

# Tackling HIV/AIDS using Nanotechnology

By

Chang Woo Lee

PASS WITH MERIT

RESEARCH PAPER  
BASED ON  
PATHOLOGY LECTURES  
MEDLINK 2010

## Abstract

Nanotechnology has a lot to offer medicine because for one thing it is able to interact on the same scale as cells. Most research conducted recently suggests that diagnostic and imaging techniques can be improved using nanotechnology. Targeted deliveries of drugs also seem possible.

This paper explores the potential use of nanotechnology in treating HIV/AIDS, including the possibility of developing an HIV/AIDS vaccine (which has never been done before), and the possible next steps that can be taken. A few problems that will need to be overcome, such as evaluating the safety of using nanoparticles, are also discussed.

## Introduction

*“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.”<sup>[1]</sup>*

*Richard Feynman, 1959*

This quote by the Nobel Prize winning physicist, Richard Feynman, marked the birth of nanotechnology. He proposed the revolutionary idea of building things from ‘the bottom up’. It was only three decades later that nanotechnology entered the public domain through the publication of ‘Engines of Creation’ by Eric Drexler (1986).<sup>[2]</sup> This document stated that ‘building machines on the scale of molecules’ could be used for everything from removing pollutants in the air to medicinal robots repairing bodies at the cellular level. With the discovery of a series of nanoparticles buckminsterfullerene, in the 90’s, the field of nanotechnology grew significantly.

Nanotechnology is the study of manipulating matter at the atomic scale. It deals with structures ranging from one to one hundred nanometres (nm) where one nanometre is  $1 \times 10^{-9}$  metre. When we consider that the size of a single virus being around 100nm, we get a clearer idea of the size of materials that are dealt with in nanotechnology.

Nanotechnology is currently used in wide range of industries. The food industry uses smart packaging with nanoparticles to detect bacteria. Nanoparticles are included in the fabrics of the clothes to protect us from UV radiation. However, how is nanotechnology relevant to medicine?

The biggest advantage of using nanoparticles in medicine lies in their size. We are talking about molecules that are smaller than a virus. They work at a cellular level. Current diagnostic instruments rely on detecting macro-scale anomalies. This means that numerous diseases can only be detected at a later stage where treating becomes more difficult. Detecting at the cellular level, where the signs of diseases first appear, will allow us to detect diseases at a very early stage.

Iron oxide nanoparticles can be used to improve the MRI images of cancer tumours. Coating the nanoparticle with the peptides that bind to cancer cells, these iron oxides can bind themselves to the tumour. Iron oxide possesses a magnetic property, which enhances the images from the Magnetic Resonance Imaging scan. This allows us to visualise the primary

tumour and any metastases to specific cell types. Similarly, nanoparticles can carry drugs, so targeted drug delivery is possible.

With the help of nanotechnology, drug delivery systems can be improved. So can the diagnostic and imaging techniques. Certain nanoparticles (notably silver) have even showed natural anti-microbial properties. The use of nanotechnology in medicine offers a lot of exciting new possibilities. On the other hand, nanotechnology is relatively new. Therefore, its toxicity in the body and the safety of its use must be evaluated, which will be discussed later.

## Discussion

Before I discuss the use of nanotechnology in treating HIV/AIDS, a thorough understanding of the disease will help us to see how nanotechnology could potentially lead to its cure and prevention.

Acquired Immunodeficiency Syndrome (AIDS) is the end-stage disease caused by a virus known as a Human Immunodeficiency Virus (HIV). AIDS is a deadly disease if untreated because HIV destroys our immune system. The immune system protects us from pathogens causing diseases. Hence, people with AIDS become more susceptible to opportunistic infections as their immune system is weakened. Even mild diseases such as common cold can be lethal. In the past 20 years, HIV has infected 60 million people and resulted in 25 million deaths world-wide.<sup>[3]</sup> In 2009 alone, 33.4 million people were estimated to be living with HIV/AIDS, with 2.6 million newly infected.<sup>[4]</sup> 1.8 million deaths were attributed to HIV/AIDS.<sup>[4]</sup> HIV/AIDS is a serious global problem. HIV is transmitted through the exchange of bodily fluids. This most commonly happens during unprotected sexual activity or the sharing of injection equipment with an infected person.

