

NANOTECHNOLOGY AND
ITS ROLE IN THE
TREATMENT OF HEART DISEASE
IN PARTICULAR, ATHEROSCLEROSIS

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Abstract

Since 1959 the research on Nanotechnology has developed hugely, and it is this research that is the basis for this paper. Using molecular machines it is possible that, in the future, both a cure and an early detection for fatal diseases will be found. Within that, current research is leading to a point where potentially fatal heart diseases, for example atherosclerosis, can be cured relatively non-invasively. In this paper a two ways in which this could proceed are discussed (CREKA-targeting micelles and nanoburrs), as well as the possible social and ethical issues surrounding such developments.

The conclusion is that further research is needed, particularly in the areas which apply to humans and the interaction of the two treatments. If the outcome of these further investigations is positive, then it is very likely that treatments based on nanotechnology can significantly reduce the impact of atherosclerosis in society.

Introduction

Nanotechnology is one of the newest ideas for application in both Medicine and Industry. It began with Richard Feynman in 1959 where he discussed writing the entire Encyclopaedia Britannia on the “head of a pin” [1]. From there, inspired by the revolutionary thoughts of Feynman, Eric Drexler wrote both an article (Molecular engineering: An approach to the development of general capabilities for molecular manipulation, 1981) and a book (Engines of Creation, 1986) exploring the potential for molecular machines that could work as small scale motors or gears. It was in his book that the word ‘Nanotechnology’ was first used as a term to describe manufacturing and engineering on a scale of a billionth of a meter.

During the 1980s, nanotechnology continued to thrive. In 1981 the Scanning Tunnelling Microscope (STM) was invented by Binnig and Rohrer which was the first time that individual atoms could be clearly identified. Although limited, it brought the field of nanotechnology forward as concepts were now both viewable and testable. Further expanding nanotechnology, in 1986, was the invention of the Atomic Force Microscope (AFM). This eliminated some of the limitations of the STM, mainly that non-conducting materials such as organic materials could be viewed through it. It was the AFM that played an integral part in the discovery of Buckminsterfullerene, or ‘carbon buckyballs’, at Rice University 1985-1986 by Richard Smalley.

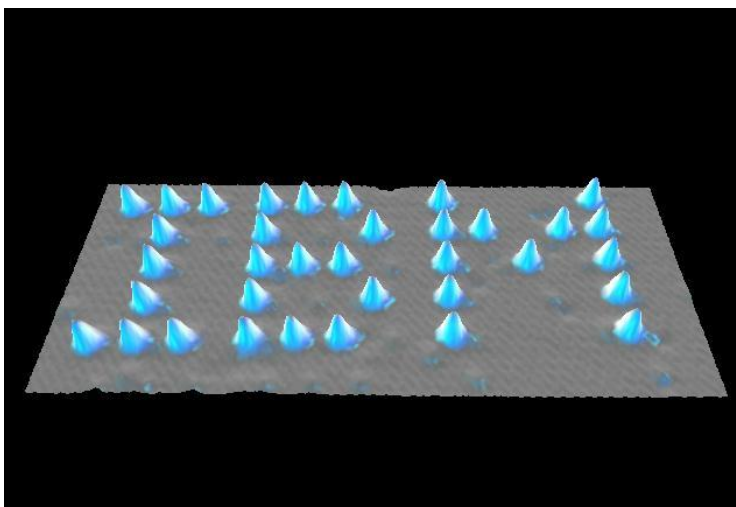


Figure 1: Don Eigler’s ‘IBM’ with Xenon on Nickel in 1989

By the end of the decade, nanotechnology had reached the stage where individual atoms were being manipulated. It began with Don Eigler's infamous stunt at IBM (shown in Figure 1) in which individual Xenon atoms on a Nickel surface were manipulated to form the letters 'IBM'. Of course, it has since grown, but this is viewed as one of the most influential steps in nanotechnology's history [2].

Nanotechnology holds great promise, and indeed has already shown to be useful, in medicine. The tiny nature of machines and particles that can be used to deliver medicine creates a way of non-invasively administering drugs to patients. Also, the tiny nature of these particles means they can be synthesised to target particular areas or types of cells (for example, damaged vascular tissue as discussed below) and thus deliver medicine directly to damaged tissue. This is particularly useful for such treatments as chemotherapy which, under normal circumstances, would destroy healthy tissue as well as cancer cells. With the use of nanoparticles, however, research has shown that specific cancer cells can be targeted and destroyed. It is also this principle which will be used in the discussion later to suggest a treatment for atherosclerosis.

