

**THE USE OF NANOTECHNOLOGY  
IN THE CARDIOVASCULAR SYSTEM  
FOR DIAGNOSIS AND TREATMENT**

**BY**

**Max Johnson  
Claire Laidlaw  
Hannah Peter**

**PASS**

**RESEARCH PAPER  
BASED ON  
PATHOLOGY LECTURES  
AT MEDLINK 2010**

**Word Count: 3,657**

## ABSTRACT

Intro/ abstract: Nanotechnology is a branch of science, which combines chemistry, biology, physics, maths and engineering. It involves particles smaller than 100nm, with a specific function at cellular atomic and molecular levels. The concept of employing nanotechnology in biomedical research and clinical practice is known as nanomedicine. Nanomedicine is poised to have an important impact on the treatment of cardiovascular disease (CVD). Nanotechnology is utilized for both diagnosis and treatment.

Advances in nanotechnology can be applied to treatment of cardiovascular disorders, in providing an alternative to blood transfusion by improving oxygenation of tissues through enhanced blood supply, through regeneration of cardiomyocytes and expansion of the vascular lumen. Prevention of myocardial infarction through expansion of arteries and facilitation of effective blood supply could save millions of pounds each year.

However, if heart attack does occur, nanotechnology with stem cells can help to increase recovery of heart tissue, which would otherwise not be possible.

## INTRODUCTION

Nanotechnology is defined as the engineering of nano sized particles. The concept of nanotechnology has been around since the 1950s, with the first technical paper discussing the potential of such engineering published in 1977 (K. Eric Drexler). The application of nanotechnology was realized with the discovery of 'buckyballs' in 1975 (R. Buckminster Fuller), a round molecule of 60 carbon atoms. Since then, research has been conducted into nanotechnology with increasing developments into engineering. In recent years its potential relevance to medicine has been acknowledged increasingly, leading to the postulation of exciting new treatments.

This paper will discuss the application of nanotechnology in the diagnosis and treatment of cardiovascular disease, and consider how, in conjunction with stem cell therapies, it may pose a viable alternative to current surgical techniques that are used to prevent and treat the effects of myocardial infarction.

### Early detection/diagnosis

The cardiovascular system is made up of the heart, which consists of four chambers: two atria and two ventricles, the blood vessels and blood. Without an effective cardiovascular system for the transportation of oxygen and nutrients to tissues, the body cannot survive. The main problems that affect the cardiovascular system are:

- Blockages in the blood vessels (in particular, the coronary arteries)
- Lack of oxygen delivery to the heart muscle resulting in myocardial infarction
- Blood loss, for example, due to trauma

The development of nanotechnology may result in more effective treatment than existing therapies for these problems.

New imaging techniques using contrast-enhancing nanoparticles have been developed to detect the early stages of cardiovascular disease (CVD; Table 1; Fig 2). These can be used even before symptoms arise.

Table 1: *Nanotechnological imaging techniques*

<b>Category</b>	<b>Nanoparticle</b>	<b>Imaging Technique</b>
Fluorescent	Quantum Dots	Fluorescence Tomography
Radioactive	Cross-linked Iron oxide	Positron Emission Tomography (PET) Scan
Paramagnetic	Gadolinium Chelated with diethylenetriamine pentetic acid	Magnetic Resonance Imaging (MRI) Scan
Super Paramagnetic	Iron oxide	MRI
Electron-dense	Gold or iron	Computed Tomography (CT) Scan
Light-scattering	Gold nanoshells	Optical Coherent Tomography
Photoacoustic	Colloidal nanobeacons	Photoacoustic Tomography



Fig 1: *a cardiac MRI scan showing the heart of a patient and one of their lungs*

The main focus of these direct imaging techniques currently is in the detection of narrowing of blood vessel walls due to the build up of fatty deposits (atherosclerosis) and of recurrence of vessel narrowing (restenosis) following treatments such as angioplasty and stents. The early detection of cardiovascular disease is beneficial, enabling early intervention through improved diet and increased exercise, monitoring of the disease process, and traditional treatments such as statins and other drugs to reduce cholesterol and blood pressure respectively.

