted nanomedicines, which perfectly combine antibody engineering and nanomedicine, is becoming a possible state-of-the-art in nanomedicine research”. By using nanoparticles as a delivery system for Herceptin, once again the rate of reaction is increased, as is the bioavailability.

But there is another factor about using nanoparticles for herceptin which triggers a whole new treatment method, endocytosis. If the nanoparticle is around 50nm, the particle can bind to several herceptin molecules, so a single nanoparticle is joined to a number of receptors by the specific binding of the antibodies. The cancerous cell then engulfs the whole nanoparticle, stopping the signalling to natural killer cells ^[12]. This method has been used to get quantum dots into the cell.

Quantum dots are crystals of semiconductor material, which were originally designed for use in electronics, but which have proved useful in nanomedicine because they transfer energy easily. These dots are often made to be fluorescent, so when a dot like cadmium selenide is exposed to a form of energy- like ultraviolet light- the dot starts to glow. This property has proved very useful in the imaging of cancer, both in surgery and as a new form of scanning.

Personally, the mechanism with which quantum dots are engulfed by cancer cells is best used for a different nanoparticle. Gold coated with mAbs will be engulfed by the cancerous cell just like the quantum dot. Then by using a process called photodynamic therapy, an infrared light can be shone through the body without heating any cells, but causes the gold to heat up to a temperature to kill the cancerous cells in close proximity.^[13]

With some more development, nanosensors could be sophisticated enough to alert a computer, or other nanoparticles when the density of a lung or breast has increased due to abnormal growth. Tumour cells are usually more acidic than other cells in the body^[14], so a pH nanosensor could also be used to detect tumours.

Ethical Issues:

The ethical issues of using nanoparticle drug delivery systems and therapies are minimal; the main concern is of the safety of the particles, as little is known about their effect on the body. Multi phase trials under stringent research methodology would need to be used as with all new medical research, so nanotechnology must be developed further, and not held back due to fear of the unknown.

Conclusion:

This paper has shown evidence of nanotechnology being implemented into oncology in several aspects, from the research stage to the diagnosis and treatment. Not only can it be used to create new treatment methods, but also modify existing treatments which are currently successful, making them even more effective and less dangerous by removing the need for toxic solvents and improving the delivery system of other drugs.

Chemotherapy is an area where development using nanotechnology can be seen but there is not enough. Paclitaxel is one of only a handful of drugs which have been trialled with nanoparticles, and the results have proved very successful, lowering the toxicity, increasing the bioavailability and overall increasing the rate of treatment. The drug delivery system needs not be a complex material synthesized in a lab; it can be something as simple as albumin, the most common protein in the blood plasma. Paclitaxel seems to be one of the first in a revolution of chemotherapy drugs, and there are plenty of other drugs which would benefit from similar development.

Other anti-cancer treatments also prove promising, developing from using nanoparticles as a delivery system to nanoparticles as part of the treatment itself, including the use of monoclonal antibodies and gold. I feel combining these can lead to a two tier approach to the treatment. Firstly allow the antibodies to bind to the cancerous cell, and allow the cell to engulf the whole nanoparticle. The gold can then be heated, killing the cell. This is tier 1, tier 2 is allowing the immune system to play a part in the treatment, synthesising the antibodies and then killing the marked residual cells. This may prove to be the most effective way of removing residual cancerous cells, because currently, the only way it can be done is by also killing a 'clear margin' of healthy cells to ensure all cells have been killed.

There are some obstacles to face e.g. with quantum dots. Cadmium selenide, which proves to be one of the most researched quantum dots, is highly toxic, restricting its use in medical imaging. However, with more research into nanotechnology, a new alternative will surely be found, proving to be safe in the body, and possess more beneficial properties, and if one doesn't exist naturally, it can be synthesised.

This paper has used several examples to show that nanotechnology is changing oncology; however the biggest problem is that more still needs to be done. But we must bear in mind that nanotechnology is a relatively new development, as the concept has been since Feynman's "There's Plenty of Room at the Bottom" talk in 1959, but it is only recently the technology has been available for nanotech engineering, which we must not forget, is simply building by atoms and molecules.

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