Heart disease is one of the most common causes of death in our society. In 2009 approximately a third of all deaths in males were due to cardiovascular disease [3]. This figure has decreased since 1961, where almost 50 % of all male deaths were due to cardiovascular disease. Despite this, cardiovascular disease is one of the largest causes of death in males, equal only to cancer [3]. Amongst the possible causes of the high death rate due to cardiovascular disease is the fact that, on average, a male has a blood cholesterol level of 5.5 millimoles per litre – 0.5 mmol/l more than the government's recommendation [4]. High cholesterol levels are especially influential as a cause of atherosclerosis, the condition caused by a build up of cholesterol, fibres and dead muscle cells as plaques (atheromas) on the lining of artery walls. Atherosclerosis causes the arteries to become narrower, thus leading to such other cardiovascular diseases as thrombosis and myocardial infarction.

At present, atherosclerosis is being approached in a number of ways. One is prevention, another is surgery. Prevention is used for patients who appear to be at high risk of cardiovascular disease and atherosclerosis. Depending on the severity of the risk increase, patients are either advised to change their lifestyle or prescribed medicines to decrease some of the risk factors (e.g. statins for high cholesterol levels). Surgery is used when critical arteries become affected by atherosclerosis, mostly the coronary artery. Again, this is done in two ways. Coronary Angioplasty is one, where a balloon is fed using a catheter to the narrowed artery via either the arm or groin and expanded, thus flattening the atheroma and widening the artery. The other is Coronary Bypass surgery, where a portion of a healthy blood vessel is used to create an alternative pathway for blood to flow to the heart, thus bypassing the narrow vessel.

Although these are only a few methods of treating atherosclerosis (another being stents), all of these have issues. Prevention is only viable for those who have an increased risk of atherosclerosis, or who have mild cases of it. It cannot help those with serious cases. The problem with the treatment for serious cases is that they are both invasive surgeries. There is an inherent risk with any surgery and the ideal solution would be to find a way to treat the

more serious cases of atherosclerosis in a non-invasive fashion. I therefore propose the use of nanotechnology to achieve this, in ways discussed below.

Discussion

For reasons stated above, atherosclerosis can be a serious threat to a person's life. And although we have ways of treating such serious cases, we have not yet reached a point where we can do this non-invasively. However, nanotechnology research is bringing us closer to a possible solution, or in fact solutions. This paper will look at two such possible solutions – one which specifically targets plaques of atherosclerosis and the other which targets damaged arterial tissue.

Using Multifunctional Micelles ^[5]

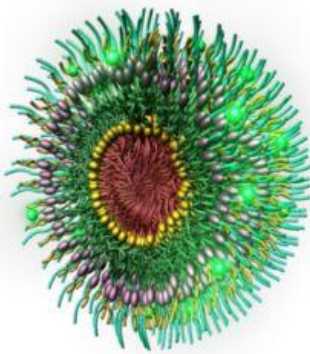


Figure 2: The micelle which the team developed

The University of California Santa Barbara have developed and tested a nanoparticle which they believe will target atherosclerotic plaques, specifically the “shoulder” region – the place where the plaque is most likely to rupture and cause further cardiovascular problems. This nanoparticle was a multifunctional micelle – a lipid based collection of molecules that form a sphere [6] (see Figure 2). On the surface of this micelle was attached a pentapeptide (cysteine-arginine-glutamic acid-lysine-alanine) that targets fibrin in and on atherosclerotic plaques. Fibrin has already been used extensively as a target for drug delivery to specific sites and it is widely recognised as being deposited in atherosclerotic plaques.

