

The Use of Nanoparticles in the Clinical Management and Treatment of Atherosclerotic Plaques

By Bronwen Crowther

PASS WITH MERIT

Research paper based on
Pathology Lectures at Medlink 2010

Abstract:

Atherosclerosis – the formation of a fat-based plaque on the internal surface of an artery - is a condition which is of increasing concern in Western society, due largely to an increased dietary intake of low-density lipoproteins and a subsequent substantial increase in the percentage of the population exhibiting high blood pressure at an earlier age. This paper discusses the prospective treatments of atherosclerosis using developing and current nanotechnology as a medication delivery system, the pathophysiology of the condition and the logistics of the considered treatment. Its aims, therefore, lie principally in the formation of a conclusion as to the suitability of the utilisation of nanotechnology in such medical instances, with reference to the ethical considerations involved in this process and drawing upon recent preclinical studies pertaining to the treatment of the plaque and promising future applications.

Introduction:

Primarily, and often specifically affecting the large and medium arteries, atherosclerosis is the most prevalent form of arteriosclerosis, and presents as a build-up of cholesterol, fatty substances, cell waste products, calcium and fibrin – collectively, ‘plaque’ - which eventually contributes to the significant reduction of the diameter of the lumen of the affected blood vessel. The formation of an atheromatous plaque (Figure 1) occurs when the endothelium of an artery becomes damaged, and the body’s immunological response causes inflammation in the area - T cells are ‘activated’ by antigen-presenting macrophages, an action which directly precipitates the production of interferon. This not only prepares the macrophages for plaque formation, but also hinders the smooth muscle and endothelial functions. ‘Activated’ macrophages produce pro-inflammatory cytokines which induce pro-coagulant and adhesive properties on endothelial cells, meaning that the macrophages become attached to the endothelium and gradually harden.

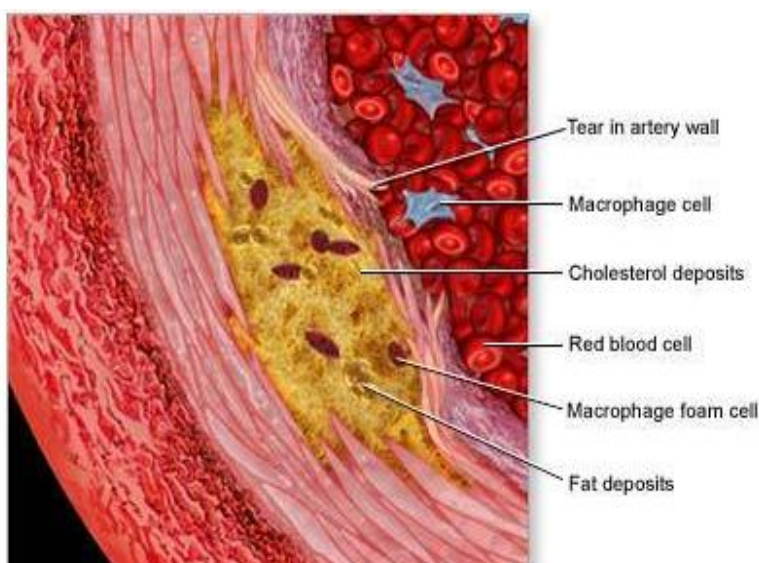


Figure 1.

<http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001224/figure/d19e1865/?report=objectonly>

With an increased fat intake in the typical diet, especially in developed countries, incidences of atherosclerosis are becoming more common, and are occurring in people of a younger age. The risk factors associated with atherosclerosis include a diet rich in low-density lipoproteins (with a higher proportion of lipid than protein), high blood cholesterol levels, high blood pressure, smoking and diabetes, all of which are proven to be closely associated with obesity – a far more common condition in the last few decades due to socio-economic changes. The physiological consequences of atherosclerosis are numerous and invariably serious – in the case of coronary heart disease, once the lumen has been restricted by more than approximately 50%, the blood flow is insufficient to the extent that arteriosclerotic heart disease, also known as angina (pain felt following exercise) develops. Coronary thrombosis is a direct consequence of arterial plaque build-up, as the brittle nature of the calcium deposits evident in the later stages of plaque formation increases the risk of cracking/breaking. This initiates the platelet response, and a blood clot is subsequently formed, which may break off and cause a pulmonary embolism, stroke or myocardial infarction.