## Nanoparticles used in CVD treatment (therapeutic nanoparticles, or nanocarriers)

Nanocarriers for targeted drug delivery and therapy are being developed. These drugs are used to prevent restenosis and have four different modes of action:

- **Cytotoxic drugs:** inhibit the growth of smooth muscle cells (eg. Paclitaxel)
- **Platelet derived growth factor (PDGF) receptor antagonists:** inhibitors of inflammatory response, these are immunomodulators eg. Steroids and bisphosphonates, eg. Tyrphostins
- **Antibiotics:** eg Fumagillin
- **Gene targeting drugs:** Other promising therapeutics affect specific gene targets responsible for thrombosis or intimal hyperplasia, eg. prostacyclin synthase and thymidine kinase

Table 2: *Types of nanocarriers used in CVD therapy*

<b>Nanocarrier</b>	<b>Therapeutic Drug</b>	<b>Experimental model</b>	<b>Outcome</b>
Neutral Liposomes	Bisphosphonates	Injured carotid artery in rats	Macrophage depletion and reduced inflammation
Cationic Liposomes	Chloramphenicol acetyl transferase (CAT)	Balloon-injured artery in pigs	Increased expression of CAT
Haemagglutinin Virus of Japan Liposomes	Tissue factor pathway inhibitor gene	Iliac artery of hyperlipidemic rabbits after angioplasty	Reduction of intimal hyperplasia
Perfluorocarbon Nanoparticles	Surface bound streptokinase	Human plasma clots	Fibrinolysis
Polyelectrolyte Nanoparticles	Gene encoding for urokinase plasminogen activator	Rat carotid artery	High transfection efficiency
Polymeric Nanoparticles	AG-1295	Balloon-injured carotid artery in rats	Inhibition of Restenosis

## Theranostic nanoparticles

Theranostic nanoparticles combine a diagnostic imaging application with a targeted therapeutic nanoparticle and are gaining great interest as a valuable advance for drug delivery. However, this application is still in its infancy. They can be used to verify the delivery of an active compound to its intended site. An example of this is a system based

on fibrin-coated perfluorocarbon nanoparticles, which can be used for acoustic or MRI imaging with targeted thrombolysis.

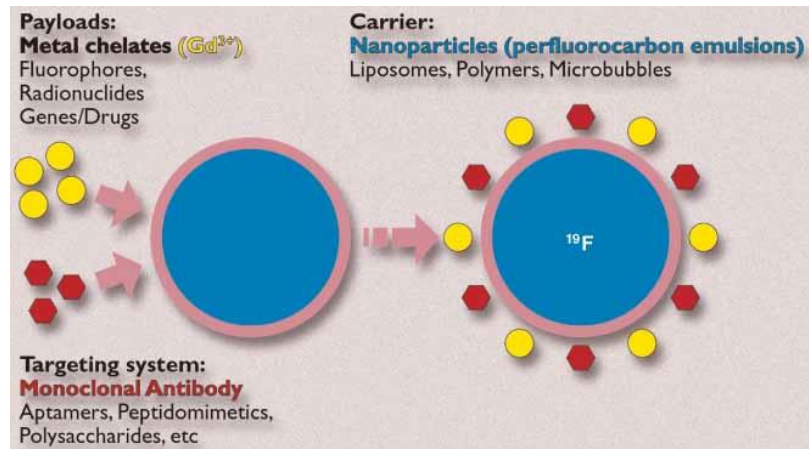


Fig 2: fibrin-coated perfluorocarbon nanoparticles

### **Nanotechnology used as CVD Indicators**

The ability to monitor for precursor signals of CVDs could potentially reduce many fatalities associated with the disease. Monitoring thrombotic or haemorrhagic events could facilitate the diagnosis and treatment of stroke and embolisms. Measuring the variations in pressure, flow of blood and biomolecule concentration, or iron concentration, could provide insights into the understanding of cardiovascular events. Currently, nanotechnology is being researched for its potential to provide CVD indicators. These have been divided into ex-vivo harvesting and detection of biomarkers and in-vivo sensors for CVD.

1. **Ex-vivo ("out of the living") harvesting and detection of bio-markers.**  
 Identification of biomarkers provides a powerful tool for screening, diagnosis, prognosis and therapeutic monitoring. Treating the underlying causes of CVDs could significantly reduce premature mortality. Examples of such biomarker detectors are nanowires, nanocantilevers and impedimetric analysis electrodes. Nanowires, offer great potential for the diagnosis of CVD by measuring pH variations, or detecting trace amounts of biological or chemical species. Nanocantilevers can be used for the detection of DNA species or circulating protein biomarkers. Impedimetric analysis electrodes have been developed to measure the concentration of D-dimer (this is a recognised biomarker of increased blood clotting activity in deep vein thrombosis (DVT) in whole blood). This device could improve the accuracy and reliability of early assessment of patients at risk of DVT.
2. **Nanotechnology used in in-vivo ("within the living") sensors for CVDs.**  
 Nanosensors are being developed for in situ rapid detection of ions such as,  $K^+$ ,  $H^+$ ,  $Na^+$  and  $Ca^{2+}$ . These sensors have demonstrated the role of  $K^+$  and  $H^+$  ions as potential indicators of the onset of acute myocardial ischemia. A multi-

nanosensor silicon needle was developed in-vitro, for the detection of myocardial ischemia during cardiac surgery by employing field effect transistors (FET).

