

NANOTECHNOLOGY IN MEDICINE AND THE USES OF
CARBON SCAFFOLDING IN RESOLVING
OSTEOGENESIS IMPERFECTA

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PASS WITH MERIT

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ABSTRACT

Our background work for our paper is the uses of nanotechnology in society and the current research being carried out to improve this fairly new scientific field. We then go on to talk about the specific application of nanotechnology in human bones and what is involved when a bone re-models itself in normal circumstances. The central topic of our paper is how carbon nanotubes can improve bone strength for a person with the condition Osteogenesis Imperfecta. We will also discuss the complications and applications of the carbon nanotubes and will end with a conclusion of whether the carbon nanotubes are effective enough to be used to treat Osteogenesis Imperfecta.

INTRODUCTION

Nanotechnology

Nanotechnology is the science and technology of manipulating particles on an atomic and molecular scale in between the range of 1 to 100 nanometres. The world is showing a rapidly growing trend of miniaturization (design or construct of an already existing object on a smaller scale). This is extended into modern day medicine, and can be seen in consumer goods such as sun cream. Sunscreens contain nanoparticles like zinc oxide or titanium oxide, while older sunscreens contained much larger particles which left a white sheen on the skin. This paper focuses on the use of nanotechnology in medicine.

Current Research

There are two approaches to nanotechnology. Firstly there is the bottom-up approach, which involves materials or devices being built from molecular components, which assemble themselves by the principal of molecular recognition (this is known as molecular nanotechnology). The other approach is top- down development where nano-sized objects are constructed from larger existences without atomic-level control.

Nanotechnology has many uses due to its nanoscopic size. A use of nanotechnology is self-cleaning glass. This involves using nanoparticles to make glass photocatalytic which is when UV radiation hit the glass, the nanoparticles gain energy causing them to break down any organic material on the glass and thus cleaning it. Nanoparticles can also cause the glass to become hydrophilic ('water loving'). Hydrophilic occurs when the water makes contact with the glass, it spreads across the glass evenly cleaning it. This happens as the nanoparticles cause the glass to distribute its charges unevenly polarising the glass. Therefore, the water will be attracted to different parts of the glass due to its dipole nature. Pilkington is an example of a company that offers this glass known as 'Activ Glass.'

Another fairly recent use of nanotechnology is using nanoparticles to enhance your clothing. This is only a premature idea as scientists are still testing its effectiveness. Fabricated clothes are coated with a thin layer of zinc oxide nanoparticles, which gives better protection from UV radiation and therefore, clothes will not fade as fast. Clothes can also become stain

resistant by covering them with nanoparticles in the form of whiskers or hairs to help repel water.

A medical use of nanotechnology, which could avoid infections, is antimicrobial bandages. The scientist Robert Burrell who created a process to manufacturing bandages using nanoparticles of silver first put this idea forward. The silver ions smother the harmful cells by blocking the microbes' cellular respiration and therefore destroying them.

Nanopores are the latest research being made. A nanopore is a very miniature hole which reads a single molecule of DNA as it goes through the hole. When the DNA molecule goes through the hole, it pulls the pore-forming protein with it, ultimately placing it in the hole and creating a strong chip-based system that is made to measure for selection and device applications. The researchers have understood that this device is fully functional and can be used to detect DNA molecules.

The future of nanotechnology is still quite uncertain. Scientists are trying to create an 'assembler' (a nano-sized building machine) which can custom build matter. If these assemblers can self-replicate, then it may be possible to manufacture products and processes on a large scale. Molecular nanotechnology appears to be the outlook of nanotechnology with the most potential to succeed.

Nanotechnology in Human Bones

Introduction

Bone is a rigid tissue which contains cells embedded in excess intercellular material. The two major components of this material are collagen and calcium phosphate. Large amount of the bone is made out of matrix. This contains organic material such as carbonated hydroxyapatite crystals and also inorganic material known as collagen. Bone is formed from hardening of the matrix entrapping cells. When the osteoblasts become entrapped they become osteocytes. The collagen and the carbonated hydroxyapatite crystals are present in regular arrangement with other components such as water and bone lining cells.

There are four major types of cells present in bone. Osteoblasts, Osteocytes, Osteoclasts and undifferentiated bone stem cells (mesenchymal), which are all found in or upon the intercellular matrix.