The British Heart Foundation reports that in the UK heart disease affects over 750,000 people and accounts for approximately 1 in 5 male deaths and 1 in 6 female. The NHS costs allocated for the treatment and prevention of atherosclerosis and subsequent heart failure are therefore immense. The BHF is currently launching a 'Broken Hearts' research campaign due to cost around £50m, perhaps indicating just how much money must be put into solving this pressing, expanding medical issue. Coronary heart disease is the largest killer in the UK, and although much is being spent on research and treatment, patients with severe heart disease have only a 40-50% chance of living for another 5 years, and around 3-4% of UK men between the ages of 35 and 74 die every year as a direct result of coronary heart disease. This clearly indicates a need for more advanced and effective treatment, and there are already-discovered methods which are currently progressing through medical trials, which may revolutionise the clinical management of this prevalent condition. One such potential treatment involves the use of nanoparticles, as seen in the research study and paper "Spatiotemporal controlled delivery of nanoparticles to injured vasculature," by Juliana Chan et al. (Massachusetts Institute of Technology; January 18, 2010).

Nanotechnology is essentially the construction of substances at a molecular level. In a medical context, in the case of atherosclerosis, nanotechnology may be used in a variety of ways; in the imaging and characterisation of atherosclerotic plaques, in the diagnosis of cardiovascular disease, and in the delivery of drugs such that they are able to be specifically targeted at the affected area. Nanotechnological methods may be used in conjunction with current treatments, such as statins for the lowering of cholesterol and stents which may be inserted into the blood vessels in order to improve and regulate the blood flow. It may be able to replace, at least partially, the use of more surgical methods in treatment, such as coronary bypass surgery. Nanotechnology is a relatively recent advancement in science -

studies into the field of nanotechnology were first inspired by the notable physicist and Nobel Prize winner Richard Feynman in 1959:

"I want to build a billion tiny factories, models of each other, which are manufacturing simultaneously. . . The principles of physics, as far as I can see, do not speak against the possibility of manoeuvring things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but in practice, it has not been done because we are too big."

This excerpt encapsulates the central ideas of nanotechnology - to build upwards from an atomic, or 'nano' level. This entails the use of individual atoms to create a variety of structures; the most notable of which include nanotubes (hollow tubes), micelles (spherical structures) and Buckminsterfullerene, or 'Bucky Balls' (Figure 2).

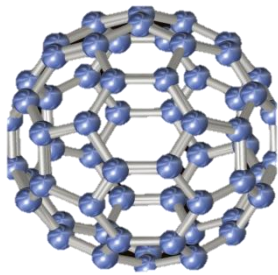


Figure 2

<http://www.nanotech-now.com/images/buckyball2-large.gif>.
Copyright Dr. Roger C. Wagner, Dept. of Biological Sciences, University of Delaware.

Since 1959, the study of nanotechnology has progressed rapidly, and the structures that scientists are able to create have developed drastically in terms of complexity and function. (Figure 3).

