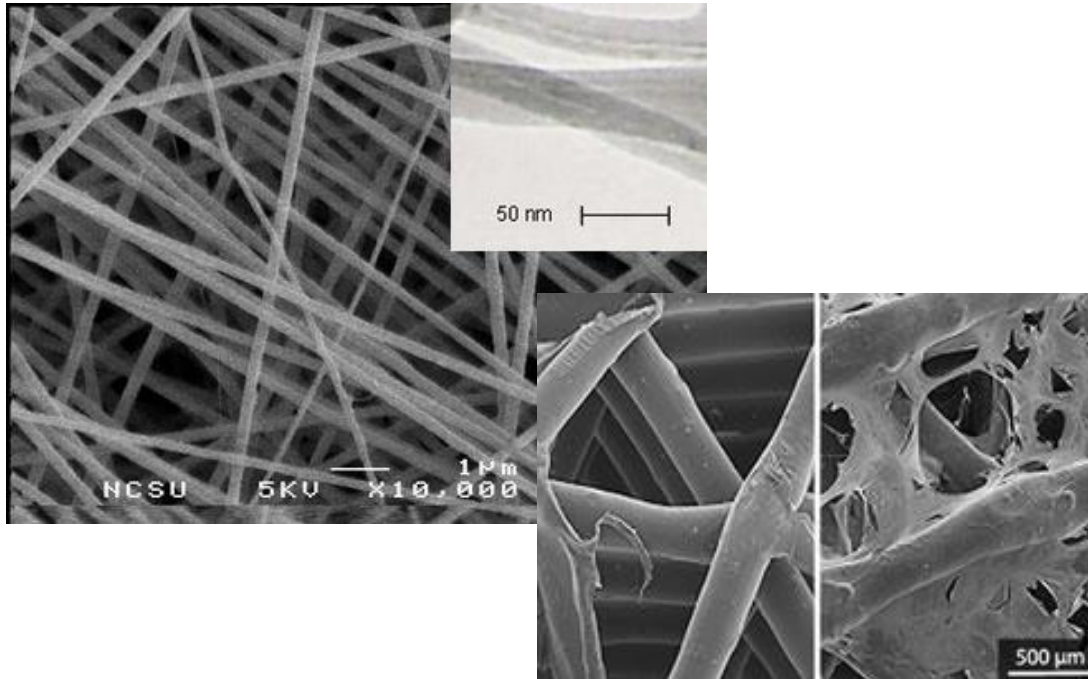


# Learning from nature; tissue engineering using nano scaffolding



BY

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PASS WITH MERIT

RESEARCH PAPER  
BASED ON  
PATHOLOGY LECTURES  
AT MEDLINK 2010 or MEDISIX 2011

## **Abstract**

Zebra fish can miraculously repair damages to their heart but unfortunately, humans cannot. Tissue engineering using nano scaffolds has the potential to allow us to repair or even re-grow not only the heart but any other limbs and organs. Therefore, tissue engineering is often known as regenerative medicine.

This dissertation will explore the prospect of using nano scaffolds in tissue engineering and identify the ideal characteristics of a nano scaffold. It will discuss the use of organic and inorganic materials for the fabrication of nano scaffolds. Additionally, techniques of fabrication like electrospinning and nanofibre self assembly will also be explored and the advantages and disadvantages of each method will be identified.

## **Introduction**

A record high 3706 transplants were carried out in 2010 in the UK. Even so, three people die each day due to the lack of a suitable organ or the lack of donors. <sup>[1]</sup> On top of this, the people who are lucky enough to get a suitable organ transplant may not subsequently lead a healthy life due to the immunosuppressant drugs that are necessary to stop the body's immune system from attacking the organ. Nanotechnology seems to be a promising field in which organs can be grown outside the body using the patient's own stem cells. This novel opportunity could eliminate the need for donor organs and the need for immunosuppressant drugs, saving many lives.

Nanotechnology, one of the most promising technologies of the 21<sup>st</sup> century can be defined as engineering at a very small scale, usually from 0.1-100 nm. Nanomedicine, a highly promising area of nanotechnology, refers to medicinal diagnostics and therapies at a molecular level. Latest research shows that various life-threatening issues could be combated remarkably well by nano medicine.

