Nanoparticulate drug delivery systems for the treatment of Atherosclerosis

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Abstract:

Nanotechnology is at the forefront of medical technology. It has a vast number of applications in the health sciences and new breakthroughs are being made constantly using it. Atherosclerosis, a disease that has plagued humankind from as early as Ancient Egypt, has now been placed into consideration in conjunction with nanotechnology to develop new ways to deliver drugs in order to treat this heart condition. The purpose of this research article is to shed light on recent developments in Nanotechnology by analyzing its recent real world applications to treat Atherosclerosis.

Traditional methods of treatment including medication, lifestyle changes, and even invasive surgeries. Out of the three methods, two of them have been identified as having complications. Oral medication and surgical procedures have proven to be successful in treating Atherosclerosis, but both can cause unintentional tissue damages amongst other problems. Nanotechnology can be used to precisely deliver drugs and even help at a cellular level by improving white blood cell responses to plaque buildup. Research has shown promising results when nanotechnology was used. The usage of liposomes developed with nanotechnology helped increase solubility of statins which helped increase absorption and bioavailability. When medication was placed inside the developed liposome, it was shielded until it reached the target site. The biochemical properties of liposomes have helped researchers attach biomolecules like

antibodies to the liposomes in order to help with targeting as well as guiding and attracting macrophages to areas of interest like plaque sites. Another nanotechnology that is being further developed for a medical application is carbon nanotubes. These nanotubes can be easily grown and have been used successfully to deliver drugs and even create nano-scaffolding to help tissue growth and regeneration. Nanotechnology has an enormous potential for medicine and when applied to the heart condition Atherosclerosis, nanotechnology has been able to prove its usefulness successfully.

Key words: Carbon Fiber, Carbon Nanotubes, Drug Delivery, Micro Motors, Atherosclerosis, LDL, Liposomes, Liposome Drug Delivery

Introduction

1.1 What is Atherosclerosis?

Atherosclerosis is a widespread disease that affects the lives of many people in the United States. There are 18 million Americans that are 20 years and older that have heart disease (4). Around 650,000 Americans die from heart disease every year, contributing up to 25% of annual deaths in the United States (4). These statistics are alarming and continue to increase every year. To prevent this number from increasing, it is necessary to discuss what atherosclerosis is and the cause of this disease.

Under normal condition, the coronary arteries have a smooth interior surface that allows blood to smoothly flow through. However, LDL or “bad” cholesterol starts to build up on the coronary artery wall (4). In order to combat this, the body’s immune system sends white blood cells to clean up LDL deposited on the artery wall (4). Overtime, the combination of white blood cells cleaning up and combining with LDL eventually forms plaque. This plaque restricts blood flow which eventually causes atherosclerosis.

1.2 Traditional methods of resolving Atherosclerosis

Current methods of resolving atherosclerosis involve using medication as a form of protection. Statins could be used for lowering LDL cholesterol for most people (9). Research has shown that taking statins over a period of a year causes LDL levels to fall by 60%, raise levels of HDL or good cholesterol, and lower the level of triacylglycerides (9). Another group of drugs that can potentially reduce atherosclerosis are fibrates. Fibrates are drugs that reduce triacylglycerides, molecules of fat that contribute to plaque buildup in atherosclerosis (9). Many other drugs can be used to treat atherosclerosis, including Niacin, blood pressure lowering, and blood clot reducing drugs (9).

These drugs have proven their effectiveness in treating atherosclerosis, but they come with faults. One problem with the drugs is non site-specific targeting. Due to non-site specific targeting, drugs can have a tendency to accumulate in healthy tissue (14). Also, when in the bloodstream, the drug will conduct bioactivity before it reaches the target site (14). A drug's effectiveness could be increased if it were protected before being delivered to a specific site.