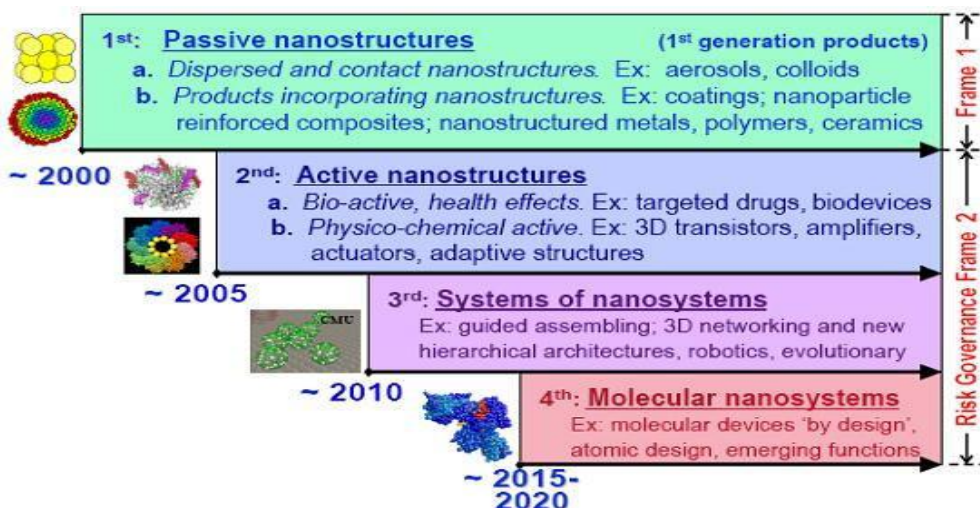


Figure 3

<http://www.crnano.org/whatis.htm>

The shape of many of these nanoparticles may be specifically altered in order to enable them to act as a delivery method for drugs, so that a more specifically targeted dose may be given. It must be stressed that the notion of nanotechnology is not necessarily to 'shrink down' the technology that we already possess. Nanotechnology aims to build *upward* instead of, as we have always done, downward, and it is this different approach that gives 'nanotech' an inherent suitability use within to the field of medicine.

Discussion:

There are, in theory, numerous methods that may be employed through the use of nanotechnology that would combat arterial plaque growth, and therefore prevent heart disease. Before, however, the study of nanotechnology is able to produce working complex biomechanical tools, scientists are using the more basic principles of nanotechnology in conjunction with therapies that have been clinically tested and are currently in use.

Fumagillin, a neovessel growth inhibitor, has recently been proven to drastically reduce plaque growth in rabbits in a study conducted by scientists at Washington University School of Medicine in St. Louis, as seen in "Endothelial avb3-integrin targeted fumagillin nanoparticles inhibit angiogenesis in atherosclerosis." (2006). The nanotechnology involved not only acted as a delivery system – a sort of biomechanical capsule - but also permitted the drug to remain on the affected site for a longer period of time, due to the nanoparticle's ability to attach itself to the plaque. Neovessel inhibitors prevent the growth of new, tiny blood vessels which provide access to the plaque for the aforementioned macrophages, effectively preventing substances that would fuel the growth of the plaque from reaching it, and restricting its progression.

In the study, rabbits were administered the drug following an 80-day high-fat diet which caused the growth of small plaques in the coronary arteries, and other arteries throughout the body. The nano-spheres used, invented by Samuel Wickline, M.D., professor of medicine, of biomedical engineering, of physics and of cell biology and physiology, and Gregory Lanza, M.D., Ph.D., associate professor of medicine and biomedical engineering, were able to be detected by an MRI (Magnetic Resonance Imaging) machine in order to monitor the progress. This may perhaps suggest the possibility of using similar nanoparticles in the future for the detection and diagnosis of atherosclerosis in humans, not just in the treatment. They were also capable of transporting more than one molecule, which leads to the reasonable conclusion that were there a more advanced drug created for the treatment of atherosclerosis, this nanoparticle would be capable of carrying that to the plaque as well as a neovessel inhibitor. If this drug were, for example an anti-inflammatory capable of reducing the size of a plaque, then not only would the growth of the plaque be slower, if not halted, but the severity of the blood vessel restriction could also be less. If implemented, this method would provide thousands with the chance to drastically reduce their risk of experiencing congestive heart failure, a stroke, a pulmonary embolism or a heart attack.

Intriguingly, fumagillin is not a currently used drug in the case of atherosclerosis due to its neurological side-effects, but the nanotechnology-based delivery system enables smaller doses to be used due to the fact that the drug is, owing to the structure and properties of the nanoparticle, able to be 'carried' directly to the plaque, thus eliminating the need to administer higher quantities of the drug. In fact, the dosage of fumagillin each rabbit received was 50,000 times lower than the total fumagillin dose used in previous experiments. This did not affect the efficacy of the drug, as illustrated through the results of

