Nanotechnology; Applications and Future Potential in Regenerative Medicine with Particular Focus on Bone Reconstruction

By
Lauren Hutchinson

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Abstract

The use of nanotechnology to create structures that are exactly the same size as cellular human structures offers enormous possibilities for the repair and replacement of human tissue, hip joints already benefit from this technology as titanium joints have been enhanced this paper will look at some of the current ideas and potential future applications of nanotechnology in regenerative medicine, together with a look at the ethical dilemmas posed by this technology.

Introduction

"...for I was never so small as this before, never" – Lewis Carroll, **Alice's Adventures in Wonderland**

It has been predicted that over the coming 10 to 15 years nanotechnology will revolutionize the practice of medicine and thus have significant impact on human health. It is expected that nanotechnology will be developed at several different levels; materials, devices and systems. Nanotechnology can be defined as the science and engineering involved in these applications where the smallest functional organisation, in at least one dimension, is on the nanometre scale, or one billionth of a metre (Silva, 2004). Over 200 companies are involved in nanomedicine research and development, thirty eight nanomedicine products are currently on the market and dozens of additional products are on the pipeline. The market of nanotechnology is expected to grow to $12 billion by 2012 (Resnik, et al., 2007).

In order to appreciate the size level at which nanotechnology operates it should be considered that the average protein is 5 nm, therefore the developments achieved through nanotechnology will be operating at a sub-cellular level. Fine nanoparticles' diameters generally range from 100 to 2500 nanometers, while ultrafine particles range between 1 to 100 nanometers wide. The attraction of this should be the fine tune control that can be obtained in the absence or reduced potential for side effects, as opposed to conventional medicine. As biological scientists further explore cells at a molecular level this has allowed engineers to develop substances which mimic these discoveries – this equals nanotechnology.

The European Commission has established a European Technology Platform on nanomedicine (ETP, The challenge). They have the shared aim of working together for the health care of Europe to match the high expectations that nanomedicine has raised so far. Their policy objectives are;

- Establish a clear strategic vision in the area
- Decrease fragmentation in nanomedical research
- Mobilise additional public and private investment
- Identify priority areas
- Boost innovation in nanobiotechnologies for medical use

The three key priorities have now been confirmed by these stakeholders;

- Nanotechnology based diagnostics including imaging
- Targeted drug delivery and release
- Regenerative medicine (ETP, 2006)

It is the last of these three key priorities, regenerative medicine, which will be the basis of this review paper. **Regenerative medicine** is the process of creating living, functional tissues to repair or replace tissue or organ function lost due to damage, or congenital defects. Regenerative medicine allows scientists to grow new organs or tissues in laboratories and replace faulty or damaged ones within the human body.

The major applications of various nanomaterials currently being used in regenerative medicine and related biomedical applications include their use as:

- Scaffolds for cell growth
- Delivery devices for various drugs, growth factors and genes
- Applications for cellular modification, isolation and tracking (Verma, et al., 2011)

Nanotechnology medical developments over the next few years have a wide variety of uses and will potentially change medicine and save many lives. Due to the relative sizes of cells and molecular machines, nanoparticles are working on a whole new level to anything else. Regenerative medicine, which this paper will be based on, will cover a range of aspects of regenerative medicine with a particular focus on bones.

Nanomaterials used in regenerative medicine are primarily made using either the top-down or bottom-up approach. These terms were first applied to the field of nanotechnology by the Foresight Institute in 1989 in order to distinguish between molecular manufacturing (to mass-produce large atomically precise objects) and conventional manufacturing (which can mass-produce large objects that are not atomically precise). A top-down approach is the breaking down of a system to gain insight into its compositional sub-systems, such as inkjet printing where externally controlled tools are used to cut, mill and shape materials into the desired shape and order. A bottom-up approach is the piecing together of systems to get higher, grander, more complex systems. It resembles a seed model whereby the beginnings are small but eventually grow in complexity and completeness; however this may result in a tangle of elements and subsystems. In relation to nanotechnology, bottom-up approaches in contrast to top-down, uses the chemical properties of single molecules to cause single-molecule components to self organize or self assemble into a useful conformation (Verma, et al., 2011).

Overall, bottom-up approaches should be able to produce devices in parallel and much cheaper than top-down methods but could be overwhelmed as the size and complexity as the desired assembly increases.