Other types of treatment for atherosclerosis include forms of surgery. There are three main types of surgery used for atherosclerosis: percutaneous coronary intervention, coronary artery bypass grafting, and carotid endarterectomy. Percutaneous coronary intervention is used to open blocked or narrowed coronary arteries, improving blood flow to the heart and relieve chest pain (5). After the surgery is performed, a stent or a small mesh tube is used to keep the arteries open (5). Carotid endarterectomy is similar to percutaneous coronary intervention, but is more specific by only applying treatment to the neck. The result from this procedure brings back blood flow to the brain and has the potential to prevent a stroke (5). Coronary artery bypass grafting is a technique where instead of removing the plaque, the narrow artery is rerouted to another coronary artery (5). The technique used to reroute the narrow artery is through the use of grafting. Like the other two techniques, coronary artery bypass grafting improves blood flow to the heart, relieves chest pain, and could prevent a heart attack from happening.

Just as with drugs, there are disadvantages with these methods of atherosclerotic surgery. For one, stenting has not been proven to help people live longer (22). Coronary artery bypass grafting, or CABG, is the best treatment in terms of extending the life of the patient with many problematic blockages (22). However CABG is a process that takes time to perform, making it not as useful as stenting for an ER situation. The ultimate problem, however, rises after the surgical procedures. The patient still has to take medicines after the procedure to prevent future cases of atherosclerosis (22). Surgery can remediate the problems caused by plaque buildup, but cannot do anything to prevent it. In other words, these types of surgeries are designed as treatments to a problem, not to prevent it.

1.3 Implications of Nanotechnology

The definition of nanotechnology refers to the study of materials at length scales below 100nm (24). To put this into perspective, a human red blood cell has an average diameter of 7 micrometers or 7000 nanometers (18). Nanotechnology has huge implications for resolving atherosclerosis due to its incredibly small size. It can be used to resolve plaques itself, detection, or be used to deliver drugs. When nanotechnology protects drugs and delivers directly to the site of the plaque, it prevents the drug from building up in areas of healthy tissue and prevents the degradation of the medicine before it reaches the targeted site (14). This allows the medicine to extend its bioactivity which leads to improved effectiveness of medicinal treatment (14). Also, the ability to detect and send drugs to the specific site makes nanotechnology able to combine therapeutic and diagnostic modalities into one agent (14). Another potential feature of nanotechnology is that it could be used to aid the immune system in combating plaque. This paper will be exploring in detail the current development of nanotechnology for atherosclerosis treatment and possible future trends.

Main Body

1.1 Introduction to Liposomes

As discussed in the introduction, an advantage of using nanoparticles for atherosclerosis treatment is the delivery of drugs. But how effective is nanotechnology in boosting the efficiency of current medicines? Let's take a look at statins as an example. Statins are a group of drugs that work by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (2). This makes statins an ideal solution to reduce the amount of LDL in the body and prevent atherosclerosis. However, statins do have some limitations that make it less effective in its treatment. Some of its limitations include poor aqueous solubility, low oral absorption, and limited bioavailability when administered orally (2). This article proceeds to explain that the newer nanotechnology allows for statins to have increased solubility, absorption, and bioavailability (2). It also allows for controlled releasing of the drug in the affected areas.

One of the technologies that can perform this feat are liposomes. Liposomes are sphere shaped vesicles that contain one or two phospholipid bilayers (23). This structure is very similar to the ones that are found in the cell membrane. This gives the liposome the ability to travel all across the body through the bloodstream and at the same time protect what is inside of it. Medicine like statin can be encapsulated inside of a liposome to protect it from the environment of the body before it reaches the targeted site. Just like how cell membranes have different proteins and sugar coated across its surface, liposomes can have different receptors on the outside lipid layer. A process called PEGylation can attach polyethylene glycol to the surface of the liposome, increasing the amount of time for liposomes to reside in circulation and the chance of targeting the plaques (1).

1.2 Attaching Antibodies to Liposomes

In addition to attaching polyethylene glycol, researches have also managed to attach antibodies to liposomes as well. When plaque begins to build up in the arteries, the endothelium becomes damaged. This in turn makes it release specific molecules which could help in liposomes in targeting it (1). The endothelium expresses many cell adhesion molecules, including VCAM1, intracellular adhesion molecule 1, E-selectin, P-selectin, alpha V beta 3 integrin, and JAMS (1). JAMS or junctional adhesion molecules are shown to be promising, as it helps direct inflammatory cells to the atherosclerotic site (1). Antibodies that are against these molecules could be attached to the liposome and sent to the plaque site. Not only would the liposome be able to deliver the drugs to the target site, it could also send signals to the immune system to further reduce the plaque. It has been researched that liposomes with anti-VCAM-1 attached to the surface managed to accumulate successfully to the site of the lesion (1). Another research has found that liposomes with anti-ICAM-1 were found to bind strongly to the endothelial cells (1). Scientists have also found that by attaching antibodies that are against E-selectin to liposomal surface causes the liposomes to accumulate on endothelial cells where the atherosclerotic lesions were located (1).