the experiment, which detail that the growth of the new blood vessels in plaques was reduced by 60-80%. This result could have enormous implications for the future of drug administration, not only in the case of atherosclerosis, but in many other areas of medicine. Patrick Winter, Ph.D., research assistant professor of medicine also came to this conclusion, stating that "This could open the door for a lot of drugs that have failed to be approved because they caused a number of side effects at a higher dose. It might pay to look at these drugs again and ask if placing them on these nanoparticles can help them be effective at a lower dose and clinically useful." Applying this knowledge to the conclusions made above regarding the prospect of the nanoparticles delivering more than one drug, we may develop these theories further - it may be possible that heart medications that have already been rejected due to side-effects present in a large dose may be suited to this hypothetical treatment method. Indeed, the research team have stated "We think fumagillin nanoparticles potentially could be incorporated into a protocol that includes lipid-lowering statin drugs or dietary changes", perhaps indicating that in the next 10 to 20 years, atherosclerosis may be vastly more treatable than it is today through a combination of treatments including ones that are nanotechnology-based.

Unfortunately, fumagillin is only functional when used in cases where the plaque being treated is in its early stages of growth – plaque in the later stages is much harder as a result of calcium deposits, and this method is more preventative than functional as a treatment in severe cases of atherosclerotic heart disease. It was also observed by the team that these effects were only short term – when the fumagillin was used with no statins alongside it, the MRI scans indicated further growth of the neovessels despite the continued administering of the drug. However, when the statin had been given for at least one month prior to the fumagillin treatment, the previously recorded five-fold reduction in neovessel growth was maintained for four weeks. It may therefore be reasonable to suggest that this drug, in conjunction with the nanotechnology delivery system, would be suitable for high-risk patients who are already taking statins for high cholesterol. This would mean being able to prescribe the drug to those who are most at risk as a preventative measure, thus decreasing vastly the risk of developing a more severe condition at a later point. From a financial point of view, this medication would, as a result of lowering the number of patients with heart failure, save the NHS much of the money that it currently spend on the hospitalisation and treatment of patients whose hearts have been rendered all but useless by atherosclerosis.

In much the same vein as the research at Washington University School of Medicine, scientists at Harvard Medical School ("Spatiotemporal controlled delivery of nanoparticles to injured vasculature," Juliana Chan et al. Massachusetts Institute of Technology; January 18, 2010.) have created a nanoparticle that they term 'nanoburrs', which are attracted specifically to the 'basement membrane' of the endothelium because they are coated with protein fragments that target specific proteins unique to this layer. This layer, and therefore these proteins, is only exposed when the lining of an artery is damaged due to the presence of atherosclerotic plaque build-up.

The drug in this research study, paclitaxel, rather than preventing the growth of plaque via the prevention of the formation of neovessels, actually inhibited the cell division itself in order to halt the expansion of the volume of the plaque. The particles which are designed to transport the drug are spheres 60 nanometres in diameter, and have a triple-layered structure; an inner layer which holds the drug itself and a polymer chain called PLA, a middle layer which consists of soybean lecithin, and an outer coating of a PEG – a polymer designed to protect the rest of the nanoparticle during its journey through the blood stream. Once the ‘nanoburr’ has attached itself to the exposed basement membrane of the arterial wall, the drug may only be released when it has become detached from the PLA polymer chain. This takes longer the longer the chain is in length, leading to the ability to regulate the speed at which the drug may be released over time. Currently, the maximum time taken for the paclitaxel to completely disperse has been 12 days, proving that scientists are truly able to create slow-release, targeted drug delivery systems.

This treatment method has been hypothesised as a companion therapy for patients having an arterial stent inserted, or as an alternative treatment for patients whose atherosclerotic plaques are situated in areas where a stent would not be suitably placed, such as at a point of divergence in the artery.

Researchers at Rutgers, The State University of New Jersey have approached the issue of plaque formation differently, proposing to use nanotechnology as a means to prevent the collection of foam cells which comprise the bulk of a plaque. During the process by which atherosclerosis becomes a problem, macrophages accumulate oxidized LDL (low-density lipoproteins) and secrete chemicals that break down nearby tissues. These then become what is known as a collection of fatty foam cells. The researchers, therefore, aim to administer a drug capable of acting as a blocker so that the macrophages are incapable of coming into contact with the LDL, thereby minimising the quantity of foam cells produced and the volume of plaque present. This contrasts with the current medical protocol of introducing statins in order to lower the quantity of LDLs in the body, rather than inhibiting their progress once they are actually in the blood stream.