The particular interests in regenerative medicine which will be discussed in further detail include regeneration of the central nervous system, hip replacements, re-growth of cartilage and nanoparticles with stem cells. The paper will look at the methods behind each of these upcoming developments, the likely benefits to patients, the NHS and society as well as the risk factors and ethical issues, posed by these new developments.

Discussion

Regenerative medicine focuses on tissue engineering. Tissue engineering was defined in 1988 as the “application of principals and methods of engineering and life sciences towards fundamental understanding of structure-function relationships in normal and pathological mammalian tissue and the development of biological substitutes to restore, maintain or improve tissue function” (htt1). Nanotechnology is currently used in the replacement of damaged tissue, but provides enhanced prospects for self-repair of tissue within the body rather than replacement.

Bone implants, for example hip replacements, have been standard medical procedures for many years now. It is estimated that the average life span of a hip replacement is only 10 to 15 years, with an ageing population it is clear that the life time of these implants must be significantly enhanced. To succeed as an orthopaedic implant material, a material must be habitable especially for bone-forming cells (osteoblasts) such that they can colonize on the implant surface and synthesize new tissue (Sato, et al., 2004). Titanium is an already frequently used material for bone implants due to its properties including high tensile strength. It has been known for a long time that titanium surfaces have to be coated to enhance bone formation, and have used materials at the micron size. Nanotechnology has already allowed for the coating of this titanium, uncoated titanium generates less bone formation for the titanium hip to bond to the surrounding pelvis, which is fundamental for a successful hip replacement. New nano-produced materials induce a roughness on the titanium which is at the same size level as the bone osteoblasts are accustomed to.

Future developments could lead to nano-sized scaffolds being developed. The construction of a scaffold for which the matrix would assemble itself has many requirements, such as it must create a tolerable, biocompatible environment that allows cell infiltration and restoration of lost tissues. The scaffold should also deliver appropriate signals for cellular regeneration in a controlled and localized manner (Verma, et al., 2011). These may still be titanium based, such as a thin layer of 30nm thick titanium which now adds a further property of flexibility to hip replacements (May, 2006), this leads to many different potentials for hip replacements such as personalised moulding in bone implants, rather than three standard sizes. Personalised hip replacements may improve function and life expectancy. High titanium strength maintained with an added property of flexibility and moulding due to nano particle’s ability to work on a cellular level. Scaffolds could even be further developed such that they are made with biodegradable polymers with or without incorporation of growth stimulants. The incorporation of growth stimulants into biodegradable polymers, which will create bone formation, whilst the biodegradable polymers dissolve, only leaving the formation of a new “self hip replacement” with no implants. Where what started as a repair with the implantation of the nano scaffold structure has resulted in true regeneration with the new “self hip”, rather than the previous short life titanium hip replacement currently in operation. This entire philosophy of joint replacements was recently described as biological joints replacing artificial joints (Dalby, 2011). With nanoparticle hip replacements the longer life expectancy would mean a cost saving to the NHS as it is common for the same person to receive 2-3 hip replacements in their lifetime, and is currently increasing with the ageing population, a saving in costs to the NHS is vital for the future.

The most promising area both now and in the foreseeable future for nanotechnology lies in its interaction with stem cells. Stem cells are single cells located throughout the body that are able to differentiate and grow into the myriad of cells and organs contained within the body. They are typically characterised as embryonic stem cells (ESCs) or tissue specific adult stem cells, e.g mesenchymal (MSC), haematopoietic and neural. As nanotechnology structures are operating at the same size level as these important single cells, this allows for the potential manipulation of these cells.

Nanoparticles may be able to bind with a stem cell and then be used to deliver stem cells to target locations, through “homing” devices within the nanoparticle. The ultimate goal would be the targeted delivery of a nano structure containing the required growth factor to initiate growth and differentiation of already existing stem cells within a patient’s body.