Osteoblasts arise from the differentiation of osteogenic cells (this is the tissue that covers the surface of the bone and in the marrow cavity). Osteoblasts are largely responsible for the formation of the intercellular material. They produce a template for the rest of the bone producing and bone remodelling cells. This template is made out of collagen and hydroxyapatite crystals. Collagen has a triple helical structure and forms the intercellular scaffolds by lining up in columns and hydrogen bonding with neighbouring strands. The intramolecular forces known as covalent bonds between the collagen molecules allow them to do this by forming microfibrils, which then link up to form fibrils. These fibrils are the scaffold that HA crystals are formed on which in turn are used for the mineralization of the bone.

Osteocytes are a star shaped cell which is abundant in compact bone. Osteocytes are only found in fully formed bones, this is because they are effectively Osteoblasts entrapped by the mineralized extra cellular matrix they secrete. Small channels called ‘canaliculi’ form towards other Osteocytes, in this way Osteocytes are maintained by the exchange of nutrients and waste products. Osteocytes form a network which monitors for damage to the bone.

Osteoclasts are cells that are responsible for the dissolution and re-absorption of the organic and mineral part of the bone. They dissolve the organic collagen and the inorganic calcium and phosphorus of the bone by secreting a number of enzymes specifically acid phosphatase and cathepsin K. When the mineralized bones are broken into sizable fragments the Osteoclasts engulf and digests them. The salvaged minerals are then released into the blood stream.

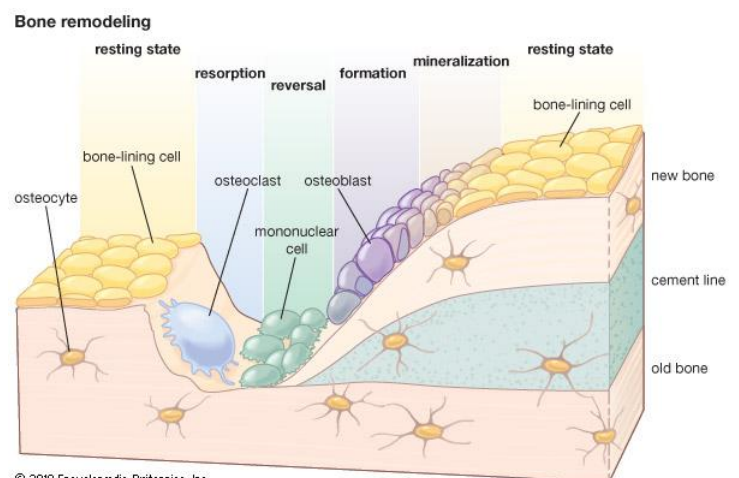
Mesenchymal stem cells possess the ability to differentiate into a number of cells such as Osteoblasts and chondrocytes (cartilage cells). These undifferentiated cells are found in the bone marrow specifically the red marrow.

Bone Remodelling

Bone remodelling is the lifelong cycle of bone destruction and re-absorption. The two main cells in this process are Osteoblasts and Osteoclasts (as mentioned above). Bone remodelling naturally occurs when human bones are first developing and temporary bone is quickly laid down, this bone is not sustainable so it is reabsorbed and new stronger permanent bone is made.

When Osteocytes detect a microfracture or damage to the bone they trigger bone remodelling mechanisms. After the detection, the second stage of this process consists of Osteoclasts moving to the area of the damaged bone by chemotaxis. They then start to break down the intercellular material by releasing hydrogen ions through the action of carbonic anhydrase. This aids cathepsin K to absorb the mineralized bone matrix into calcium and other required substances. Osteoclast cells release the calcium ions they absorb into the blood stream to meet the body’s metabolic needs. This process helps to keep the skeletal structure tensile. When the Osteoclasts have re-absorbed as much as the damaged area necessary, Osteoblasts then start to form more extracellular matrix by synthesising collagen and then mineralization of the remodelled bone.

These Osteoblasts then become entrapped in the densely packed intercellular material and become Osteocytes. This cycle is repeated many times to fulfil the body’s metabolic needs of calcium ions and other necessary substances.



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OSTEOGENESIS IMPERFECTA

Osteogenesis Imperfecta (OI) also known as Lobstein Syndrome is a rare hereditary disease of the connective tissue. OI arises from a genetic defect that causes defective or no collagen production. OI is characterised by a mechanically weakened tendon, fragile bones, lax joints, skeletal deformities and in extreme cases prenatal death. There are 8 types of OI, Type 1 is the most common however symptoms vary from person to person. Type 5, 6, 7 and 8 are not relevant to this paper, as the abnormalities are not caused by the collagen.

