

The use of Nanotechnology to control thrombus formation
caused by CHD; by inhibiting Von Willebrand Factor.

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PASS WITH DISTINCTION

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Abstract

Nanotechnology is an ever-progressing and exciting field that is being applied to medicine. The main development areas are drug delivery, therapeutic techniques, diagnosis and imaging. The unique properties of nanoparticles make them ideal for such treatments, with little side effects and life-changing results. Currently the main areas of focus are Cancer, Cardiovascular Disease and Genetics. Our paper proposes a possible treatment for those suffering from Coronary Heart Disease and more specifically, atherosclerosis, and are therefore at high risk of forming a fatal thrombus, resulting in myocardial infarction. We postulate that using current developments in nanotechnology for medicine, a drug delivery system could be engineered to diagnose and treat high levels of Von Willebrand Factor glycoprotein, which is essential to the aggregation of platelets in the process of thrombosis. If this treatment were successful, the number and medical cost of patients admitted for a myocardial infarction would decrease, thus giving more time to treat the cause of the disease and offering a better quality of life for many.

Introduction

On the 29th December 1959, an American physicist called Richard Feynman gave an after-dinner talk at the American Physical society exploring how things could be built and manipulated on a molecular scale. Feynman stressed that, *“In the year 2000, when they look back at this age, they will wonder why it was not until the year 1960 that anybody began seriously to move in this direction”*. However it was not until 1974, when a man called Norio Taniguchi first used the term “nanotechnology” when referring to technology with the precision of the nanometre. From then on, the term “nanotechnology” has developed into something we know to be incredibly exciting and revolutionary to medicine and industry across the world.

Since the development of microscopes within IBM in Switzerland during the 1980s, we have witnessed a huge leap in advancements on a molecular scale. The first paper on nanotechnology was published in 1981 by K. E. Drexler, where he discussed the design of protein molecules using nanotechnology, which paved the way for molecular manufacturing. In this paper, Drexler highlighted the current problem of the time of handling atoms in bulk rather than individually, so imperfections were always obtained as a result of the devices shrinking in order to handle the matter. He noted that as long as we stuck with this method, we will be *“unable to reach the ultimate level of microtechnology — the structuring of matter to complex atomic specifications”*. Drexler made the point that it was possible to design protein molecules with complex atomic structures, which would side-step obstacles faced by the current nanotechnology of the time.

Then came a discovery that really pushed nanotechnology forward in 1985 – the buckyball. Its full name is ‘buckminsterfullerene’ after the architect who designed a dome based on the structure of this molecule, Richard Buckminster Fuller, and is formed of 60 Carbon atoms joined in hexagons and pentagons to make a ball structure – hence the name “buckyball”. Being an extremely stable molecule, due to its low levels of reactivity, the buckyball could be key to the progression of nanotechnology. Coupled with its insolubility, the buckyball has been under close attention, and has been put forward as a component for drugs delivery, or isolation, as it has a hollow middle.

Along with the buckyball, another exciting development in nanotechnology was Carbon nanotubes (as shown in Fig1), which are rolled up sheets of graphite with the wall of the nanotube only one atom thick. Their hollow nature allows them to act as pipes for drugs delivery or insulation, and the fact that it is graphite also allows it to conduct electricity. Their long thin shape allows them to penetrate membranes such as cell walls, and act almost like needles in their drugs delivery. Their electrical resistance also changes when molecules attach to their surface, so scientists are developing this as a detection method.

