

The Production and Application of Collagen Mimetic Peptides in Regenerative Medicine

BY

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Abstract

Nanotechnology is an exciting and rapidly developing field at the cutting edge of modern medicine. The ability to manipulate substances at an atomic level offers new ways of treating disease and has brought about the potential for the body to effectively and efficiently repair itself with the aid of artificially produced nanostructures.

This paper looks at nanofibres that are manufactured to imitate the function and composition of collagen, a substance that occurs naturally within the human body, and the role that they can play in the regeneration of human tissue. The formation of a synthetic extracellular matrix has the prospective use of guiding cells into certain forms, thus being able to guide the cells into, in effect, regenerating certain tissues in the body. These artificial Collagen Mimetic Peptides can also be used for surgical and detection purposes.

INTRODUCTION

Regeneration is one of nature's most complex and desirable phenomena. It is a trait that is not unique to any individual species and, in the animal kingdom, is displayed particularly remarkably by members of the Echinoderm phylum, such as starfish. In humans, however, the capacity for phenotypic plasticity in response to trauma is limited, with the liver being the only internal organ that can reform and resume full functionality without any medical intervention. New advances in nanotechnology, however, have prompted a breakthrough that could provide an effective environment for tissue growth with the aid of an artificial scaffolding structure.

Although several polymeric materials can be used for the fabrication of bio-scaffolding, for example Chitosan and hyaluronic acid, the primary focus of this paper will be exploring the exploitation and development of a synthetic polymer, collagen mimetic peptide. Collagen mimetic peptides (CMPs) are chains of amino acids that display the same properties as natural collagen. They can be made up of a triple helix of Glycine, Proline and Hydroxyproline in the sequence $-(\text{Pro-Hyp-Gly})_x-$.^[1] CMPs can be formed using the methods of electrospinning, self assembly and phase separation, with each method offering different products suitable for different uses. The scaffolding, irrespective of the method of formation, all provide a good base for cell bonding, differentiating and propagation due to their high surface area to volume ratio and similar properties to the natural Extracellular Matrix (ECM), and so are extremely useful in the development of new tissue.

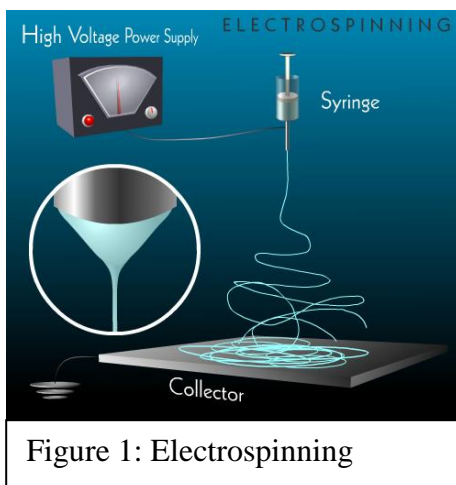


Figure 1: Electrospinning

Electrospinning uses an electrically charged, spinning tip to draw very fine fibres from liquids. It does this by applying a high enough charge to the liquid so that the electrostatic repulsion offsets the surface tension so that the droplet of liquid stretches and, if the molecular cohesion is high enough, a charged jet of fluid is propelled from what is known as the Taylor cone. This then dries midflight and, as the charge migrates to the fibre's surface, the new fibre is elongated by a whipping process caused by electrostatic repulsion at kinks in the strand. This is then collected on a grounded plate as a sinew with nano-scale dimensions.(Fig 1)

As shown by the work of L. Buttafoco and team, (2006)^[2], electrospinning can induce an aqueous solution into producing collagen or CMP that is thick enough-around 200nm-to

support a confluent layer of muscle cells to grow on a purpose designed mesh. However, as pointed out in the work of L.A Smith and P. X Ma (2004)^[3], nanofibres created using the method of electrospinning are primarily large and therefore suitable for the wider diameter parts of the extracellular matrix.