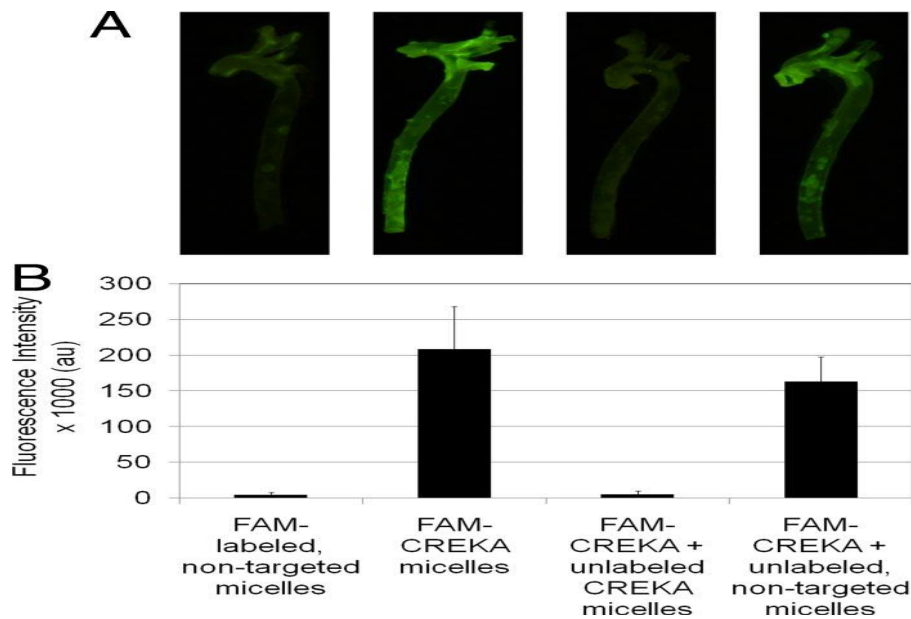


Figure 3: Results of CREKA-targeting study

The team first induced atherosclerosis in mice by keeping them on a high-fat diet. They then injected the pentapeptide (known as CREKA) micelles into the mice. They fluorescently labelled these micelles so that they could track their progress through the mice. They consequently found a number of key things about their CREKA micelles which could prove crucial in the treatment of atherosclerosis.

The first was that the CREKA-targeted micelles collected around atherosclerotic plaques in the aortic tree. When using similarly labelled, but non-targeting, micelles there seemed to be no particular collection in the aortic tree. To further cement their conclusion, the team preinjected either non-labelled CREKA-targeting micelles or non-labelled non-targeting micelles and injected the labelled CREKA-targeting micelles again. This time there was a great decrease in fluorescent intensity in the aortic tree for the non-labelled CREKA-targeting micelle, but very little for the non-targeting (see Figure 3). This gave the conclusion that the CREKA-targeting micelles were able to target specific atherosclerotic plaques especially in those areas prone to atherosclerosis.

The second was that the CREKA-targeted micelles did not cause clotting in the vessels of the mice. This is a particularly good feature of these micelles as, with the treatment of atherosclerosis, further clotting is undesirable. This makes CREKA-targeted micelles better than the nanoparticle that has been previously developed for targeting tumours – CREKA iron oxide nanoparticles – which have been shown to induce clotting in the lumen of the targeted vessels. This was proven by a further study in which the CREKA-targeting micelles were injected into a mouse with a specific tumour – no clotting was induced.

The team used these findings to test if their micelles could be used to deliver thrombin inhibiting drugs to the site of atherosclerotic plaques. Thrombin has been found to both induce clotting and increase the progression of atherosclerosis by causing smooth muscle cells to bind to Low Density Lipoproteins. If thrombin can be inhibited, then the progression of atherosclerosis can be halted and its adverse effects avoided. The production of thrombin

is induced when an atherosclerotic plaque ruptures – the specific place where the CREKA-targeting micelles were found to concentrate.

The drug Hirulog, a small synthetic protein, is a direct thrombin inhibitor and has fewer side effects than its counterpart, Heparin, which inhibits thrombin indirectly but can cause major bleeding in patients. Hirulog was attached to the CREKA-targeting micelles and injected into the mice. It was found that CREKA-targeting micelles still concentrated in areas of high atherosclerotic plaque formation, and also that antithrombin activity was significantly increased in these areas. This was especially noticeable in the difference between the new antithrombin activity in wild mice and the new antithrombin activity in the mice with atherosclerosis. If antithrombin activity can be increased then it is possible to provide a cure for thrombosis and atherosclerosis.

The downside to CREKA-targeting micelles is that they were given a circulation time of 3 hours. If a patient is in a critical position then 3 hours is potentially too long to be waiting for treatment to work. In this case surgery would still be a better solution. However, it was not concluded from the findings if this was the minimum circulation time for the micelles, or if a shorter time could create the same positive effects. Further research would need to be done into how the length of circulation time affects the effectiveness of the micelles and the Hirulog. It is probable that a compromise would have to be reached between allowing the longest possible circulation time for full effectiveness of the micelles, and offsetting the positive effect of the micelles by using too much time for them to come into effect.