Another potential target that liposomes can use is LDL receptor-1 or LOX-1 (1). The reason is because this receptor creates upregulation on the endothelium after the release of inflammatory cytokines due to oxidative stress (1). When this happens, oxidized LDL or bad cholesterol can bind to the receptor and thus eventually build up plaque in the artery. Scientists have tested a version of liposome that had an anti-LOX-1 antibody on its surface on mice with LDL receptor deficiency (1). The result of this study showed the liposomes administered intravenously were successful in targeting the LOX-1 receptors in the mice.

1.3 Using Phosphatidylserine to aid Macrophages in Targeting Plaque

In addition to attaching antibodies onto liposomes to trigger an immune response, liposomes can be used to aid macrophages in targeting the plaque. A molecule known as phosphatidylserine (PS) has been found by researchers on the surface of apoptotic cells and serves as an attractant to macrophages (1). This means that if a synthetic version of phosphatidylserine could be made and incorporated onto the surface of liposomes, then it would lead to an increased reaction of macrophages to the plaque sites. Scientists have tested the reliability of liposomes going to atherosclerotic plaques by using Watanabe heritable hyperlipidemic rabbits (1). The researchers tested the liposomes by using the *in vitro* method and encapsulating In-nitrilotriacetic acid onto the liposomes (1). The In-nitrilotriacetic acid was used by the scientists to detect the liposomes in the body of the rabbit via single-photon emission computed tomography or SPECT (1). After 48 hours, the researchers performed a SPECT procedure and found the atherosclerotic regions easily identified. The size of the liposomes that were used in this experiment was reported to be under 100 nm, as these liposomes were intended to be engulfed by the macrophages reaching the plaque site (1).

1.4 Transporting Stem Cells Using Liposomes

As discussed in the introduction, common repair methods of atherosclerotic damage include stenting and CABG. However, the problem with these surgical procedures is that they are reactionary rather than preventative. The previous sections have indicated that liposomes are very reliable in terms of accurately locating arterial plaques. A way to help repair plaque is through the incorporation of stem cells. The benefits of using stem cells is tremendous, as it can differentiate into neo-intima smooth muscle cells or endothelial cells that can perform recovery of vessel function (1). Direct injection of stem cells to the lesion site, however, has led to disappointing therapeutic results (1). When using direct injection for stem cell applications, the inaccuracy and delay of the treatment procedure is no different than CABG or stenting. First, the doctor needs to know the location of the atherosclerotic plaque site. This usually happens when the plaque has already started to become a problem in the patient. Second, the direct injection could occur not directly at where the plaque is located at. The third and final problem is that even though the plaque may be resolved in that specific area, it will not help resolve a plaque issue located in another area in the body.

It turns out that liposomes can be made into stem cell carriers as well. As discussed in the previous section, liposomes are very accurate in determining where arterial plaques are located at. This solves the current problem of directly injecting stem cells to the lesion site. Not only can the liposome carry drugs and antibodies to reduce plaque, it can also carry stem cells to repair the damages caused by the plaque. Plus, the liposomes can also locate and repair plaques in areas that doctors cannot detect.

To do this, liposomes have to include two antibodies, CD34 and ICAM-1. CD34 is an antigen that will be used for the surface of stem cells and ICAM-1 will be used for navigating plaque areas (1). When the liposomes that contain the stem cell reach the plaque location, the stem cells will be released by using a sonoporation technique. This sonoporation technique involves a 1-MHz low-amplitude continuous ultrasonic wave that breaks the gas bubbles sandwiched between the lipid bilayers of the liposom (1). This will break open the liposome and allow the stem cells to attach to the damaged endothelial wall to perform repairs.