In order to create strands of a smaller size, a different technique must be employed. The Self Assembly method is omnipresent throughout nature and is how a number of structures in our bodies form, for example haemoglobin forms from individual primary polypeptides and ribosomes form inside the nucleolus from ribosomal proteins and RNA. The cells have receptors that guide them to corresponding parts of the extracellular matrix, so forming an artificial ECM would be a very efficient way for tissues to be cultivated. For example, as stipulated in S. Zhang's 2003 paper, *Fabrication of novel biomaterials through molecular self assembly*^[4], a combination of the otherwise weak van der Waal forces, hydrophobic and hydrophilic interactions, ionic bonds and, most notably, hydrogen bonds allow biological structures to seemingly self-assemble to form functional structures. In the laboratory, beta pleated sheets can be produced in an aqueous solution, similarly to protein folding inside the body, or *in vivo*. This works as the hydrophilic part of the molecule seeks out water so water mediated hydrogen bonds can form, whilst the hydrophobic part seeks is repelled from any water. If this could be refined, it could allow cells to rearrange themselves into the correct location for the formation of tissue and organs.

The final technique, phase separation, was pioneered in 1999 by Ma and Zang^[5] and involves first dissolving the polymer, before thermally inducing a liquid phase separation. The polymer then turns into a gel, which gives structures formed by phase separation a porous nature. This can be controlled using temperature until the desired level of permeability is reached. When this has been achieved, the solvent is extracted from the gel using water, before finally freeze drying the gelatinous CMPs under vacuum to form foam (Fig.2). This process is relatively simple, fast and cheap compared to the others previously listed and offers a unique product; the gel can be used to create a scaffold from 50-500nm, in which macropores can be integrated using sugars or salts. This improves mass transport, cell distribution and tissue formation. The shape of any anatomical part can be recreated using a simple mould.

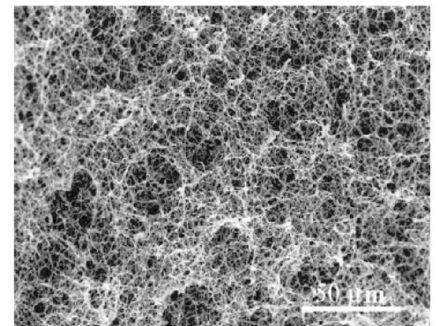


Figure 2: Scanning electron micrograph of nanofibrous foam synthesised using the phase separation technique.

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DISCUSSION

The potential for tissue regeneration using nanostructures is an area of science that is being extensively researched. Currently, tissues from the bladder to the neural cells can be developed, with the prospect of artificial cartilage soon becoming a reality. With sufficient progress, growth of nerve cells could help cure paralysis, and the growth of an organ could save the life of a terminally ill patient. However, these are only a few concepts at the beginning of what looks to be a bright future.

The scope for development in the field of regenerative medicine is enormous and, potentially, there is no limit to what a team of scientists could recreate. Using our substantial current medical capabilities as a starting point, it could be possible in the future to progress into regenerating whole organs-rather than just individual tissues-in one go. Using a complex ensemble of nano-scaffolds arranged within and around each other, it is theoretically feasible to suggest that each part of the scaffold could guide cell development uniquely, thus forming the different parts of each organ, but, simultaneously, the intertwined scaffolding would encourage development that would, combined, help the organ grow as one.

To a certain degree, the foundations for this kind of pioneering science have already been laid down. As detailed in a news article by Wyatt Andrews and published by CBS News (2008)^[6], he examines evidence and learns that it is possible for simpler organs, in this instance the bladder, to be grown using a patient's own cells and to be ready almost immediately for transplantation. It is in the construction of more complex organs, such as the heart and kidneys, where the future of regenerative medicine could be the most interesting.

The heart would be an especially challenging project for the reason that, as a muscle, it is myogenic. This means that it can contract and relax of its own accord and does not rely upon any impulses from the central nervous system to do so. Herein lays the challenge.

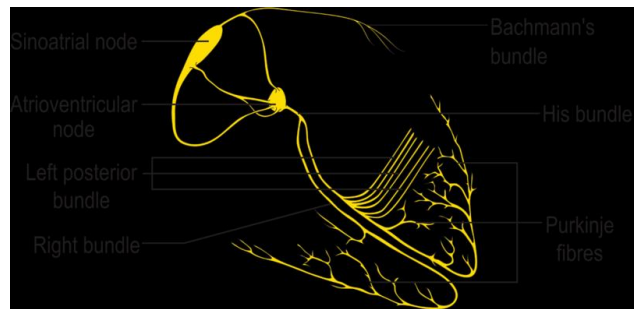


Figure 3: The Isolated Heart Conduction System

Whilst it is possible to grow muscular tissue, giving a heart the means to function naturally would be extremely difficult. The heart beats due to electrical impulses that pass through the centre of the heart that start at the sinoatrial node, travel through the atrioventricular node and then into the Purkinje fibres (Fig.3). This is what causes the contraction. Whilst possible to grow muscle cells, to grow these highly specialised myocardial cells (cells which are bi-nuclear, which have more mitochondria but fewer contraction-causing myofibrils) would require a sample of the cells to initiate the replication, which would then need to be grown on a scaffold that was integrated inside the cardiac skeleton. To do this, different scaffolds would need to be connected to different cells, but at the same time would have to grow alongside each other so that the organ would develop fully.