It is with this CREKA-targeting micelle that a treatment for atherosclerosis can be developed. By testing it on humans and approving it, we can advance the treatment for both atherosclerosis and thrombosis (and therefore myocardial infarction) through the use of the inhibitor drug Hirulog. The many benefits of micelles, outlined above, mean that they are excellent for progressing our medical treatment of atherosclerosis and, although not discussed here, tumours.

This research shows that there is great potential for CREKA-targeting micelles in the treatment of atherosclerosis and the prevention of its adverse effects. The problem faced now is that these findings are only reported in mice at present and therefore might not translate into the same positive effects in humans. However, if the same conclusions can be drawn for humans as for mice, then CREKA-targeting micelles would be an excellent solution to the current problem.

Using Nanoburrs ^[7]

MIT and Harvard Medical School have produced a new nanoparticle which clings to the wall of the arteries [8]. They have been called nanoburrs for the resemblance to burrs – the nanoburrs have tiny protein fragments which allow them to attach themselves to target proteins like the ones on the arterial wall. These nanoburrs were 60nm, made of a lipid shell interface surrounding a polymer core (see Figure 4).

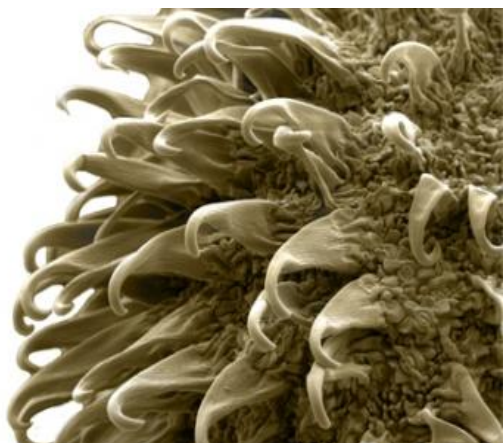


Figure 4: Graphical image of a nanoburr

In a similar way to the experiments on micelles, the nanoburrs were labelled fluorescently. They were then injected into rats with injured left carotid arteries. The carotid arteries were injured by continually pushing a balloon catheter into the artery, inflating it and withdrawing it which removed an endothelial layer to expose basement membrane. Basement membrane is exposed when vascular tissue has been damaged and is used as the target for these nanoburrs.

For the *in vivo* technique, the nanoburrs were injected and left to circulate for 1 hour. Four times as many nanoburrs were in the injured left carotid artery than in the uninjured right one. This showed that the nanoburrs were effective in targeting the damaged tissue in the carotid artery. To check that systematic delivery would be possible, the rats were injected again with the nanoburrs, this time via a tail vein. Similarly positive findings were achieved, with twice as many nanoburrs found in the injured carotid artery than in the uninjured one.

The *ex vivo* technique used balloon-injured rat aortas to test the effectiveness of the nanoburrs. The nanoburrs were incubated with the abdominal aortas for 5 minutes under constant pressure and then washed out to ensure any nanoburrs that had not attached themselves to the arterial wall did not show up on the fluorescent imaging. Twice the number of nanoburrs attached themselves to the arterial wall than the scrambled peptide and non-targeted did. Similarly, when repeating the experiment with uninjured aortic tissue, four times as many nanoburrs attached themselves to the injured tissue than the uninjured tissue.

The nanoburrs have an added positive side in that the drug that it carries can be released slowly over a period of several days – the longest achieved at present being 12 days. This would mean that patients would not have to endure repeated injections, but would need even as little as one to receive a full dose to recover. The drug is situated in the core of the nanoburrs and is released when it detaches from the specific polymer chain by a process called ester hydrolysis. The team have been able to alter how long this process takes, and thus how long it takes to release the drug, by changing the chain length on the polymer. In the research it was found that approximately 94% of the drug (in this case Paclitaxel) had been released by the 12th day whereas when using a stent only about 10-20 % is released. If

this progresses as hoped, the nanoburrs would only need to be injected once – with the recommended dose of treatment – saving both time and money.

There seemed to be no particular problems with the nanoburrs in reference to induced clotting or other adverse side effects. Of course, these tests were performed with the vascular tissue of rats, and it remains to be seen if the nanoburrs would have any adverse effects on the human system.