2.1 Introduction to Carbon Nanotubes

Nanotubes made out of carbon are at the forefront of medical technologies. Usually created from hydrocarbon derivatives like petroleum and even recycled plastics, carbon nanotubes have been produced and used since as early as 1960. The first usage was in aircraft engines but as the technology has developed, carbon fiber has been able to be applied in medical technologies. From prosthetics to drug delivery, carbon nanotubes have been an ideal mechanism for tissue repair, growth, and structural support.

Carbon nanotubes are relatively thin. They grow in a kind of buckyball type pattern that is one carbon atom thick. They can be grown in a lab and if needed sterilized for medical applications. According to John Myers, nanotubes made of carbon are smaller than carbon fibers because they are made of carbon at the molecular level and are less than two nanometers thick (7), This makes carbon nanotubes ideal for drug delivery and tissue therapy.

2.2 Application of Carbon Nanotubes

In a recent study done in 2017, researchers found that carbon nanotubes have the potential for aiding in cardiovascular tissue therapy. When used as a “matrix enhancement additives to chitosan, collagen, and polylactide etc.” nanotubes led to what was described as a “mechanically strengthened scaffold for growth of soft tissue and skeletal muscle tissue constructs (8).” This breakthrough discovery has been in the works since 2012 and started out with growing cardiomyocytes on structuctures called carbon nanomaterial scaffolding. When the original growth structures, mainly gelatin, were enhanced with carbon nanostructures, researchers discovered new benefits like enhanced cell growth and properties of the growing cardiomyocyte tissue.

Drug delivery is one of the vital applications of carbon nanotubes. Carbon nanotubes have been proven to help treat and diagnose atherosclerosis and are created by rolling up sheets of graphene. Drugs and therapeutics are attached either to the outside or the inside of the tube and then released into the target site (13). In one study, carbon nanotubes were used to deliver a drug that stimulates macrophages in atherosclerotic plaque. The stimulated macrophages were observed to target and destroy the dead and dying cells responsible for atherosclerosis (21). The nanotubes were so efficient that when tests were conducted in mice, the macrophages were observed to only target dead and dying cells while leaving healthy cells alone.

Carbon nanotubes have the potential to aid with imaging of cardiovascular tissue. In a study conducted by researchers, the heart of a mouse cadaver was imaged with success. The usage of carbon nanotubes reduced the necessary amount of X-Ray source emission. This means that the usage of carbon nanotubes in imaging cardiovascular tissue proved to be safer and more efficient than usage without carbon nanotubes(11). According to a study published in the Journal of American Heart Association, carbon nanotubes have been found to be successful in another type of imaging called fluorescence imaging and have been used in a cancer treatment procedure called photothermal ablation. Hisanori Kosuge, the author, states that “carbon nanotubes are promising “theranostic” agents for vascular inflammation and atherosclerosis (15).”

In an extensive review on drug delivery via carbon nanotubes, a conclusion was drawn that single-walled carbon nanotubes have garnered high interest by the medical research field due to their small size, stable structure, and non-polar nature. (17). The structure of nanotubes has proven to be efficient in carrying drugs across cell membranes and in carrying large dosages of drugs.

2.3 Drawbacks of Carbon Nanotubes

There are potential dangers to the usage of carbon nanotubes in drug delivery and therapy. If inhaled, the nanotubes can be directly transported by the bloodstream and into remote organs. This is dangerous as drug delivery using single and multiwalled carbon nanotubes can potentially cause the delivery of drugs into the wrong area, causing fatal side effects. The nanotubes themselves can also be toxic to the cardiovascular tissue that they are not supposed to affect. The side effects of nanotubes both single and multi walled are not directly studied and thus need to be accounted for before usage in treating cardiovascular disease in humans. A study on the effects of multi walled carbon nanotubes on human umbilical tissue showed negative signs. These negative signs include decreased cell survival, DNA damage, and increased reactive oxygen species. Another problem that arose was negative results in atherosclerosis treatment. The study found that using multi walled nanotubes required more study in treating atherosclerosis as negative results were more than likely (12).