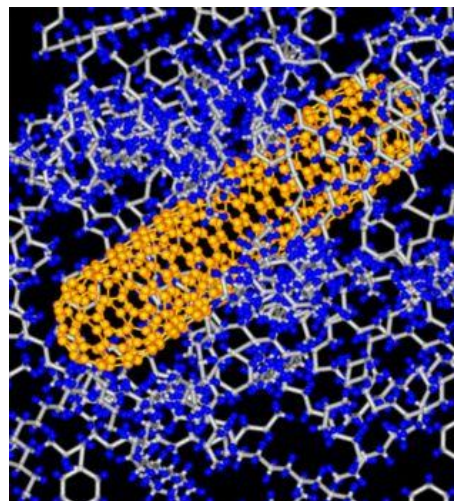


Figure 1 - Carbon Nanotube – Photo courtesy of NASA

Currently, nanotechnology is becoming a prominent tool in areas of medicine including; drug delivery - where nanotubes are designed with attached molecules that are then to be attracted to diseased cells e.g. cancer cells, and then deliver drugs to the affected area quickly. The earlier a disease is located and treated the more likely the full recovery. Nanoparticles that deliver chemotherapy drugs are currently under development. Along with this, nanoparticles are being used in therapy techniques, for example the hollow structure of buckyballs makes them ideal for trapping free radicals during an allergic reaction to prevent inflammation. Nanotechnology can also be applied to diagnostics and imaging, using nano-engineered molecules like quantum dots (qdots) to locate cancer tumours.

Anti-microbial techniques were one of the first applications of nanotechnology in medicine; nanocrystalline silver was used to treat wounds. One of the characteristics of nanoparticles is that they contain nitric oxide gas, which is known to kill bacteria, making them extremely useful in fighting microbes. Incorporating detection with drug delivery, nanoparticles are able to release anti-microbial drugs to kill any invading pathogens.

As well as all the incredible advances that nanotechnology has already given us, it could be the breakthrough we need to combat one of the western world's biggest killer – Coronary Heart Disease (CHD). CHD can develop over many years, and is a multifactor disease, but is often self-inflicted by activities that cause hypertension such as heavy smoking. The hypertension can be especially damaging in the coronary arteries, causing wear to the endothelial walls. This encourages the growth of smooth muscle, which consequently can result in fatty substances such as LDLs being deposited on the endothelial walls, forming an atheroma. This condition is atherosclerosis. As the atheroma develops, it narrows the lumen further and increases the risk of rupturing and initiating the process of thrombosis, forming a thrombus that constricts blood flow even further, and can result in a myocardial infarction (heart attack).

At the end of 2010 the British Heart Foundation published the most recent statistics on CHD;

“It is responsible for 82,000 deaths in the UK each year, an average of 224 people each day. There are nearly 2.7 million people living with heart disease in the UK. The UK spends £3.2 billion each year on healthcare costs for heart disease.”

Of these myocardial infarctions is the biggest killer, with around 124,000 attacks each year in the UK alone, this equates to someone dying as a result of heart attack every six minutes.

There are effective medical treatments available for CHD e.g. Statins, beta-blockers and ACE inhibitors, but developments are being made in applying nanotechnology to monitor and treat this disease. Current areas include the problem of plaque build up of the endothelium of the artery walls, which can lead to atherosclerosis and thrombosis.

An area of treatment for CHD is controlling clot formation by the use of anticoagulants. An example would be warfarin. Warfarin is used to treat thrombus formation and prevent a potentially resultant myocardial infarction (heart attack). However, drugs such as warfarin can carry some serious side effects such as severe bleeding, muscular aches, flu-like symptoms, along with fatigue and hair loss. This therefore has left scope for the use of nanotechnology and its unique treatment advantages – one of many being that it often targets a very specific area, and therefore has little or no side effects.

We have examined how nanotechnology can be applied and manipulated in medicine thus far, so we shall now discuss how various concepts and techniques of nanotechnology may be developed further specifically to the aspect of controlling thrombosis.

Discussion

We suggest that the principles and current development of nanotechnology in medicine could be applied to control levels of Von Willebrand Factor (VWF) at the site of thrombus formation in major arteries, thus preventing fatal clots resulting in Myocardial infarction.

The purposes of a clot are to prevent further bleeding when a rupture occurs. It is a complicated process, where chemicals are released from damaged endothelium wall that instigate a cascade of reactions. Simply - Pro-thrombin is converted into thrombin, which then converts fibrinogen into fibrin that forms the 'mesh' that accumulates platelets and red blood cells forming a clot. Von Willebrand factor is a glycoprotein that 'sticks' the platelets together.