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This same technique would need to be applied for the construction of blood vessels, for example the vena cava, the valves and also the chordae tendineae, meaning a number of different interconnecting scaffolds would need to be in place each with a different type of cell. If this treatment were to be perfected, it could replace the need for artificial pacemakers. Furthermore, if cells from the individual needing treatment were used, it would mean that there would be no chance of rejection, which is a risk if transplantation is considered, as the organ would be an exact genetic match for the individual. It would also render the transplant waiting list obsolete as, if within a few weeks, a patient could have an organ custom made and ready for surgery, there would be no need for transplants at all.

Even if not intended for clinical use, the use of a full human organ grown in a lab would be extremely useful. Pharmacologists would be able to conduct research into the effects on

drugs without putting any person at direct harm. This could potentially be used on a large scale for drug testing, but it could also be used on a more personal level to diagnose any allergies a person may have.

In respect to nerve cells, it would be very complex, but it is possible that biodegradable nano-scaffolds could be integrated with stem cells or specialised nerve cells in order to promote the growth of new nerve cells, which could then be used to bridge the gaps in damaged neurones or as a replacement for neurones with faulty synaptic sites. In 2010, it was shown by Hiroshi Kawabe in the Journal 'Neuron'^[7], that the enzyme Nedd4-1, usually an enzyme that controls protein degradation by ubiquitination, is essential for dendrite growth in neurones. If a porous nano-scaffold infused with the enzyme was placed upon a neurone, it could stimulate neurone repair. This could help reconnect and repair the whole nervous system, giving hope for a cure for paralysis and possibly motor neurone disease. The nerve cells grown outside of the body could be implanted into the body by surgery and then connected to the original neurones using a nano-scaffold. After the success of nerve reattachment, the artificial structures would degrade and be excreted from the human body as waste.

Despite the clear advantages of organ growth in labs over transplantation of a donated organ, the operation to insert the new organ would still require major surgery. Another direction regenerative medicine could take is to achieve not growth of organs *in vitro*, but *in vivo*. In the future, nano-structures made from CMPs could be implanted using keyhole surgery, straight to the part of the body that was damaged or affected. By placing the scaffolding onto the organ, it would encourage and manipulate the organ's cells to grow and attach to the artificial extracellular membrane, which would in effect regenerate the organ whilst it was still inside the body. This would be very effective and would not inhibit a person's daily life, allowing them to continue as normal.

It would, of course, be possible to replace the natural extracellular membrane with an artificial one made from CMPs using this method as well. If a person's ECM was damaged during their life, for example by acute ischemia, it could be replaced by using a non-degradable, artificial alternative. As displayed by L. Buttafoco's paper on the electrospinning of collagen, other substances, in his case elastin, can interwoven amongst the collagen to give a strong and supportive mesh which still has the properties of the ECM. In the future, it is possible that this could be inserted into the body to repair damaged framework or to strengthen existing weaknesses. On top of this, there is the prospect that the natural fibres could be mixed with artificial ones, maybe even carbon nanotubes, creating a more effective, strengthened ECM to prevent further damage.

CMPs also have another useful trait: they can infiltrate the structure of natural collagen at certain points. By attaching gold nanoparticles to the end of CMP strands, S. Michael Yu and Martin Pomper (2008)^[8] were able to tell the exact point of crossover between synthetic and natural collagen. In the foreseeable future, this discovery has the potential to highlight any breaks in the ECM. The gold particles show up on transmission electron micrographs (Fig 4) so would show any damage, indicated if particles were not situated at regular intervals or if they were arranged in a different pattern. Any breakages could again indicate ischemia and an abnormally large quantity of gold particles could perhaps indicate the presence of any tumours or blockages in arteries. Upon detection, appropriate action can be taken, for

example removing the tumour or, upon damage, using a CMP scaffold to stimulate repair or by directly using artificial collagen to fix the damage if it is to the ECM itself.