Nano scaffolding is an extremely important development in tissue re-growth and regeneration. The technique initially developed by a group of scientists at Sheffield University UK, is also studied by the US military amongst others. <sup>[2]</sup> The method requires making nano scaffolds; a 3D microstructure of ultrafine (polymer) fibres which provides a microenvironment in which cells can grow, divide and re-create damaged tissue or even more complex structures. The polymer provides a lattice for the new cells which move up through the fine scaffolding. <sup>[3]</sup>

In 2006 researchers from the University of Sheffield used nano scaffolding to repair skin damage for people with third degree burns. The researchers attached a person's skin cells on to a nano scaffold and the cells grew over it. The skin-covered scaffold was placed on the wound where it bonded with the patient's body. The scaffold then apparently disappeared – it was digested and reabsorbed by the patient's body (this will happen with any nano scaffold provided that it is biodegradable). <sup>[4]</sup>

In December 2008 at the 26<sup>th</sup> Army Science conference in Florida, Dr John Parmentola, director of research and laboratory management for the US Army explained that they had used nano scaffolding to re-grow the tip of a man's finger, restoring everything he had lost – the nail, the bone and tissue. He also added that the US Army were able to re-grow whole bladders for people with

bladder damage. Furthermore, the technology allowed them to repair the wall of a woman's uterus as well.<sup>[4]</sup>

## **Discussion**

### **Ideal characteristic of a nano scaffold**

In order to achieve the desirable clinical outcome, there are many challenges that a nano scaffold has to overcome. For example, it is crucial for a nano scaffold to be biocompatible, mainly so that it does not become toxic to living cells. Additionally, it should have a high porosity and an adequate pore size to enable efficient movement of cells and diffusion of nutrients, oxygen, metabolites and waste products.<sup>[6]</sup> Also, it should be biodegradable so that surgical intervention is not required to remove it after the body has formed its own matrix.<sup>[7]</sup> The scaffold should be able to provide structural integrity within the body by encouraging cell adhesion, cellular interaction, proliferation and migration of cells.

Simple diffusion is favoured by a high surface area to volume ratio. Therefore, it can be hypothesized that a tissue being engineered would benefit from a nano scaffold with a high surface area to volume ratio as well. This is because it will allow a larger part of the tissue to be in contact with the surrounding, enabling efficient diffusion. Also, it will allow more of the cells in the scaffold to interact with each other if the tissue is being engineered outside the body and with other cells and tissues if tissue is being engineered inside the body.

The properties of the nano scaffold are evidently determined by the method of fabrication of the scaffold and the material used to do this. Just like different reactions have different catalysts, the engineering of different tissues requires different nano scaffolds. This is due to the surface chemistry exhibited by the scaffolds. This essay will now look at some nano scaffold fabrication techniques and some materials used for this.

### **Materials used for fabrication of nano scaffolds**

Materials which are both organic and inorganic, including metal oxides, have been investigated. Natural biodegradable materials seemed especially desirable because the body would have been capable of getting rid of the by-products when the scaffold breaks down. Natural polymers like collagen, chitosan and fibrin gel, on the whole have shown some positive results for example, cell compatibility. Unfortunately, there are still immunogenicity issues in these polymers and they lack mechanical strength to support the growth of tissues.

Contrastingly, inorganic or synthetic biodegradable polymers can be fabricated to produce nano scaffolds with adjustable physical and mechanical properties. Their degradation rate can easily be controlled and this is an increasingly desirable factor because different cells form their extra cellular matrices (ECM) at different rates. Some examples of these biopolymers include, poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL), and

poly(glycolic acid) (PGA). These are all poly- $\alpha$ -hydroxy esters that de-esterify in the body as the polymer degrades in to simple metabolites. <sup>[6]</sup> For example, PLA is a polyester which degrades inside the human body to form lactic acid – a naturally occurring chemical easily removed from the body.

Most of these materials were already familiar in the world of medicine before the advent of making nano scaffolds for tissue engineering; they were employed as biodegradable sutures. <sup>[7]</sup> Polymers are strong and can be made into different shapes and structures like film or fibre.