Table 3: *Nanotechnology used in in-vivo CVD sensors*

<b>Sensor Targets</b>	<b>Technology</b>	<b>Applications</b>
K <sup>+</sup> and H <sup>+</sup> ions	FET	Myocardial Ischemia
Na <sup>+</sup> ions	Fluorescent nanosensors	QT syndrome, heart failure
Ca <sup>2+</sup> ions	Boron-doped silicon nanowires	Multiple CVDs
Nitric oxide	Single walled carbon nanotube	Hypertension ischemia/reperfusion
Blood pressure	In <sub>2</sub> O <sub>3</sub>	Pressure monitoring
Blood Flow	Piezoelectric-bioMENS	Stenosis in heart bypass

### **Expansion of the arterial lumen**

Arteries are the blood vessels which supply blood to organs. Disease processes such as atheroma can lead to narrowing of the arteries, known as stenosis. Atheroma involves deposition of fibrous tissue, fatty deposits and calcium in the vessels. There are three important consequences of stenosis. First, it can result in reduced blood flow and therefore reduced oxygen delivery to tissues. Thus, since the flow through a tube is proportional to the fourth power of the radius, halving the diameter results in a reduction to a sixteenth in flow. Second, localized narrowing of a blood vessel can lead to turbulent flow with development of blood clots that can break off, causing important downstream blockages, or emboli. Third, the atheromatous plaque can ulcerate, which also causes clot formation. Stenosis and turbulent flow can be corrected by dilating the blood vessel, in a process known as angioplasty. An internal splint known as a stent may also be introduced to help brace the artery open.

An angioplasty involves a balloon-tipped catheter being inserted into the narrowed artery. The balloon is inflated using a liquid at much higher pressure than that in the vessel, thereby expanding or dilating the atheromatous artery. The balloon is then deflated and released once the artery is sufficiently expanded. Stents are used in conjunction with angioplasty; they are introduced into the artery in a 'crimped' form surrounding the balloon and expand with the balloon. The stent then stays in place whilst the balloon is deflated, bracing the artery open and preventing restenosis (Fig 1).

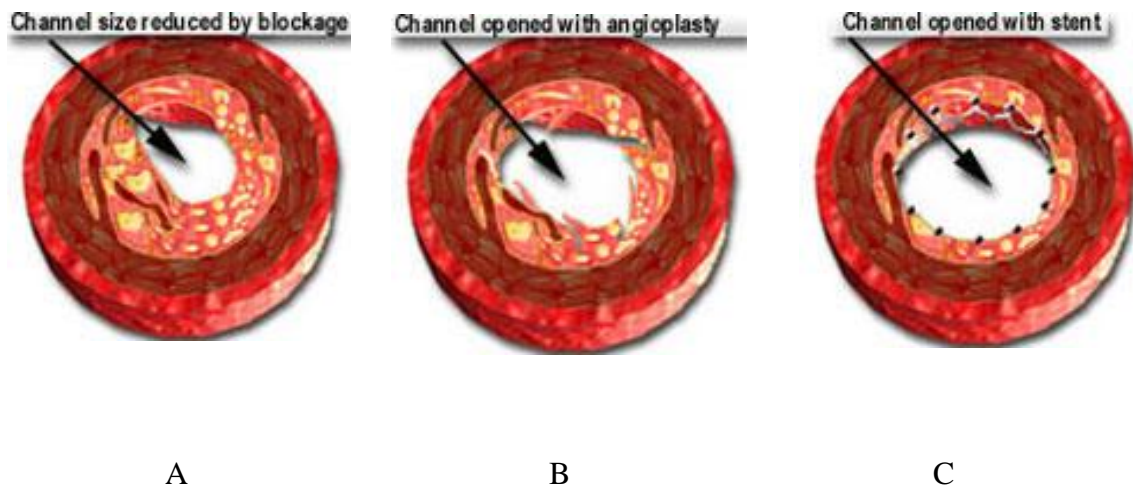


Figure 1:

*Cross section of an artery showing untreated atheroma (A); atheroma treated with angioplasty (B); atheroma treated with stent (C)*

The use of a stent has the advantage that it gives a smooth opening to, and prevents ‘recoil’ of the artery which can lead to the blockage returning. However, stents are not appropriate in all cases: it may be difficult to manoeuvre them around tight bends in blood vessels, especially if there are calcium deposits which make the vessel wall solid and brittle, and they cannot be used in very small blood vessels.