Firstly use nanotechnology to detect and measure excess levels of Von Willebrand Factor:

The National Heart, Lung and Blood Institution in the U.S has established a programme involving scientists and researchers from leading universities and institutes in America, making up centres for The Program of Excellence in Nanotechnology, dedicated to investigating the possibilities of using nanotechnology for heart and lung related illnesses. Of these, one of the aims within the research at Emory University Georgia Institute of Technology is to find ways of making qualitative analysis for plaque *in vivo* (inside the body), i.e. detecting its presence. Currently quantitative analysis can only be achieved using samples of body fluids. For example, Nanosphere Inc. has developed high sensitive protein diagnosis using gold nanoparticles. Gold nanoparticles have properties that have proven for them to be most efficient in diagnosis:

- They can be functionalised with oligonucleotides (short pieces of DNA or RNA with sequences complementary to target sequences of clinical interest) or antibodies specific to the protein of interest.
- They are extremely stable – have a long self-life and are non-toxic.

Their properties also enable them to be used in drug delivery. They have already been incorporated into drug delivery systems to treat cancer, its advantage being that molecules can be attached to enable the nanoparticles to travel through the bloodstream without being attacked by the body's immune system. This therefore makes them ideal nanoparticles for a method of detecting VWF. While this can be achieved *in vivo* using current developments, further developments need to be made to enable quantitative analyses *in vivo*; thus enabling treatment to only be administered when levels are calculated as higher than normal i.e. forming a fatal clot blocking an artery.

Diagnosis using nanotechnology:

A possible way of achieving this could be to use the ideas of the recent discovery and development of an artificial pancreas/nano-chip. Professor Tejal Desai of bioengineering, at UCSF, has used bioMEMS technology (biological microelectromechanical systems) to create a nano-porous chip/capsule that can be implanted into the body. It builds upon current developments of lab-on-chip forms of diagnosis, using it therapeutically. The capsules contain millions of pancreatic cells, when blood sugar flows inside, it stimulates the cells to produce insulin and control the body's blood sugar levels.

This idea could be applied to create a mechanism that detects levels of VWF. A capsule/nanochip implanted near the coronary arteries, at sites that show early signs of atherosclerosis, which contains gold nanoparticles as part of *in vivo* diagnosis. To retrieve the gold nanoparticles for analysis, methods used by Nanosphere, Inc. could be built upon; magnetic nanoparticles with corresponding antibodies for target protein are used to 'pull' the complex towards a magnetic field to then be analysed. Developing a similar process *in vivo* and to be able to quantitatively analyse the level of VWF present, once achieved would then stimulate the release of nanoparticles, stored in the chip, which then treats by inhibiting VWF. Tests would be needed to ascertain what level of VWF correlates to its presence being too high and thus a fatal clot is forming. Only when this calculated level is detected will the treatment then be simulated to release.

Developing Quantitative analysis *in vivo* could benefit not only heart disease, but theoretically could be applied to diagnosis and therapeutic techniques of many other illnesses.

Alternatively the same processes of detection and diagnosis could be applied to target Factor VIII levels as they correspond to levels of VWF. Factor VIII, when inactive, binds to VWF during circulation to form a stable complex. During thrombosis it disassociates and is then involved in a cascade of reactions that converts fibrinogen to fibrin, forming the 'mesh' of the blood clot. If Factor VIII is not bound to VWF it rapidly degrades; therefore the detection of high levels of factor VIII, would suggest that a corresponding high amount of VWF is present. However again tests would be needed to recognise what level, when detected, would correlate to a fatal clot being formed. It would be extremely important to ensure the results of these tests were accurate, as it would prevent any risk of releasing the inhibiting treatment too early, preventing a clot from forming at all.

Practicality would need to be considered for this alternative i.e. is detecting and creating a nanoengineered particle that correspond to Factor VIII more simple in structure and more cost effective to create, than a nanoengineered particle that corresponds to VWF glycoprotein?