Inorganic materials such as bioglass, ceramics and metal oxides have also been investigated as well, mainly due to the fact that biopolymer nano scaffolds present some unresolved issues as well. For instance, the body has a local inflammatory response to the polymer. However, for soft tissue applications such as the engineering of skeletal muscle or cardiovascular tissue, polymeric nano scaffolds are superior to that of nano scaffolds made from ceramics or metal oxides. This is because polymers have mechanical and chemical properties similar to that of the extracellular matrices of soft tissues. On the other hand, when a bioglass nano scaffold was seeded with osteoblasts, positive cellular proliferation was observed. <sup>[6]</sup> This shows that bone regeneration favours inorganic scaffolds as opposed to organic polymers.

### *Nano scaffold fabrication techniques*

#### *Lyophilization technique*

The lyophilization technique a form of freeze-drying, <sup>[8]</sup> was originally used for the preservation of biomaterials. It involves extracting the solvent (usually water) from a biomaterial by firstly freezing it and then under a vacuum subliming the solid solvent into a vapour leaving behind a porous solid minus the solvent. This process is aided by heat.

Nano scaffolds can be made using this technique. Firstly, a synthetic polymer is dissolved in a solvent (for example PLA in dichloromethane) and water is added to the polymeric solution in order to form an emulsion. Next the emulsion is cast into a mould and frozen quickly. This is usually done by immersing it in liquid nitrogen. The frozen emulsion is then freeze dried to separate the solvent and water from the polymer. This last stage is what forms the porous solid. The thickness of the scaffold can be controlled by the amount of solvent, water and polymer used. Although the pore size is relatively small, the porosity is often irregular and there is no way of controlling this <sup>[9]</sup>. Another drawback of this technique is that involves using an organic solvent. Therefore it is important to ensure that the solvent is completely removed in order to avoid the death of cells once they are seeded on to the nano scaffold.

#### *Solvent casting and particulate leaching (SCPL)*

Much like the aforementioned technique, SCPL involves dissolving the polymer in an organic solvent and then casting it into a mould with porogen particles. The porogen can be an organic salt like sodium chloride, saccharose crystals, gelatine or paraffin spheres. After the polymeric solution is cast, it is allowed to evaporate fully. Then the mould with the solid polymer is immersed in a liquid bath that is suitable for dissolving the porogen particles; water in the case of sodium chloride,

saccharose and gelatine, or an aliphatic solvent like hexane for the paraffin. Once the porogen is fully dissolved, a porous polymeric structure is obtained.<sup>[7]</sup>

The size of the porogen particles determines the size of the pores and the polymer to porogen ratio is directly proportional to the porosity of the nano scaffold. Therefore, this approach allows a regular and predictable porosity in the nano scaffold. However, the range of thicknesses for the nano scaffold is limited. This technique also involves using an organic solvent and the drawback of this is mentioned above.

### Nanofibre self-assembly

Nanofibre self-assembly is a method that enables the synthesis of nano scaffolds that are extremely similar in scale and chemistry to that of a natural in vivo extracellular matrix. When in physiological concentration, salts like saline, tissue culture fluid, physiological solutions or human body fluid such as cerebrospinal fluid, self-assembling peptides spontaneously undergo self organisation to form well ordered structures.<sup>[10]</sup>

EAK16-II (fig. 1[i]), a 16 amino acid peptide, contains alternating alanines and alternating charges on the glutamates and the lysines.<sup>[11]</sup> In general, a scaffold made from self assembling peptides consists of  $\beta$  sheet ionic peptides with alternating amino acids and 50% charged amino acid residues. The periodic repeats of alternating ionic hydrophilic and hydrophobic amino acids results in the  $\beta$  sheets having distinct polar and non-polar surfaces. The well-defined sequences allow the peptides to undergo ordered self assembly. A number of self assembling peptides that encourage cell attachment, cell survival, and cell differentiation are RADA16-I (fig. 1 [ii]) and RADA16-II. The difference between them and EAK16-II is that the glutamates and the lysines on the EAK16-II are replaced by arginine and aspartic acid.<sup>[11]</sup> This class of peptides is made up of natural amino acids only. They undergo spontaneous assembly into well-ordered nanofibres and scaffolds; the diameter of the nanofibres being approximately 10 nm and the pore size of the scaffold being between 5 and 200 nm. The nanofibre density correlates with the volume of peptide solution used. These scaffolds also consist of over 99 % water and are very similar to natural extracellular matrices including collagen.<sup>[12]</sup>