These new molecules have been termed nanolipoblockers, and are in the form of a ‘micelle’ structure, meaning that they consist of nano-chains which have been designed such that their polar nature causes them to arrange themselves so that the ends cluster around a point. “We're employing the tools of nanotechnology – specifically tailoring the structure of the molecule, changing groups on the ends of the chains and closely analysing which forms of the particles bind to the different macrophage receptors” stated Kathryn Uhrich, Rutgers’ professor of chemistry and chemical biology.

It should be mentioned that in addition to these atherosclerosis-specific functions, nanotechnology also provides new techniques for imaging, diagnosis and other methods of treatment, such as by combining nanoparticles with an MRI contrast. Molecular beacons, magnetic nanoparticles, gold nanoparticles, quantum dots, polyketals and hydrocyanine

dyes are all biomechanical tools developed by nanotechnology, and they will drastically change the diagnosis and treatment of many conditions and diseases in the future.

The financial and ethical considerations of research into such a prevalent and deadly condition are many. The current research so far discussed within this paper indicates that the techniques used are predominantly preventative – it seems that once atherosclerosis has progressed to a later stage it becomes much more difficult to treat. The use of these preventative methods, though it may save the NHS money in terms of palliative care and surgical procedures on patients who would otherwise have developed congestive heart failure, the cost of such elaborate, specifically-designed nanostructures begs the question of how much exactly this would cost to implement as a treatment recognised and utilised by the national health service.

Gang Bao, the program's director and the Robert A. Milton Chair in Biomedical Engineering in the Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, has stated that "By using nanoparticle probes in vitro and in vivo, we hope to be able to detect early-stage cardiovascular disease, but many important issues such as detection specificity, toxicity and safety still need to be addressed." This suggests strongly that as an emerging method, nanotechnology is still not fine-tuned enough for use in a clinical environment. Even if it were, the moral implications of some of the aspects of the research are enough to dissuade some from favour of it.

Before many of the methods involving nanotechnology are able to be used, a stem-cell based procedure must first be performed in order to establish the extent of the damage caused to the vasculature. Stem cells are often gained through either embryonic or umbilical means, and the ethical beliefs surrounding this practise are contentious. The discarding of an embryo is seen by many faiths and cultures to be equivalent to the murder of a child, and as such many would object to the use of nanotechnology on moral grounds. Animal testing, always a contentious issue, may also be a factor in the judgement of the ethics of the research into atherosclerosis. The rabbits and rats used in the pre-clinical trials of these new drug delivery systems would have been exploited and eventually killed, an idea that some find morally repugnant and unacceptable.

The idea of having fully automated - though simple and tiny - machines inside the human body raises the question of where we 'draw the line' with nanotechnology. Richard Feynman spoke of "A billion tiny factories", but what of the emissions that would be produced? The consequences of filling humans with 'machinery', apt to break down or malfunction, could have devastating implications in the years, decades, or centuries to come, as nanotechnology advances and the structures that we are capable of building are no longer passive transportation devices, but tiny surgeons relied upon for the body's functionality.

Conclusion

Research into cardiovascular disease, and discoveries of new clinical managements and treatments, is occurring internationally and progressing quickly. As mentioned previously, charities, universities and individual scientists are combating a condition which seems uncontrollable, fuelled by a decrease in the quality of diet consumed in Western society. However, as many of these effects are at least partially self-inflicted, there are those who believe that time, money and resources would be better spent in other areas of research.

The controversial nature of the research, as well as a lack of public funding, have led to a severe hindrance in the progress of study, though breakthroughs in the field are not by any means scarce. The meticulous nature of drugs trials and pre-clinical studies ensures that any new method of the treatment of atherosclerosis will not be in regular use for at least another 5-10 years. Though there may be a certain amount of moral scrutiny over debatable issues such as the use of embryonic stem cells and the questionable future nature of biomechanics, heart failure is so common that the current technology and treatment available is inadequately equipped to tackle the problem it presents.