HIV is a retrovirus. It contains its genetic information in the form of RNA. After the virus enters its target cell, its RNA is copied by a viral enzyme called reverse transcriptase to make a piece of complementary DNA (cDNA). Another viral enzyme cuts the cellular DNA and the viral cDNA is inserted or spliced into the gap in the cellular DNA. HIV is then replicated in the infected cells. The problem is that the reverse transcriptase used to copy the HIV's RNA is very error prone. It makes about one error (mutation) each time it copies a piece of viral RNA, accounting for the high genetic diversity of HIV.

HIV has another unusual property which worsens the problem: after entering the target cell, it can remain dormant for many years. During this 'latent' phase, our immune system cannot recognise the cells containing inactivated, dormant HIV. This ability to establish a 'viral reservoir' that cannot be detected is one property of HIV that makes it such a problem.

There is currently no cure for HIV/AIDS and Highly Active Antiretroviral Therapy (HAART) is the only current treatment available. Despite the improvements made in the last decade, HAART still cannot overcome the unusual properties of HIV. HAART involves three or more antiretroviral drugs taken simultaneously by the patient.<sup>[5]</sup> The first problem HAART faces is that however effective, it cannot eliminate cells already infected with the virus. It also cannot eliminate the viral reservoirs. Therefore, it only prolongs the onset of AIDS by 10-30 years. It does not cure. The second problem is the poor patient compliance to the drug regimen. The patients are required to take the medication daily for a lifetime. As this is often too demanding, they fail to adhere to the treatment schedule. This allows some virus to develop resistance. Unfortunately, even with good compliance, due to the extremely high mutation rate of HIV-1, it can still develop resistance.

Nanotechnology can be used to reduce the amount of antiretroviral drugs needed to be administered. Various studies have demonstrated the use of nanotechnology to deliver drugs. An experiment carried out by Dou *et al* (2006) has demonstrated that the antiretroviral drug Indinavir can be delivered to various tissues in nanosuspensions.<sup>[6]</sup> A study done by Baert *et al* (2009) also showed the drug Rilpivirine being delivered to the cells in nanosuspensions.<sup>[6]</sup> More interestingly, in Baert's study, a single-dose administration of the drug in

nanosuspensions resulted in sustained release over 3 weeks in mice and 3 months in dogs.<sup>[7]</sup> Hence, nanotechnology allows smaller dosage of drugs being administered at a time. This would reduce the toxicity of the HAART and improve patient compliance to the treatment.

Unfortunately, drug therapy for treating HIV/AIDS has limitations. The HIV can become resistant to all the antiretroviral drugs. Scientists are working hard to synthesize new drugs to overcome this problem. However, at some point in the future, we may run out of alternative drugs.

In 2008, a team of international scientists led by Kumar and Ban used short interfering RNA (siRNA) to suppress HIV-1 spread at the cellular level.<sup>[8]</sup> siRNA are long double-stranded RNA strands that are cut apart into much shorter strands. The two strands then separate. One strand degrades. The other strand however is used to recognize a specific RNA strand. When this 'guide strand' finds its targeted RNA, it cleaves to the RNA and prevents the RNA from being translated into a protein. It simply stops a particular RNA, and hence gene from being expressed.

The study used a single-chain antibody to deliver siRNA molecules. These siRNA molecules used targeted genes of T helper cells (a type of T cell), which produced peptides that were used by the HIV-1 to enter the cell. The idea was to silence the T helper cell's messenger RNAs coding for these peptides, making the T helper cells 'HIV resistant'. The result was positive. It showed a dramatic suppression of HIV infection in a mouse. siRNA molecules were shown to halt the T cell destructions in the mice, stopping the virus completely.

Although promising, the scientists still faced problems. The capacity of an antibody to carry the siRNA was limited. Furthermore, an antibody is a relatively large protein. When injected into the human body, the immune system could attack these foreign antibodies, stopping them before they can start to function.

A group of scientists led by Mark Saltzman at Yale University (2009) discovered that you could pack thousands of siRNA molecules into a nanoparticle.<sup>[9]</sup> We could combine this study with the previous study to come up with an effective HIV/AIDS treatment.

T helper cells contain unique peptides that are only found on their surface. For example, there are the CD4 receptors and nanoparticles can be coated with peptides that only bind to the CD4 receptors. We can use this to ensure that they only affect the T helper cells. This is a form of targeted delivery. As shown by Saltzman's team, nanoparticles have the capacity to carry thousands of siRNA molecules. This solves the limitation faced with the use of a single-chain antibody. In addition, this means clinicians could reduce the number of substances introduced to the body, making the treatment less toxic. Smaller amounts of substances also mean that the patients are more likely to adhere to the treatments. Furthermore, unlike the antibodies, nanoparticles can evade the body's immune system owing to their microscopic size. It seems almost certain that siRNA will be delivered. A study must be done, however, to show that the siRNA-delivery by the nanoparticles will actually work. In fact, the nanoparticles have already been shown effective at delivering siRNA.