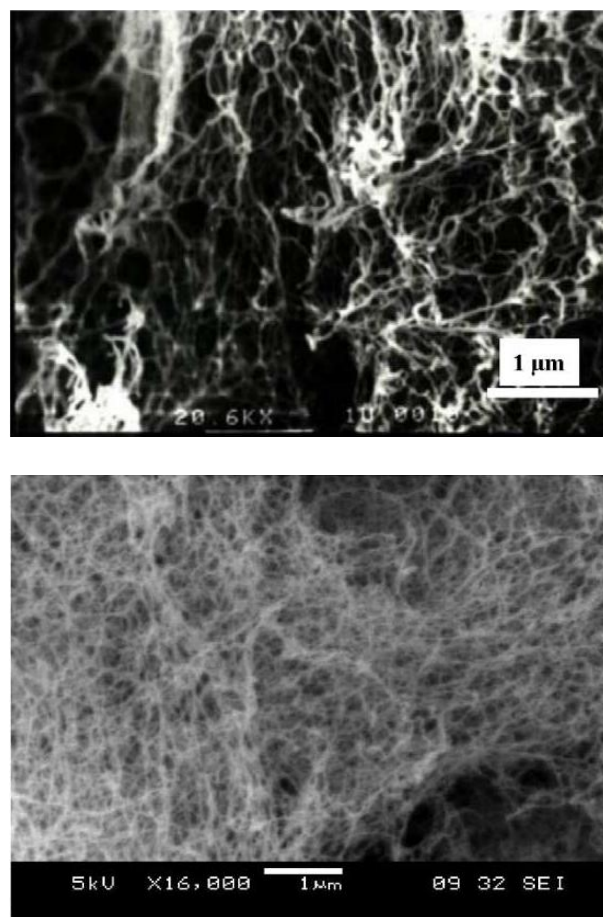


Fig. 1 SEM images of self assembling peptides. (i) EAK16-II (ii) RADA16-I. These peptides form nanofiber scaffolds with nanopores.

## Electrospinning

Electrospinning is already a well-established technique that allows the manufacture of nanofibres (the basis of a nano scaffold) out of polymers. When a high enough voltage is applied to a liquid droplet, the liquid becomes charged. This results in the electrostatic repulsion counteracting the surface tension of the liquid causing the droplet to stretch. At a critical point known as the Taylor Cone, a stream of liquid erupts from the surface.<sup>[13]</sup>

Nanofibre fabrication makes use of this technique. A polymer solution is injected at a constant feed rate through a nozzle or needle which is charged to a high voltage (10-30 kV); this set up is illustrated in fig. 2. The high voltage is applied to establish a charged jet of liquid which is attracted to the earthed collector. During this process, the solvent evaporates leaving dry polymer fibres. Fig. 3 illustrates some nanofibres that were electrospun<sup>[14]</sup> I hypothesise that the earthed collector could be of any shape and the nano fibres will take on this shape. For example the collector could be moulded in to the shape of a specific organ so that the proliferating cells will have extra support and to some extent knowledge as to how and where to grow.