As with the micelles, the problem that researchers now face is whether we can translate the results with rats to humans. The results achieved were for the carotid artery which does not have the same implications on cardiovascular disease, although it is important for the progress of medicine nonetheless. Despite the aortic results being *ex vivo*, and therefore not as conclusive to future solutions as desired, it still showed a lot of promise for this area and the treatment of cardiovascular disease and atherosclerosis. The use of nanoburrs could allow us to provide drug treatment over a period of about 2 weeks to patients with damaged vascular tissue – which often leads to atherosclerosis – with minimally invasive techniques.

Nanoburrs, like micelles, could offer us a future solution to atherosclerosis. If the research is used to provide an effective treatment for humans, considering the advantages found in the research, then future treatment of atherosclerosis looks both economically viable and incredibly hopeful.

A further suggestion for the treatment of atherosclerosis is not using just one, but both of these solutions in conjunction with each other. Obviously the necessary research would be needed to ensure that one did not inhibit the other, but providing this is not the case, we may have procured a great solution. The nanoburrs are used to target damaged vascular tissue and release medicine that will fix this and any atherosclerotic plaques that may have formed, whilst the micelles are used to halt the progression of atherosclerosis and thrombosis. Together they could act as both a prevention and a cure for atherosclerosis.

Having discussed two possible solutions to the aforementioned problem with treating atherosclerosis, the social and ethical issues must also be addressed.

Social and Ethical issues

The obvious ethical issue with both of these ideas is of course the way in which the research was carried out, that is, with the use of animal testing. The testing of new drugs and techniques on animals has been a common feature of ethical debate since it first began. The problem with each of these tests is that animals have been purposely injured in order to test the treatment method stated. These can both be seen as quite cruel, especially in the nanoburrs research where their methods are almost inhumane.

However, the problem with arguing against animal testing is that, without it, we would not be at the stage where both treatments are ready for human testing. If animal testing were not used then potentially fatal side effects (and this is the same for any drug) that had not been discovered could cause untold damage to the human testers. For example, the fact that the CREKA-targeting micelles do not induce clotting was tested using animals but there

would have been no way of knowing if they had been safe for human use without these tests.

Of course, this opens up the argument used by many animal rights activists: what decides that humans are more important than animals? Inevitably it is for society to decide if using animal testing to progress medical and scientific research is ethically right. As it stands, the majority viewpoint is that it is ethically right because, although a few animals may suffer, the potential to save many human lives outweighs this.

It is hoped that nanotechnology will be able to provide low cost solutions to medical problems. Indeed, if nanoburrs have the same positive effect on humans as they did with rats then it will be possible to have fewer injections of a suitable drug to treat vascular damage and atherosclerosis. This benefits society because if fewer injections are needed then fewer treatments by nurse or doctor will be needed meaning that they will have more time to treat other patients. Also, this would mean that such treatments would cost less for society and the government, leaving more money for other treatments and research.

The CREKA-targeting micelles would also benefit the economy and society in general. If we can use micelles as a form of treatment for atherosclerosis then fewer surgical procedures would be needed. This would mean more operating theatres and surgeons would be available for other procedures. This of course would also be cheaper than extensive surgery, especially considering post-op care would not be needed.

So both suggested solutions show social and economic benefits. However, if nanotechnology in medicine continues progressing in the way it has, we may have to address the issue that skilled doctors and surgeons may become redundant. If it becomes a case where all that is required is an injection of a particular medicine – like the nanoburrs – then the staff requirement will be little more than a team of qualified nurses. This might cause problems if surgeons have less opportunity to practice, and so less experience when cases arise when they do require surgery. However, this level of advancement is unlikely to happen any time in the near future and so, for now, it may remain as an issue to be dealt with later.

Conclusion

Although atherosclerosis is potentially fatal and a serious threat to our society, by using nanotechnology we may reach a reasonable cure for it. Using both nanoburrs and micelles, as outlined above, the plaques that define atherosclerosis and damaged vascular tissue which precedes it may be diminished. Not only this, but the use of nanoburrs in particular is economically advantageous due to the decreased number of surgical treatments. As with most research, the research done to propose these ideas has raised a number of ethical issues, specifically that of animal testing, but I believe that with the possible advantages that lie ahead because of this research that the issues are far outweighed. In the not too distant future, it is probable that nanotechnology in the form of nanoburrs and micelles will provide the non-invasive cure for atherosclerosis that we desire.

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