Saltzman's research further involved the use of nanoparticles to deliver the siRNA. Using genetically engineered mice that produce a green fluorescent protein, he tested the effectiveness of the nanoparticles at delivering the siRNA. It worked perfectly. Nanoparticles

were transferred to the vaginas of female mice and the particles were shown to have spread the siRNA molecules deep into vaginal tissue and into the uterus – most proteins stopped fluorescing in the region suggesting the expression of fluorescent proteins were blocked by the siRNA molecules. Therefore, the idea of making T cells resistant to HIV-1 using nanoparticles could be possible. What's more, it was found that the nanoparticles released their cargo slowly over a month. This controlled-release delivery system, with its ability to carry thousands of siRNA, will allow the patients to receive their treatment only once a month. This is much better compared to HAART, which requires the daily consumption of numerous pills.

This technique may have one potential problem, however. It works by preventing the expression of certain peptides on the surface of the T cells. These peptides may serve a particular purpose. It may be a receptor molecule for hormones, for example. For the safety of patients, a thorough study of these peptides' function would be necessary. Alternatively, a new technique will have to be developed, which would silence the genes in a controlled manner.

A month ago, a successful clearance of HIV infection by simply boosting the function of cells vital to the immune response was demonstrated by Pellegrini *et al* in their study.<sup>[10]</sup> During their research, they discovered a gene called SOCS-3. SOCS-3 becomes highly activated in an overwhelming infection like HIV, and suppresses the immune response especially in the T cells. Interleukin-7 (IL-7), a naturally-occurring immune hormone, was found to switch this gene off. They use IL-7 to boost the immune system in humanized mice with HIV-like condition, and a successful clearance of the infection was shown.

Strengthening the body's immune system is another possible approach in treating HIV/AIDS. Using this study, we could simply introduce IL-7 into the body, hoping to boost the T cells. However, as IL-7 is a hormone, we do not know how it would affect other cells. In addition, if IL-7 is added straight into the body, the body may even work to remove it, because the level of IL-7 is unnecessarily high the liver would metabolise the hormones, making the treatment ineffective. Delivering IL-7 directly to the T cells specifically may be a better idea. Nanotechnology can be used. How the nanoparticles can be made to target the T cells has been discussed already. This time IL-7 could be carried instead of siRNA molecules or antiretroviral drugs.

The optimum amount of IL-7 needed to be delivered would need to be investigated. The wrong amount of IL-7 delivered to the T cells could make the treatment ineffective. Too small, it may not work. Too large, T cells may become hyper-activated, which may induce an autoimmune response (the immune cells attacking body cells).

Only two months ago, a research led by C. Neff (2011) demonstrated the use of a molecule known as a chimera to both stop the HIV replication inside infected cells and neutralize free-floating HIV.<sup>[12]</sup> Chimera is a mixture of two different types of RNA: siRNA and an RNA sequence known as an aptamer. siRNA, as discussed already, is used to knock out the viral genes used by the HIV-1 to replicate. The aptamer binds to a protein called gp120, which is found on the surface of HIV and HIV-infected cells. The aptamer played a role in delivering the siRNA to infected cells in this study. However, aptamer was also shown to neutralize the free-floating virus in the blood.

This study, although similar to Kumar and Ban's, is different. They both utilize the siRNA molecule. In the case of Kumar and Ban, they used siRNA to silence the T cells' genes to make them HIV resistant. On the other hand, in the case of Neff *et al*, siRNA was used to silence the viral gene stopping the HIV-1 from replicating inside the cell. Effectively, siRNA replaced the antiretroviral drugs in attacking the virus.

The use of siRNA is far better than the use of antiretroviral drugs. It only binds to the viral gene and silences it. The virus is not killed in the process, but only made harmless. Hence, the virus simply cannot develop resistance. Furthermore, the aptamer used in the study even neutralized the free-floating virus in the blood.

Using nanotechnology, we can take this even further. We could use nanoparticles alongside the chimera used in this study. Nanoparticles can replace the aptamers for delivering siRNA molecules. Saltzman's study has already shown the ability of the nanoparticles to carry thousands of siRNA molecules.<sup>[9]</sup> We can then coat the nanoparticles carrying siRNA molecules with the aptamers. Aptamer's ability to specifically bind to the gp120 proteins will guide the nanoparticle to the HIV-1 particles and the virus-infected cells. In addition, when travelling in the blood, the nanoparticle will be able to neutralize any free-floating virus with aptamers on its surface. The study showed that the antiviral effect of the chimera lasted only about a week, which means that the patients would need to have regular injections. Since this approach involves nanoparticles, their controlled-release delivery system will allow the treatment to last for longer periods of time.