Conclusion

Liposomes have the potential to resolve a lot of problems caused by atherosclerosis. The components of a liposome are relatively simple, consisting of a spherical lipid bilayer. This lipid bilayer is very similar to the ones found in cell membranes, allowing for the liposome to have good transport around the human body. Liposomes can also serve as a carrier for drugs that can reduce plaque and stem cells that can promote repair. It can also have antibodies incorporated into the lipid bilayer, which allows for accurate plaque navigation and detection. All of these reasons make liposomes a very promising nanotechnology development for detection and treatment of atherosclerosis.

Carbon nanotubes have been an astonishing breakthrough in a medical application that range from prosthetics made from carbon fibers to arm chair configured drug transport tubes. Fullerene or carbon nanotubes have greatly aided in the experimental therapy of cancer and now atherosclerosis. Research into carbon nanotubes has been showing promising results and have proven to effectively deliver drugs in mice test subjects. When atherosclerosis resolving drugs were attached and sent into the test subjects, the drug was effective in stimulating the body's macrophages which in turn started to resolve the atherosclerosis plaque buildup and dead cells.

Targeting and treating diseased tissue and removing plaque blockages has been the direct challenge for nanoparticle research in atherosclerosis treatment. Nanotech research has proven the tremendous usefulness of the particles in a cardiothoracic application. From drug delivery all the way to immune response boosting, nanotechnology has given medical professionals and patients a new hope for the future.

Future Trends

Metallic nanoparticles and carbon nanotubes are two very futuristic technologies that are being developed for usage in the very near future. New molecular structures for the particles and tubes to be attached to are being discovered and tested. These structures that are currently being tested are far less toxic and more biocompatible. They also have the ability to precisely target anything that needs to be targeted (6). Majority of research and development in nanomaterials has been put towards cancer therapy as it is a serious pressing matter. Nanotube research has however has proven to be effective for more than just cancer. Atherosclerosis treatment is one such developing area in which nanotubes have had a high rate of success. Carbon nanotubes have conventionally been used in their single wall state to deliver drugs but new multi walled nanotubes have been synthesized and have shown promise in the field. The only problem is that there is not enough testing and research into the side effects of both carbon nanotubes and metallic nanoparticles. The small amount of research unfortunately has provided insight into the detrimental effects if misused. Another developing field of nanomaterials in the medical field is vaccines. Polymer based nanomaterial has shown promise in the area of drug delivery and according to a study, nanoparticles with low cytotoxicity can protect the antigens of degrading environments in our body leading to higher effectiveness of uptake (16).

Carbon nanotubes have been recently in the light for their potential as nanomotors in drug delivery and to transport enzyme molecules in the body (10). Current research has been experimenting with different drug and tube combinations to see which combination is the most effective and the least destructive. The nanomotors can be used in conjunction with other nanotechnologies like nanoparticles to be able to be guided via magnetism in order to transport and move materials where they need to go in the body (25).

Another application of gold nanoparticles that is being developed is radiation therapy. Another application towards cancer therapy, gold nanoparticles have been applied with conventional radiation therapy which can potentially enhance the probability of the therapy targeting and destroying diseased tissue instead of healthy tissue (20).

Despite how much promise liposomes show in terms of reducing atherosclerosis plaque, there are some drawbacks in practical application. The blood flow in arteries has a tendency to apply shear stress to the endothelial wall (1). When this happens, the nanoparticles could be washed away from the targeted site. In addition, the shearing action of the blood flow reduces the time of interaction between the targeted plaque and the particles. Scientists are currently working on liposomal formulations that will release their cargo or payload at the plaque site reliably, while being confronted by the increased shear stress caused by abnormal blood flow (1).

Using stem cells to treat atherosclerotic plaques has been shown to be an effective treatment when coupled with liposomes. It is possible to extract stem cells from children or adults. But as we get older, the number of stem cells can diminish as much as 100 to 10,000 fold in different tissues (19). Another issue is that older stem cells undergo genetic mutation that reduces its effectiveness in repairing your body (19). As of right now, scientists are proposing using remnants of placenta and umbilical cord after the birth of the baby as a resource of stem cells (19). The placenta and umbilical cord are rich resources of stem cells that have not been genetically altered. Many scientists and companies are interested in this potential supply, especially when the stem cell industry is expected to grow to $170 billion by this year (19).

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