Secondly, inhibit Von Willebrand Factor – The Treatment:

Currently, Rutgers has developed nanolipoblockers (a type of nanoparticle) that targets specific receptor molecules on white blood cells to stop LDL cholesterol attaching to them. The particle is engineered into a structure known as a micelle. Similarly scientist and engineers at US Santa Barbara have developed a micelle with a peptide on its surface that binds to the surface of plaque. In addition it has the flexibility to diagnose and deliver drugs to treat *in vivo*. This idea could be applied to blocking VWF from aggregating platelets. During the process of thrombosis VWF A1 domain binds to platelets GPIb – receptor. A nanoengineered molecule like the nanolipoblocker could be designed to fit either the domain or receptor to prevent the other attaching, possibly using Sulfatides as a template; research from the journal of Thrombosis and Homeostasis published in 2003 reveals Sulfatides' ability to inhibit platelet adhesion by binding to VWF A1 domain overlapping the GPIb-binding site.

Ethical and technical concerns:

However there could be some technical and ethical concerns regarding this treatment.

Most predominantly is the danger of controlling and blocking VWF. It is an essential component to the process of thrombosis; low amounts could mean excessive bleeding, and the inability to heal damaged tissue. The necessity to having the right amount is highlighted by those who suffer from Von Willebrand disease, where they have a deficiency or dysfunction of VWF, resulting in a lifetime of haemophilia related problems, unless treated. Although by only blocking the A1 domain, VWF's A3 domain that binds to collagen is still functional. By releasing just a certain calculated amount of nanoparticles when the diagnosis reading is detected at very high levels of VWF/ Factor VIII, it will still allow platelets to aggregate and adhere to the collagen repairing the damaged artery wall; whilst preventing the clot to continue forming to an extent that inhibits blood flow to the heart.

Many reliable tests would need to be performed in order to establish the safest amount of treatment that should be released from the nanochip at one time; taking into account any possible time lag for levels of detected VWF to return to a 'normal' level, therefore no excess treatment is stimulated to release when it is not required. With regard to the ethical dimension of this treatment one would need to ensure that people remain focus on prevention of the disease rather seeking easy solutions to the problem. In addition cost efficiency of the treatment would need considering, would the money be more beneficial elsewhere? The implication and necessity of the treatment, i.e. who takes priority in receiving the treatment considering the high number of those that suffer from CHD in the UK alone? Finally the appropriateness of how far we are willing to have foreign pieces of technology present in our bodies.

Conclusion

To conclude, our new idea, targeting Von Willebrand Factor to prevent fatal thrombus formation causing a myocardial infarction, lies within current development using nanotechnology to treat CHD. Specifically we have addressed the areas of diagnosis and therapeutic techniques. It builds upon nanotechnology research in other areas for medicine and applies it to treating CHD e.g.

- Artificial pancreas - nanochip that both detects and stimulates the release of insulin stored in the chip, treating diabetes.
- Engineered gold nanoparticles that are undetected by the body immune system, for diagnosis.
- Nano-engineered particles – micelles that target specific proteins for drug delivery.

All of these are incorporated into our idea; a nanochip that qualitatively measure and detects high levels of Von Willebrand factor, which then stimulate the release of nano-engineered micelles targeted to inhibit WVF from aggregating platelets. Theoretically this treatment would be very beneficial in reducing the number of patients admitted to hospital each year along with deaths from myocardial infarction. The treatment by controlling thrombosis in coronary arteries and preventing fatal clots will allow more time to then treat the disease.

We acknowledge that this treatment is not without fault;

Firstly, initially implanting the chip within the coronary system in areas under high pressure would require delicate surgery, unless developments could be made enabling chip to be injected and remain in the area of interest.

Secondly there is the problem of how the chip would remain in the target area under high pressure. A possible solution may be to attach molecules to the nanochip, which would then bind to the endothelial wall.

From our explorations and research so far we believe there are real possibilities in this approach to treating CHD; it would be exciting to see how this could be developed further.

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