This technique enables us both to neutralize and prevent the HIV from replicating inside the infected cells. If it works, then it will be a better treatment than HAART. We don't need to worry about drug resistance or patient compliance for the reasons discussed above. Unfortunately, it does not kill the infected cells. It does not deal with the cells infected with dormant virus – the viral reservoir – another major problem for treating HIV/AIDS. Therefore, it will not cure HIV/AIDS.

The next step will involve finding a way to actually kill both the infected cells and hence remove the viral reservoirs. Tackling the viral reservoirs may not be a problem. It is understood that macrophages, a type of white blood cells, are the major HIV reservoir cells. Macrophages have various receptors on their surface such as Fc receptors, mannose and formyl peptide. If we utilize this fact, the elimination of viral reservoir may become possible through the delivery of antiretroviral drugs or siRNA molecules by the nanoparticles to the macrophages. Investigating the effect this would have on the healthy macrophages would be necessary before we could use this method for the safety of patients.

Despite all these potential treatments for HIV/AIDS using nanotechnology, one big challenge still remains. The majority of those infected with HIV/AIDS and the most vulnerable populations reside in developing and economically poorer countries.<sup>[3-4]</sup> However effective the new HIV/AIDS treatments using nanotechnology may be, these people will not be able to afford them. Therefore, prevention of HIV/AIDS would be the best solution to the HIV/AIDS problem. The most realistic solution would be developing a safe and effective HIV/AIDS vaccine.

An HIV/AIDS vaccine has been a challenge and a goal since the discovery of the disease. Vaccines work by raising an immune response that gives future protection. 'Memory'

immune cells can then defeat the pathogens more effectively from any future infections by recalling their previous experience.

The difficulty of the challenge is partially due to the virus's high genetic variability and its ability to evade the immune system. However, the challenge really lies in generating the killer T cell (KTC) response, which is pre-requisite for an HIV/AIDS vaccine. Generating a KTC response requires the microbes to infect a special type of cell called the antigen presenting cell (APC). These cells process microbe's antigens – the 'identity' protein – and load them into molecules on their surface called MHC class I. The microbe's antigens are then presented to the KTCs. With the assistance from the T helper cells, memory KTCs can then be generated. Currently, there is only one type of vaccine that can do this: attenuated vaccines. Attenuated vaccines utilize a weakened ('attenuated') form of the microbes. The viruses in the vaccine are still infectious. They are just too weak to cause the disease in healthy individuals. The problem with using attenuated vaccine with HIV/AIDS is, some HIV can mutate and regain its strength. In recent clinical trials of HIV/AIDS vaccines developed by Merck and NIH, some vaccinated individuals showed an enhanced rate of HIV acquisition.<sup>[12]</sup> Therefore, with HIV/AIDS infecting APCs with viruses is not a good idea. Instead, we will need to deliver the antigens to the APCs. The challenge associated with delivering any exogenous antigen to APCs is that exogenous antigens require specialized 'cross-presentation' in order to be presented by MHC class I, which in turn would activate the KTCs.

Nanoparticles have potential to overcome this problem. Firstly, nanoparticles can be designed to effectively target APCs. APCs express a variety of receptor molecules, which we can use for targeting. Secondly, antigens required to be delivered can be encapsulated in their core. Nanoparticles can then directly enter the cytoplasm of the APCs and release the antigens once inside the cell. In this way, we can avoid the need of 'cross-presentation'. Antigens are released inside the cell. The released antigens will be processed by the APCs and be presented on the MHC class I. Thirdly, the controlled-release systems of nanoparticles will allow a sustained release of various agents. The release of antigens in a controlled-manner could lead to a prolonged and stronger initiation of the immune response – ideal for vaccine delivery.

In a study by Wang *et al* (2007) the polystyrene nanospheres successfully induced dendritic cells (a type of APCs) mediated immune responses in mice.<sup>[13]</sup> Production of efficient antibodies against HIV were detectable in the genital tract as well as specific KTCs in the spleen. With the help of nanotechnology, the invention of a safe and successful HIV/AIDS vaccine may well become possible.