Altering variables such as distance between collector and source, magnitude of applied potential difference and solution can dramatically vary the characteristics of a nano scaffold fabricated by electrospinning.<sup>[13]</sup>

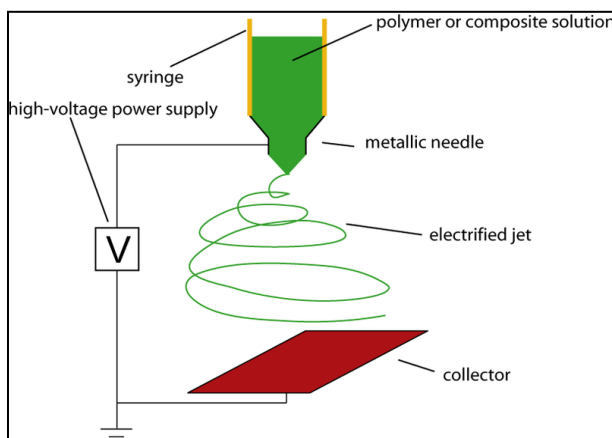


Fig. 2 Electrospinning set up.

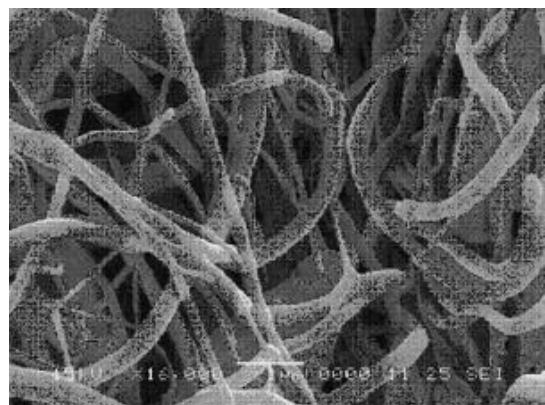


Fig. 3 Electrospun nanofibres

Numerous other techniques of fabricating nano scaffolds have been investigated by various scientists. Most techniques had some advantages but unfortunately many were overwhelmed with disadvantages as well. For example, the use of textile technology for the preparation of non-woven meshes of different polymers to make nano scaffolds was also investigated. Although a fibrous structure was a useful medium in which to grow different cells, it was unable to keep supporting the cells and form a 3D environment.

The techniques discussed in this dissertation especially electrospinning and nanofibre self-assembly have an increased number of advantages against minimal disadvantages; all of which are discussed above. Having looked at this it can be understood that overall there isn't one ideal technique in

which to fabricated nano scaffolds to fit all situations. Nano scaffolds have to be fabricated after assessing the situation. This is an advantage to a patient because it means that he or she will receive individually-based treatment. Firstly, having understood how self assembling peptides work, it is clear that this technique of fabrication of a nano scaffold has more potential for tissue engineering in vivo. For example, I believe that it may prove to be very useful in the regeneration of nerves. It is on the whole better to minimise surgery and therefore, injecting self assembling peptides in to the area in which nerve regeneration is needed is considered to be a much safer alternative.

There are several formidable barriers that have to be overcome to achieve axonal regeneration after any sort of injury to the central nervous system (stroke or trauma). To start with, the formation of scar tissue after tissue injury needs to be prevented if the nerve is to be regenerated successfully. Also, after neuronal injury, gaps in the nerve are formed due to the phagocytosis of dying cells after injury. The reconnection of nerves in the CNS after trauma seems to be possible via self-assembling peptides because they eliminate the main two drawbacks of nerve regeneration. Self-assembling peptides spontaneously form nanofibres creating a scaffold-like, tissue-bridging structure. Due to the nanoscale of the peptide fibers it is possible to be almost certain that there will be interaction between the peptide scaffold and the neural tissues on both sides of the lesion. This will therefore allow the movement of cells on to the scaffold which will then proliferate and in the process repair the nerve. Experiments on young and older animals using the peptide RADA16-I show that it discourages or prevents the natural formation of scars that usually occurs at an early stage of an injury.<sup>[15]</sup> All in all, self assembly of peptides to form nanofibres seems to be a hugely promising prospect for nerve regeneration in vivo. However, it is not always possible to regenerate tissues and or organs in vivo. For arguments sake if a patient is in need of a liver or a heart, the new organ cannot be grown in vivo because the body will not be able to support two organs that are the same. Also, the process will be less complicated were the organ was grown ex vivo and then transplanted. For example, growing the new organ ex vivo ensure that the new organ will get all the necessary nutrients, oxygen and blood supply in the correct amounts. In addition, it will also be easier to monitor the growth of the organ when in ex vivo (it minimises the amount of radiation that the patient is subject to.) This is where electrospinning comes into play.