Nanoparticles in the near future may regenerate damaged joint cartilage. At present damaged cartilage can be treated in three options, a) ignoring and hoping for the best which normally results in a further loss of cartilage, b) have the damaged cartilage removed which would limit exercise for their life and may cause onset osteoarthritis or c) have a joint replacement which is an invasive and expensive procedure however the best current option. “Unlike bone, cartilage does not grow back, and therefore clinical strategies to regenerate this tissue are of great interest” says Samuel I. Stupp, Director of the Institute for BioNanotechnology in Medicine. In the case of regenerating damaged cartilage, researchers at North-western University are the first to have designed a biologically active nanomaterial that improves the growth of new cartilage on the site of the damaged tissue (Stupp, et al., 2010). The technique activates bone marrow stem cells resulting in the production of type 2 collagen-based cartilage, which is minimally invasive, doesn’t require expensive materials or a difficult procedure. Once the nanoparticles have been injected into the site of damaged cartilage at the joint, the nanomaterial will fasten itself to the affected area and assemble a solid structure called a matrix. The matrix mimics the normal extracellular environment and attracts and binds a specialist growth factor on the damaged cartilage area which stimulates cartilaginous stem cells to grow and differentiate. After around a month, the matrix will have been replaced by actual cartilage growth and the matrix degrades down into nutrients. The development of this technique has so far been restricted to only animals though it is currently in the process of approval for testing in human subjects.

An ethical issue related to regeneration of cartilage is the concern that the use of nanomedicine will be for physical enhancement rather than therapy creating social injustice. Nanomedicine can easily be used for physical enhancement as any new medical technology that can be used to diagnose, prevent or treat disease can also be used to enhance the function or appearance of the human body or the human mind (Resnik, et al., 2007). For example application of nanotechnology to neurology could help reduce or replace memory loss or enhance human memory. Enhancement can be considered morally wrong as it may produce unfair competition, aggravate existing socioeconomic inequalities, as if only the rich can afford treatments it will be passed on through the family and widen the inequality.

Any discussion of regenerative medicine would be incomplete without the holy grail of regeneration of the central nervous system, as neurons in the brain and spinal cord have very limited potential for healing and reorganisation. Major brain and spinal cord injuries are currently serious problems that have no effective treatments. The transplantation of stem/progenitor cells may provide an effective treatment for central nervous system (CNS) injury due to the self-renewing and multipotential nature of these cells. *In vitro* cultivation of nerve axons is an ongoing field of research. Webber *et al* have described a mouse model where they injected nanofibre gel and saw re-growth of nerves and restoration of hind limb function (Webber, et al., 2010). The nanofibre gel is a peptide sequence which attaches and then attracts the progenitor cells which grow and differentiate into neurons. Nanomaterials offer a number of properties that are of interest to the field of neural tissue engineering. Specifically, materials that exhibit nanoscale surface dimensions have been shown to promote neuron function while simultaneously minimizing the activity of cells such as astrocytes that slow down central nervous system regeneration. Due to the complexity of tissue regeneration in the CNS, versatile solutions are necessary for the successful treatment of nerve injury. The inability of neural tissue to spontaneously regenerate, particularly in the CNS, is as much due to the presence of inhibitory signals which stop a chemical reaction or process as it is due to the absence of stimulatory signals which excite a chemical reaction or process. Research carried out by Bregman et al 1997, has shown the for bioactive molecule strategies nerve growth factor and brain-derived neurotrophic factor have been shown to improve axonal growth after spinal cord injury in rats. The success of using nanomaterials to repair a damaged central nervous system is still in very early stages of development and due to the complexity of the central nervous system many techniques and applications will need to be applied in order to repair the damaged CNS, although there has been some success within a few of the different components, all of the procedures need to be brought together (Seil, et al., 2008).

The same principles as explained before in regenerating damaged joint cartilage in theory could be applied to spinal cord damage. In cartilage regeneration, nanoparticles are injected at the site of damage where they fasten themselves and create a matrix, which binds specialist growth factors and stimulates stem cells to grow and differentiate into cartilage. This principal could be applied where a nanomaterial could be attached to the damaged spinal cord and cause MSCs to differentiate. The manufacturing process in nanotechnology exists, the knowledge gap is what growth factors or extra-cellular matrix is required in the environment to result in differentiation that would lead to repairing damaged nerves.