Angioplasty, meanwhile, may cause damage through the crushing of the deposits in the arterial wall, with consequent activation of a healing response, which in turn can induce re-narrowing or restenosis. The introduction of stents virtually halves the risk of restenosis following angioplasty from 40-50% to 20-30%. Despite this effective use of stents, between 1:5 and 1:3 patients will experience potentially fatal restenosis. This failure is because the artery can reject the stent as a ‘foreign object’, through the body’s immune response. This immune response involves two mechanisms: the first is the short term, normal automatic response to the damage caused when the artery is being expanded, which results in formation of a blood clot in the damaged area; the second takes around five to six months and is the formation of scar tissue around the stent. Both cause the artery to be narrower and less flexible.

### **How nanotechnology can help prevent restenosis after angioplasty**

Nanotechnology may be used in two ways to prevent restenosis after arterial expansion. The more established technique involves using a stent with nanoparticles on its surface to reduce the risk of an excessive wound healing response. The second, which is still being developed, aims to remove entirely the need for stenting.

Nanoparticles coating the surface of stents are employed to prevent excessive wound

healing. As nanoparticles are a similar size to most molecules in the body, they are not readily rejected. Nanoparticles compressed onto the surface of the stent create a rough surface to which the endothelium does not react, thus reducing scar tissue formation, while the stent itself becomes part of the endothelium and is less likely to become dislodged or ulcerate, and reduces the risk of clot formation.

The development of treatment which will obviate the need for arterial stents is being investigated, based on the principle of drug administration through nanoparticles called 'nanoburrs', which are 60 nm diameter and consist of three layers. The centre layer is an anti-scarring drug with a polylactic acid (PLA) polymer chain, the next is a fatty layer made of soybean lecithin, and the outer layer is a polymer (polyethylene glycol; PEG) which protects the particle as it passes through the bloodstream. When endothelial ulceration results in exposure of the arterial wall, the base layer of the artery where atheroma deposits build up is revealed. The nanoburr attaches via the molecules on its outer layer to target proteins lining the base layer of the artery. Once attached, the outer layer of the nanoparticle breaks down; the drug separates from the PLA polymer chain through an ester hydrolysis reaction, and allows healing of the damaged area. This nanoburr drug administration means that the effect is confined to the arterial area that is prone to splitting, which is usually at the join of atheromatous and healthy vessel tissue. It can also be used in the areas where stents can not be used so the nanoburrs enter the blood stream and target the area without the need for surgery.

Another benefit of this nanotechnology drug delivery system is that those administering the drug can control how long it takes for the drug to break free from the carrier molecules and therefore the length of time over which a single dose will act. This sustained release is achieved by changing the length of the PLA polymer chain: the longer the chain the longer it takes for the drug to be released. This system can be used in conjunction with stents to assist the healing process by using drugs which inhibit cell division such as Tacrolimus or 5FU, thereby reducing the extent of scar tissue formation, and so reducing the risk of restenosis.

### **What tests have/need to be done?**

Development of a drug involves multiple phases. In the first, pre-clinical phase, laboratory and animal studies are undertaken to demonstrate a potential biological effect and identify the dose regimen. Only when this has been done may clinical trials in humans be performed, usually in Phase I, II and III trials. In Phase 1, the safety of the drug is established, often in healthy humans and using small doses; this phase is essential before proceeding with more extensive investigations. Phase 2 consists of tests of the clinical effectiveness of the drug, usually undertaken on larger groups of patients, and attempt to identify a clinical effect rather than merely its safety; this phase will not usually give results that are powerful enough to prove the efficacy of the drug. Phase 3 studies involve randomised controlled trials on large groups of patients, designed to avoid bias that may lead to results which are not related to the treatment being tested; this phase aims to prove or disprove the clinical therapeutic value of a new drug.

The first nanotechnology treatments for coronary stenting are currently at the stage of Phase 3 trials which means that they may become mainstream clinical treatments within the next few years.