## Conclusion

Nanotechnology can significantly improve the treatment and even allow the prevention of HIV/AIDS. The current HAART system can be improved using nanotechnology. Delivery of drugs in a controlled-release manner can reduce the toxicity and improve patient compliance. Nanoparticle's abilities to carry substances and target specific cells using peptides that bind to specific surface proteins on the targeted cells allow many things to become possible. Drugs can be directly delivered to the infected cells and the viral reservoirs. Hormones such as IL-7 can be delivered to T cells to boost the body's immune system to fight off the infection. siRNA can be delivered more effectively using nanoparticles compared to the current use of viral vectors or antibodies. Effective delivery of siRNA in turn allows stopping the replications of HIV in the infected cells by silencing the viral genes. Nanoparticle siRNA delivery could also be used to make T cells HIV resistant.

A safe and effective HIV/AIDS vaccine could be developed with the aid of nanotechnology. Instead of using potentially dangerous attenuated virus to bring about the killer T cells' response, nanoparticles could be used. Their ability to target specific cells (the antigen presenting cells in this case) and to release antigens in controlled manner may provide a good alternative to activate the killer T cells' response.

However, studies must be made, before we can safely introduce nanotechnology into treating patients. The safety of using nanoparticles must be evaluated. As nanotechnology is relatively new to the field of medicine, the toxicity of nanomaterials currently remains unknown. For example, it was recently observed that nano metal particles chromium and cobalt increased the DNA damage across a barrier via a 'never-before-seen' cell signal process.<sup>[14]</sup> Most importantly, all the nanotechnology-based studies undertaken so far for the treatment of HIV/AIDS did not involve the use of human subjects. Therefore, the stability of nanoparticles in physiological conditions in humans and whether they would be as effective remains unknown. Ultimately, we must ensure the safety and effectiveness of the use of nanoparticles in humans. On the whole, however, it seems nanotechnology has enormous potential in the treatment of HIV/AIDS.

## References

- [1] There's Plenty of Room at the Bottom by Richard Feynman (1959)  
<http://www.zyvex.com/nanotech/feynman.html>
- [2] Drexler, K. E. (1986) Engines of Creation: The Coming Era of Nanotechnology, Non Basic Stock Line; Reprint edition (1 Oct 1986)
- [3] HIV/AIDS statistics  
<https://www.unaids.org/en/>
- [4] Global summary of the AIDS epidemic (December 2009)  
[http://www.who.int/hiv/data/2009\\_global\\_summary.png](http://www.who.int/hiv/data/2009_global_summary.png)
- [5] Combination Therapy for HIV/AIDS  
<http://www.tht.org.uk/informationresources/hivandaids/treatmentforhiv/>
- [6] Dou, H *et al* (2006) Development of a macrophage-based nanoparticle platform for antiretroviral drug delivery  
<http://bloodjournal.hematologylibrary.org/cgi/content/long/108/8/2827>
- [7] Baert, L *et al* (2009) Development of a long-acting injectable formulation with nanoparticles of rilpivirine (TMC278) for HIV treatment  
<http://aac.asm.org/cgi/content/full/54/5/2042>
- [8] Kumar, P & Ban, H *et al* (2008) T Cell-Specific siRNA Delivery Suppresses HIV-1 Infection in Humanized Mouse  
Cell, Volume 134, Issue 4, 577 – 586
- [9] Saltzman, M *et al* (2009) Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA  
Nature Materials, 8, 526 – 533
- [10] Pellegrini, M *et al* (2011) IL-7 Engages Multiple Mechanisms to Overcome Chronic Viral Infection and Limit Organ Pathology  
Cell, Volume 144, Issue 4, 601 – 613
- [11] Neff, C. P. *et al* (2011) An Aptamer-siRNA Chimera Suppresses HIV-1 Vial Loads and Protects from Helper CD4<sup>+</sup> Cell Decline in Humanized Mice  
Science Translational Medicine, Volume 3, Issue 66
- [12] Trials of NIH HIV Vaccine Candidate Scaled Down after Failure of Merck Vaccine  
<http://www.medicalnewstoday.com/articles/101708.php>
- [13] Wang, X. *et al* (2011) Induction of Potent CD8<sup>+</sup> T-Cell Responses by Novel Biodegradable Nanoparticles Carrying Human Immunodeficiency Virus Type 1 gp120  
Journal of Virology, Volume 81, Number 18, 10009 – 10016
- [14] Tiny tech sparks cell signal find (2009) BBC News  
<http://news.bbc.co.uk/1/hi/sci/tech/8344815.stm>