As mentioned earlier, it can be assumed that electrospun nanofibres can be collected into a mould the shape of an organ in order to give the nano scaffold a specific shape. In theory it should be possible to build a scaffold of the heart with the valves and the outer structure only. Additionally, introduction of heart stem cells on to the scaffold and giving the cells and the scaffold an in vivo experience should enable the cells to differentiate, proliferate and form a heart with its own extracellular matrix. Although, this may seem like 'star-trek' science it can in fact be backed up by research carried out at the University of Minnesota. Dr. Doris Taylor and her research team grew a beating rat heart in the laboratory. They did this by removing all the cells from a dead rat heart leaving behind the valves and outer structure as scaffolding. Next they injected on to the scaffold new heart cells from new born rats. This can be interpreted as the equivalent of injecting stem cells on to the scaffold. Within two weeks, the cells produced a beating heart which conducted electrical impulses and pumped a minimal amount of blood. Dr Doris Taylor also stated that this method could be used to grow human hearts by taking stem cells from a bone marrow of a patient and placing them on a cadaver scaffold.<sup>[16]</sup> The problem with this is that it raises many ethical issues. These ethical problems will be for obvious reasons very similar to those presented by transplanting and

xenotransplanting. Some people believe that it is unethical to 'disturb' the dead even if the person has left consent to do so. Therefore, using fabricated nano scaffolds is a much better method of growing new organs in many ways.

## **Conclusion**

As mentioned in the introduction 3 people in the UK die everyday due to the lack of a suitable donor organ. This dissertation explored the possibility of eliminating the need of donor organs by using nanotechnology for tissue engineering. Nano scaffolds provide a 3D environment in which cells can grow, proliferate, differentiate and migrate. This gives us the opportunity to grow organs ex vivo and transplant them in to people who need them. Even better, the cells that are injected on to the scaffold can be the patient's own stem cells. Therefore, this technique not only reduces the pressure to find more donors but also eliminates the need to use immunosuppressant drugs.

The focus of this dissertation was how to make these nano scaffolds and what should be used to make them. The answer to this is that it depends on which tissue is being engineering. For example, as far as materials are concerned, polymeric materials are superior to inorganic materials such as bioglass or ceramics when trying to engineer soft tissue. The growth of hard tissue like bone is supported more by nano scaffolds made by inorganic materials such as metal oxides as opposed to those made by polymeric materials.

Techniques of fabrication also depend on the situation. For example, if nerve regeneration is necessary then the method of nanofibre self assembling is favourable because it eliminates unnecessary surgery. This can be done by injecting self assembling peptide solution into the patient. The peptides will spontaneously assemble themselves to form nanofibres and in the process scaffolds. This will provide support for the axonal cells by bridging the gap between the two sites of lesion of a nerve. However, if a completely new organ needs to be grown it makes sense to do it ex vivo and then transplant it. This can be done using scaffolds made from electrospinning polymers to form nanofibers. The electrospinning technique is unique such that in theory the final shape of the scaffold can be determined by the shape of the earthed collector. This gives us the opportunity to make scaffolds the shape of specific organs and hence provide extra support for proliferating and differentiating stem cells.

The reason that cells grow, proliferate, differentiate and migrate on to the nano scaffolds is because the scaffolds exhibit surface chemistry and other properties similar to that of the natural extracellular matrix of the cells. The nano scaffolds provide sufficient structural integrity for the cells therefore, they promote cellular adhesion. The scaffolds are at a nanoscale and therefore have a large surface area to volume ratio including high porosity and adequate pore size. This encourages diffusion of all necessary substances and cell migration as well. Lastly, it is important for the nano scaffolds to be biocompatible and sometimes biodegradable. This is mainly so that they do not become toxic to the cells once the cells are placed on to the scaffolds.

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## **Images**

1. Fig 1: "Designer self assembling nanofiber scaffolds for 3 D tissue cell cultures," 8<sup>th</sup> March 2011. <http://web.mit.edu/lms/www/PDFpapers/Zhang,%20et%20al%20SCB.pdf>
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