### **Regeneration of the heart tissue post myocardial infarction**

Dr. Patrick C. H. Hsieh, currently the Assistant Professor at the Cheng Kung University in Thailand, has applied nanotechnology to stem cell therapy, and by making stem cells 'stick' to the walls of the heart, has demonstrated the differentiation of cardiomyocytes. Through this, a viable treatment of cardiomyopathy has been developed. Other areas of nanotechnology used in conjunction with stem cells are being explored for their potential application to medicine, including the possibility that carbon nano tubes (CNT) may be able to act as delivery devices in the blood stream. This area of research is being headed by Dr. Valerie Barron, a Senior Researcher at the National Centre for Biomedical Engineering Science at the National University of Ireland (NUI).

After a myocardial infarction or cardiovascular incident occurs, the body releases stem cells from the bone marrow into the blood, where they differentiate and repair the damaged heart tissue, differentiating into cardiomyocyte cells. However, this process is stopped by the body within approximately 7 days of the incident, by which time the damage to the heart has been only partially repaired. Dr. Hsieh's research applied nanotechnology to medicine to increase the efficiency of the stem cell repair of cardiomyocytes following such a cardiovascular incident. Early trials failed, because the mesenchyma cells that were injected into the heart were carried away from the target area by the blood, the stem cells could be introduced with a nanofibre hydrogel, which may increase the chance of their 'sticking' to the walls of the myocardial tissue due to its adhesive and unreactive nature. The stem cells could then mature, multiply and differentiate *in situ*, effectively commencing the regeneration of the heart.

This combination of nanotechnology with therapeutic introduction of stem cells could increase the efficiency of stem cell retention by the target tissue from <1% to almost 100%, and makes stem cell therapy for damaged myocardial tissue a viable medical treatment option. The use of nanofiber hydrogel would allow the integration of stem cells into the heart without causing diastolic dysfunction, due to the unreactive nature of the Nanofibre hydrogel. The myocardial viability and the systolic functions can be improved significantly from their reduced state following myocardial infarction. Because the stem cells are prevented from floating away by the nanofibre hydrogel, they are able to differentiate and divide, producing new cardiomyotoc cells to repair those that were damaged in the incident.

The procedure being developed is less invasive than other standard procedures that are currently in place, such as balloon angioplasties and bypass surgery. It may be administered through laparoscopy-aided injections which would cause considerably less discomfort to the patient, since it only requires small incisions to be made into the abdomen, as opposed to open surgery. This would also decrease the chances of the procedure introducing infection.

The concept of stem cell therapy often raises objections, due to the ethical issues that surround the use of pluripotent fetal stem cells. However, treatments such as the application of stem cells in conjunction with nanofibre hydrogel only require the use of autologous mesenchymal bone marrow stem cells. These stem cells may be harvested from the patient receiving the treatment. Not only does this avoid raising such ethical issues, but it also avoids the possibility of any immune response by the patient, who is being treated with autologous cells. The procedure itself could be prepared and administered quickly, and represents an effective way to treat patients rapidly that are most at risk of recurrent heart failure.

The potential for the application of carbon nanotubes (CNTs) to regenerative medicine has been exciting the medical profession for many years, and research into their effects on mesenchyma cells has begun. CNTs have already been observed to migrate to specific environments, particularly to the cells of cancerous growths, which has led to the suggestion that they might be used for cancer treatment with specific, targeted drug delivery. Moreover, they have also been observed to enter cells without causing disruption to adipogenesis; to result in differentiation of preadipocytes into adipocytes; in chondrogenesis, in the differentiation of stem cells to form cartilage; and in osteogenesis, in the differentiation of stem cells to form bones. This means they have the potential for use in manipulation of the differentiation pathways of these mesenchyma cells from the bone marrow, thus representing a controllable way of cultivating cardiomyocytes for use in treatment of cardiovascular disease.

The method by which the CNTs enter the cell without causing any form of immune response or disrupting any of the functions of the cell may also allow them to be harnessed as nanovehicles for specifically targeted medicine delivery. This potential for CNTs could be applied to many, varied treatments, such as the removal of atheromas from arteries, the treatment of coronary heart disease (CHD) with specific chemicals released to target areas by the CNTs, or even in the application of chemotherapy to specific, target areas of cancerous growth, instead of current treatments where the whole body is exposed to cytotoxic drugs such as Alimta, which can have seriously debilitating side-effects.