One major issue with nanoparticles is that due to their enhanced reactive area, which can be seen in figure 1, there can be twice as many OH- reactive sites. Compared with conventional material, nanophase materials have an increased percentage of atoms at the surface. This has benefits to nanoparticle applications in regenerative medicine as the increase in percentage of atoms, higher surface areas and altered electron delocalisation influence protein interactions and important for controlling consequent cell adhesion (Sato, et al., 2004). However this benefit also acts as a hazard as their enhanced reactive area, ability to cross cell and tissue barriers and resistance to biodegradation strengthens their cytotoxic potential relative to molecular or bulk counterparts (Verma, et al., 2011).

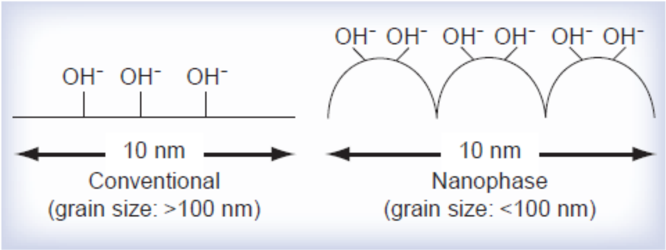

Figure 1, Exposed hydroxyl groups in conventional and nanophase materials. (Sato, et al., 2004)

Nearly all of the beneficial features found on nanomaterials also cause the greatest concern in attempting to understand what will happen within the human body. The creation of materials that are now smaller than the cells they are sitting next to; could be expected to result in increased phagocytosis and or endocytosis of those materials. This will lead to distribution of the material to a more distant site than originally intended. All of these processes could lead to increased toxicity. In regenerative medicine there is the added complication of using nanomaterials to be indistinguishable from self. This positive benefit will also make it nearly impossible for tracking of the foreign material.

The European Commission has requested the independent experts of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) to look at and evaluate “the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies”. They concluded that:

- There is insufficient knowledge and data of nanoparticles including their detection and measurement and their outcome (especially in the determination) of nanoparticles in humans and in the environment
- All aspects of toxicology and environmental toxicology related to nanoparticles and for risks assessments for ecosystems as well as humans to be carried out
- The risk assessments may need to be expanded and developed so that they cover all aspects sufficiently to do with nanoparticles (EMA, 2006).

For the development of nanotechnology to have a large impact on medicine the nanoparticle procedures have to go through a risk-benefit assessment. At one end of the spectrum, there are potentially life-saving drugs and medical devices where the only risks might be to the person whose life is being saved; at the other end of the spectrum, there are materials which through as yet unknown biodistribution pathways could result in mass environmental exposure (Goldman, 2005).

Conclusion

In conclusion, nanoparticles are only a small way down the pipeline; each current development still has a long way to go before we will see the procedures in action at hospitals. However each procedure has an exciting and new prospect and aspect that could revolutionize medicine. As each idea and nanomaterial is developed the knowledge of nanoparticles grow and therefore the uses for the particles grow too.

From the current research taking place at the moment for nanoparticles in regenerative medicine all the techniques and idea interlink with one another for example the technique to regenerate joint cartilage can be applied to any regeneration throughout the body if the correct nanomaterial and growth factor can be developed.

Nanotechnology is currently in use in the enhancement of titanium implants so that bone formation leading to adhesion of the implant is increased through the increased surface area. Scaffolds are in development which will allow bone to form around them as they are porous structures on the same scale as the normal cellular structure. New matrixes and scaffolds are being designed which will initially promote differentiation of stem cells and then slowly dissolve into nutrients locally. This will result in new bone with no foreign implant left in situ. Bone and cartilage regeneration are success stories which will provide techniques which it is hoped can be transferred to other body systems, such as the central nervous system.

There are also some ethical issues which need to be covered concerning nanoparticles, firstly a new product on the market has a high price, which then will fall over time due to competition from generic and similar products. When nanomedicine first becomes available on the market it will have a high price, which will drop in time however due to the complexity and uniqueness there will be less competition meaning that the price will fall at a slower rate. This could mean that economically disadvantaged people may not be able to afford the new on the market nanomedicine which would lead to health inequalities. This could be a significant problem within countries such as the US (Resnik, et al., 2007). The Human Genome Project has shown that having rapid new advances in medical knowledge freely available has resulted in more specific applications being developed faster. It is hoped that nanomedicine which is heavily reliant on technological engineering will adopt a similar process.

However, despite the challenges that lie ahead significant evidence now exists the nanoparticles for use in regenerative medicine are an important growing area of research that will possibly revolutionize medicine in the future.

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