The other 4 types of OI are:

Type 1 is an abnormality where the collagen is normal but there is not enough collagen made. The main symptoms of this type are that the bone is easily fractured, there are lax joints and there is defective sclera.

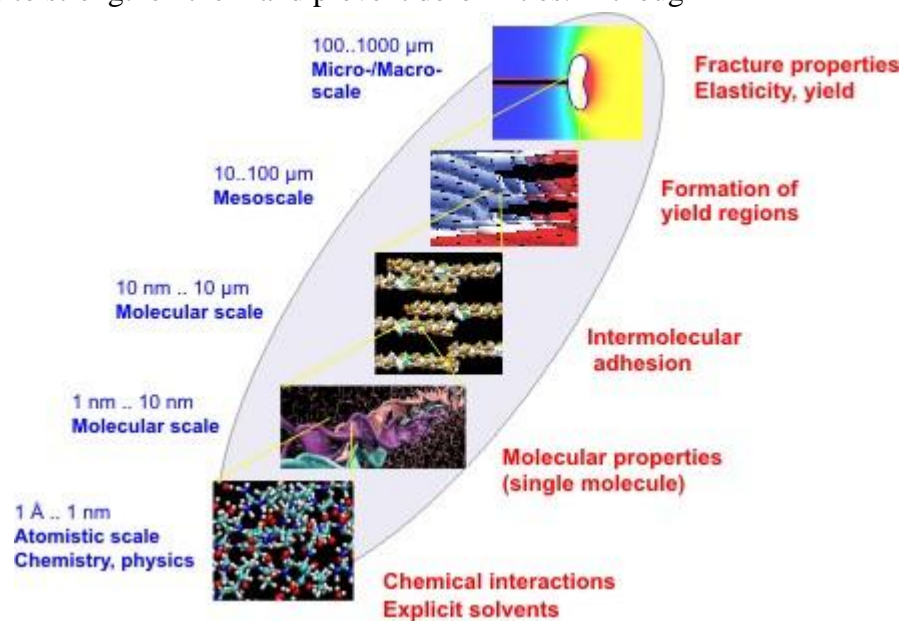
Type 2 is an abnormality where the collagen is not normal and there is not enough collagen made. The main symptoms of this type are deformed extremities, death in perinatal periods and severe bone fragility.

Type 3 is an abnormality where the collagen is not formed properly. There is a good quantity of collagen made but the molecules are faulty. The main symptoms of this type are bone fragility and progressive deformity.

Type 4 is an abnormality which is similar to Type 3, the only difference is that the collagen made is not of high quality but it is not completely faulty. The main symptoms of this type are bone fragility, short stature, early hearing loss and short deformity.

Fractures may be present at any age, but usually in the first five years of a person's life. Many patients with OI have multiple fractures of various ages. The more the person fractures his/her bone, weaker the bones get and that increases the tendency of higher number of fractures. This can affect a person with severe pain and disability may occur.

There is no known treatment for this disease yet. The current treatments are only used to prevent or control the symptoms. The current treatments consist of care of fractures, extensive surgical procedures and physical therapy. These treatments are trying to maximise a patient with OI's mobility and developing optimal bone mass and bone strength. Most of OI patients are advised to exercise so that their bone and muscle get stronger. As the bones and muscles get stronger there will be fewer chances of fractures. All these types of precautions require a lot of time and a lot of money spent. There is surgical procedure which is frequently considered for people with OI. This treatment involves inserting metal rods through the length of the long bones to strengthen them and prevent deformities. Although surgical procedure is the most common it still requires a lot of expenses and the patient goes through severe pain