The electrical conductivity of CNTs means their presence in cardiomyocytes will not disrupt the waves of excitation that pass through the heart from the sino-atrial node (SAN). This property allows CNTs to be used in electroactive cell repair, through control of the differentiation pathway of the mesenchyma cells into cardiomyocytes. Logically, the electrically conductive nature of CNTs would also allow them to be applied to stem cell repair of the nervous system, which could be applied to patients who have suffered trauma, such as accidents resulting in damage to the spinal chord. Again, this process would use stem cell therapy, but again would avoid controversy, using only mesenchyma cells harvested from the bone marrow of the patients themselves.

With the use of both nanotechnological techniques in tandem together with stem cell therapy, the hydrogel would ensure that the stem cells retained their position in the heart

so that the mesenchyma cells are able to repair the heart without threat of floating away, while the application of CNTs would ensure that the mesenchyma cells harvested from the patient's bone marrow would differentiate into the intended cardiomyotoc cells. Both of these therapies used together would effectively reduce the failure rate of treatment, through preventing undifferentiated cells floating away from the target tissue in the blood stream, and through preventing mesenchyma cells differentiating into other, inappropriate cell types.

## **Conclusion**

Nanotechnology has the potential to lead to significant advances in the diagnosis and treatment of cardiovascular disease which, as the number one killer worldwide, represents an area of medicine where improvements are urgently needed.

Nanotechnology in the treatment of myocardial infarction could result in improved recovery after heart attack, and with respect to stents will require less invasive surgery than is used in current practice, as it will reduce the incidence of restenosis or indeed remove the need for use of stents altogether.

In order for its potential to be realized, careful consideration of both the cost and efficacy of this relatively new field of engineering needs to be taken into account. Research into nanotechnology is inherently expensive, but its potential benefits should not be underestimated, and further research should be supported as it is likely ultimately to prove cost effective.

## BIBLIOGRAPHY

<http://www.who.int/mediacentre/factsheets/fs317/en/>[Soft  
<http://www.physorg.com/news/2011-02-cardiac-stem-cells.html>[Soft  
<http://www.nanowerk.com/spotlight/spotid=6883.php>[Soft  
<http://www.nanotechwire.com/news.asp?nid=10761>[Soft  
[http://www.nanomagazine.co.uk/index.php?option=com\\_content&view=article&id=1167:stem-cell-therapy-for-heart-repair-with-self-assembling-peptide-nanofiber-hydrogel&catid=38:nano-news&Itemid=159](http://www.nanomagazine.co.uk/index.php?option=com_content&view=article&id=1167:stem-cell-therapy-for-heart-repair-with-self-assembling-peptide-nanofiber-hydrogel&catid=38:nano-news&Itemid=159)[Soft  
<http://www.nano.org.uk/news/1041>[Soft  
<http://my.clevelandclinic.org/heart/disorders/heartfailure/stemcells.aspx>[Soft  
[http://current.com/technology/89232027\\_nanotechnology-based-stem-cell-therapies-repair-damaged-heart-muscles.htm](http://current.com/technology/89232027_nanotechnology-based-stem-cell-therapies-repair-damaged-heart-muscles.htm)  
<http://clinicaltrials.gov/ct2/show/NCT00428662?term=nanotechnology+stent&rank=1>  
<http://www.radiologyinfo.org/en/info.cfm?pg=angioplasty>  
<http://www.heartsite.com/html/stent.html>  
[http://www.scientistlive.com/European-Science-News/Nanotechnology/Treating\\_cardiovascular\\_disease\\_with\\_nanotechnology/22563/](http://www.scientistlive.com/European-Science-News/Nanotechnology/Treating_cardiovascular_disease_with_nanotechnology/22563/)  
<http://heartdisease.about.com/cs/angioplastystents/a/restenosis.htm>  
<http://www.nanowerk.com/news/newsid=14391.php>  
<http://www.dailymail.co.uk/health/article-1299787/They-supposed-save-lives-blood-transfusion-heart-attack.html#ixzz1Flmp3pZ4>  
[http://www.rch.org.au/bloodtrans/adverse.cfm?doc\\_id=5323#Citrate\\_toxicity](http://www.rch.org.au/bloodtrans/adverse.cfm?doc_id=5323#Citrate_toxicity))  
Biana Godin 2010 'Emerging applications of nanomedicine for the diagnosis and treatment of cardiovascular disease' Trends in Pharmacological sciences Volume 31 issue 5 pages 199-205