It may be seen that many of the potential solutions presented and discussed within this paper are not entirely functional – the effect on the growth of neovessels by the drug fumagillin, for example, are only temporary, and the toxicity of some nano-devices used in the administering of drugs is unknown in the long-term. These doubts may give cause for concern that nanotechnology may not be enough on its own, especially in the treatment of atherosclerosis, the effects of which are currently all but irreversible. In conjunction with statins, other cardiovascular drugs and differing nanotechnology-based methods, however, ‘nanotech’ shows great promise in the field of medicine. If Fumagillin performs more favourably when used in addition to a regular dose of statin, how would it respond to use alongside a nanolipoblocker? It is these sorts of tests that are yet to be conducted, and these sorts of tests that will revolutionise our medical approach to heart disease in the future.

In terms of ethics, it may be considered that the sheer number of people who will experience heart failure in the years to come take precedence over the uncertainty of future techniques that have not even been invented as of yet. There will come a point at which science must create an ethical boundary of what is acceptable to use inside our own bodies, but when the moral choice lies between retaining our biological purity or our lives, then the outcome of the situation is inevitable.

New research into treatment for atherosclerosis, especially through nanotechnology, has the potential to save thousands of lives, and may even change the way doctors practise medicine, revolutionising current medical approaches to not only cardiovascular disease, but countless other diseases as well, preventing potentially millions from a death by untreatable and painful disease.

References

Journal-based References:

- Juliana Chan, Liangfang Zhang, Rong Tong, Debuyati Ghosh, Weiwei Gao, Grace Liao, Kai Yuet, David Gray, June-Wha Rhee, Jianjun Cheng, Gershon Golomb, Peter Libby, Robert Langer, Omid Farokhzad.. "Spatiotemporal controlled delivery of nanoparticles to injured vasculature," (Massachusetts Institute of Technology; January 18, 2010).
- Feynman, R. "There's plenty of room at the bottom" (1959)
- Winter PM, Neubauer AM, Caruthers SD, Harris TD, Robertson JD, Williams TA, Schmieder AH, Hu G, Allen JS, Lacy EK, Zhang H, Wickline SA, Lanza GM: "Endothelial avb3-integrin targeted fumagillin nanoparticles inhibit angiogenesis in atherosclerosis." *Arterioscler Thromb Vasc Biol* 2006; 26: 2103-2109.
- Plourde NM, Kortagere S, Welsh W, Moghe PV. Structure-activity relations of nanolipoblockers with the atherogenic domain of human macrophage scavenger receptor A. *Biomacromolecules*. 2009 Jun 8;10(6):1381-91.

Web-based references

- Figure 1, Plaque structure.
<http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001224/figure/d19e1865/?report=objectonly> (Reviewed by: David C. Dugdale, III, MD, Professor of Medicine, Division of General Medicine, Department of Medicine, University of Washington School of Medicine. Also reviewed by David Zieve, MD, MHA, Medical Director, A.D.A.M., Inc)
- Atherosclerosis pathophysiology
<http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001224/>
- Introduction to nanotechnology
<http://www.crnano.org/whatis.htm>
- Figure 2, structure of Buckminsterfullerene
<http://www.nanotech-now.com/images/buckyball2-large.gif>. Copyright Dr. Roger C. Wagner, Dept. of Biological Sciences, University of Delaware.
- Figure 3, progression of nanotechnology
<http://www.crnano.org/whatis.htm>

- Washington University Medical School research
<http://medicineworld.org/cancer/lead/7-2006/nanotechnology-and-atherosclerosis.html>

- British Heart Foundation 'Mending Broken Hearts' appeal
http://www.bhf.org.uk/research/mending-broken-hearts-appeal/why-mend-broken-hearts.aspx?pid=p&sc_cid=MBH-EX-25&utm_source=MBH-AW&utm_medium=MBH-AW&utm_campaign=MBH-AW-G10_010211&gclid=CJzW3v2ky6cCFcoa4QodXxXwDA

- Research on nanoburrs
<http://www.understandingnano.com/nanomedicine-nanoparticles-nanoburrs-targeted-drug-release-artery.html>

- Nanotechnology specific to atherosclerosis
<http://www.news-medical.net/news/20101005/NIH-awards-contract-to-develop-nanotechnology-tools-for-atherosclerosis-treatment.aspx>

- Nanolipoblockers
<http://www.understandingnano.com/nanolipoblockers-white-blood-cells-ldl-plaque.html>