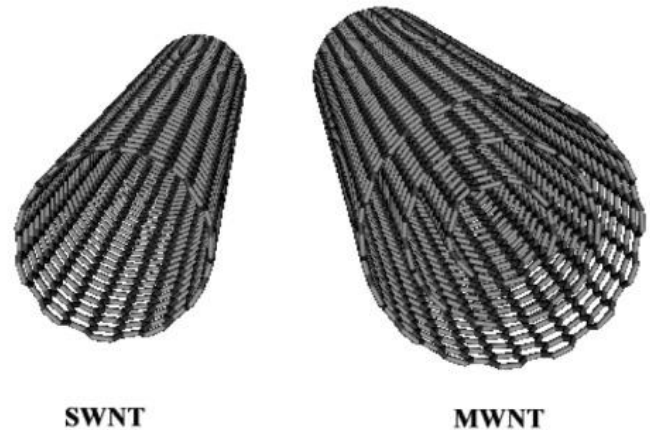


DISCUSSION

Introduction to Carbon Nanotubes

Figure 1

Carbon nanotubes(CNT) are allotropes of carbon with a cylindrical nanostructure. This is analogous to sheets of graphene rolled up into tubes, and were discovered by S. Iijima in 1991. Carbon nanotubes are the strongest known substance to man, this is due to the SP₂ bonds in between the carbon atoms. These bonds are much stronger than SP₃ bonds found in alkanes. These specific bonds give CNT their unique strength and flexibility. The nanotubes are categorized into single walled and multi walled carbon nanotubes (SWNT and MWNT).



SWNT tend to have a diameter around 1nm, this small diameter however does not stop the nanotubes being many million times longer. They possess a single cylindrical wall one atom thick and are extremely elastic withstanding sharp bends and can even be bent into small circles.

MWNT are effectively many SWNT's with different diameters within one another, the separation between the nanotubes is close to the separation of layers in naturally occurring graphite.

Application to Osteogenesis Imperfecta

Carbon nanotubes show great promise for use in the condition of Osteogenesis Imperfecta, both in mechanical strengthening and encouraging cell growth. As described earlier in the paper, this condition is caused by either a lack of or production of faulty collagen. This leads to a range of symptoms but this paper will concentrate on the strengthening of the brittle bones typical of OI.

Due to the similarities in size between collagen fibrils and CNT, many have proposed that CNT be used as a replacement for collagen and in turn to form the basis of the intercellular scaffold. Studies have shown that mature osteoblast cells proliferate rapidly on the walls of SWNTs and less successfully on MWNTs. However electrically neutral CNT with no functional group favoured proliferation of the bone cells (3). The other main focus of bone

regeneration is the formation of HA crystals. Research in 2005 showed that the self-assembly of HA crystals was achievable on chemically functionalized SWNTs (1). Individually these studies are very promising for the biocompatibility of CNT, but they do conflict. For optimum bone regeneration the conditions need to be tailored for each stage in bone formation, but this is not currently achievable as osteoblast proliferation favours CNT with no net electric charge while the formation of HA is optimal with a net electric charge (due to the attraction of more calcium ions). More research into finding a compromise of CNT would be needed to maximise the efficiency of this potential treatment.

The mechanical strength of CNT would also be desirable for patients with Osteogenesis Imperfecta. The lack of collagen leads to incredibly brittle bones, the unique strength of CNT could greatly increase bone strength and greatly decrease fracture rate. This would be much more convenient than conventional surgery where metal rods are implanted along bones. Although effective, the treatment is complicated by the extra weight added to the bones and chance of infection during the initial surgery. CNT are much lighter and encourage natural regeneration of the bone which is preferable to a mechanical support which is not always biocompatible.

Bone fractures are common to patients with OI, they are not only a regular occurrence but fractures take much longer to heal. Perhaps CNT could be used as a replacement scaffold to decrease the healing time and with SWNTs being a similar size and shape to collagen molecules, the cells could potentially rearrange them to ensure the fracture is repaired in the right shape. The CNT could also increase comfort for the patient by allowing more movement, this is due to their exceptionally high tensile strength. The substitute scaffold could be introduced during the granulation stage of bone healing; this is the stage before cartilage cells appear and before osteoblasts attempt to produce woven bone. This would leave a few days for the treatment to be initiated.

Treating bone fractures with CNT would require inhibition of the production of the faulty collagen. This could be done via halofuginone, a plant alkaloid (12). This chemical has been known to inhibit the production of type 1 collagen; small doses have proved efficient in significantly lowering the synthesis of collagen. However, a method of delivering this drug to the specific area would have to be developed.

Complications of Carbon Nanotubes

The biocompatibility of carbon nanotubes is somewhat disputed. For any orthopaedic treatment the nanotubes must cooperate seamlessly with the native cells. This would require extremely biocompatible CNT. This paper focuses on the effect of CNT on the human cell metabolisms.

Firstly, osteoblasts are secretory cells. The fusion of secretory vesicles to the plasma membrane and release of the products depend on the functioning of the cell ion channels (6). For treatment, CNT would be required to not interfere with any cell ion channels, however research into SWNTs have shown that they possess the ability to block potassium ion

channels (1). Research will have to be carried out in vivo to evaluate whether the chosen CNT would hinder the ion channel activities as this is essential for the deposition of HA and in turn mineralization of the bone.

Carbon nanotubes have been tested on human epidermal skin cells to attempt to evaluate their toxicity. This research, in 2003(8), showed that after 18 hours of exposure, peroxidative products accumulated, free radicals were formed and antioxidants were depleted. This indicated oxidative stress (excess free radicals unable to be neutralized by antioxidants, increases rate of ageing) and cellular toxicity. However, skin irritation by CNT is controversial. Another study obtained results indicating that SWNTs did not pose a dermatological effect (9). Fullerene soot with a high concentration of SWNTs was applied to volunteers with susceptible allergic skin and no inflammatory responses or irritation were reported. This points towards biocompatibility, however they clearly contradict each other therefore more research would need to be carried out. Future research should concentrate on the evaluation of pure CNT rather than fullerene soot used in the previously mentioned study, perhaps different results would be found.

Almost all CNT are non-biodegradable, this leaves open to the question of what would happen to the nanostructures during bone remodelling. As described above in this paper, during bone remodelling the lamellar bone is stripped down by osteoclasts secreting enzymes and acids then absorption of the by-products occurs. During this process collagen is broken down, however if CNT are not biodegradable, how will the osteoclasts adapt when part of the intercellular material is not broken down. Perhaps the CNT could occupy this area permanently even during bone remodelling. Either way it is still unclear what part CNT would play during the process of bone remodelling.

During synthesis, toxic metal catalysts are used to manufacture CNT. These are present in unrefined carbon nanotubes but have been removed for refined or 'pure' nanotubes. These catalysts are always transition metals such as nickel, cobalt or iron based. So far these have been the most successful catalysts but upon introduction of CNT to cells they have proved to be toxic and induce inflammatory responses. Therefore, either CNT must be refined or more biocompatible catalyst particles must be developed.

Cui et al (10), set out to evaluate biocompatibility of SWNTs by investigating the effects of SWNTs on human HEK293 cells. The researchers cultured the cells in a media containing different concentrations of SWNT. Cui et al concluded that cell growth was inhibited via SWNT induced apoptosis (programmed cell death) and decreased cell adhesion (due to the inhibition of adhesion related proteins). Another study found that SWNTs induced apoptosis; however this study was on human fibroblasts (11) which make it considerably more significant as fibroblasts are present during bone healing after fractures or damage. Tian et al, also found that unrefined CNT are less toxic than their refined counterparts, this leads to speculation about how to deal with the non-biocompatible metal catalysts which have been linked to decreasing cell viability while testing for the biocompatibility of CNT.

CONCLUSION

Nanotechnology displays great potential for use in everyday life, however the possibilities nanotechnology displays for medicine are by far the most intriguing. Nanotechnology opens up a new door for medicine, instead of miniaturisation it allows the scientific community to build up, imitating the basic internal and external structure of cells. As discussed this is epitomized in bone tissue engineering, conventional techniques for strengthening bones are crude, although nonetheless effective. They replace the bones strength with metal supports. Nanotechnology has the potential to provide an alternative to this, instead of repositioning the strain onto metal supports it could mimic the extracellular bone matrix, by the use of carbon nanotubes, to regenerate the strength of the bone. This paper has explored the potential applications of this proposed treatment in Osteogenesis Imperfecta (also known as brittle bone disease).

Due to the extreme properties of carbon nanotubes and also their similarity to collagen molecules they are one of the most promising areas of nanotechnology for medicine. The great mechanical strength and ability to allow osteoblasts to proliferate on the CNT walls mean they could replace the lack of/faulty collagen in patients with Osteogenesis Imperfecta. However, important factors such as biocompatibility still have not been completely assessed for both single walled and multi walled carbon nanotubes. Research studies are contradictory; some suggest single walled nanotubes can block ion channels, as osteoblasts are secretory cells they rely heavily on functioning ion channels for fusion of secretory vesicles to the plasma membrane.

Overall, the applications of CNT in bone related diseases are potentially groundbreaking however there are complications hindering the advancement of this field. Before use as a treatment further research should be carried out in vivo and in vitro to completely assess the biocompatibility of CNT and use of a non-toxic catalysts would be desirable.

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