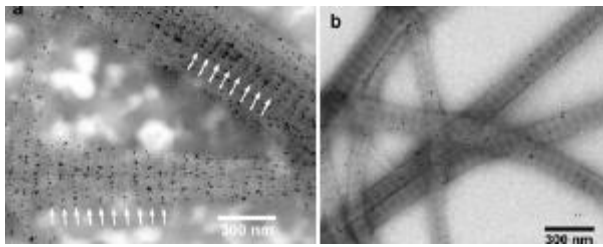


Figure 4: Type I collagen fibre after incubation with gold nanoparticles. The white arrows indicate positions of nanoparticles.

One final application could be the development of foetal surgery to encourage correct formation in the foetus. A cleft lip and palate can be identified after around 16 weeks of pregnancy and, although treatable, it can cause distress to both the child and the parent.

At present, the cleft can be fixed after birth using an artificial plug or by using a Latham Appliance, a clip that gradually pulls the roof of the mouth shut. In the future, it could be considered that implanting a nano-scaffold into the roof of the foetus' mouth may be a possible step that could eliminate the cleft before the child was even born.

Using a CMP structure, the scaffold could be manoeuvred into place and fastened to the roof of the mouth, where growth could resume after prompting from the artificial extracellular matrix. Bone is currently regenerated using bone donation from cadavers, which has a high rate of rejection or absorption. The donated bone is first carved into the correct shape, before it is surrounded by BMP-2 morphogenetic protein and periosteum tissue, usually taken from the patient's leg. This practically fuses the new bone to the old, using stem cells to fill in the gap and act as the adhesive. Using an artificial ECM as a scaffold instead of a bone, however, would reduce the rate of rejection and would encourage the growth of a completely new bone, which is much sturdier than attaching an old bone to another. The periosteum tissue could be cultured and the BMP-2 protein could be added as a supplement to promote the growth. Both the protein would be inserted at the same time as the nanostructure itself. This procedure could remove the need for any artificial devices and, importantly, this system has the potential to be done on a small scale before birth which could prevent discomfort later (Fig. 5.)

However, this situation would present huge ethical dilemmas. For some, it would not seem right to operate on a foetus whilst still in the womb. It is also possible that this procedure could increase the risk of miscarriage, as with disturbing the foetus with other prenatal tests such as amniocentesis. For others, though, it would seem like a practical solution that could treat a child and avoid any distress or disfigurement after birth. If uncomfortable with the procedure, it could also be performed after birth at the standard time of between 6 and 12 months.

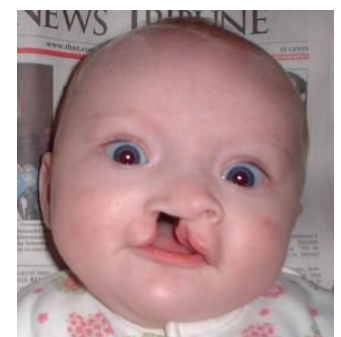


Figure 5: Girl with Cleft Lip, 6 months

On a whole, there are other ethical debates about regenerative medicine using nanotechnology. The first of these debates centres on the use of stem cells in some of the techniques, for example in constructing a new heart, nerve cells or in bone regeneration. There are two types of stem cell, embryonic stem cells and somatic stem cells. Somatic stem cells are multipotent cells that can be obtained from the bone marrow of adults or the

umbilical cord. It is agreed by most that there are no ethical problems with using these donated cells for research and for medicinal purposes.

Embryonic stem cells, on the other hand, present medics with a huge dilemma. They are pluripotent, which means that they can divide into almost any type of body cell, making them more versatile than the multipotent somatic stem cells, which have a limited range. However, the way in which they are obtained, by Somatic Cell Nuclear Transfer, means that after inducing replication by placing a somatic nucleus inside an ovum, the blastocyst that develops is then isolated after about 5 days and the cells are harvested, resulting in the destruction of the embryo. This is seen as unethical by many for two main reasons. Firstly, some people believe that this could eventually lead to full scale human cloning, as SCNT is the first step in the process. Secondly, the ova must be obtained from humans for it to work. Most of the excess ova are spares that are not needed in IVF treatment. Pro-life campaigners do not believe that any embryo should be experimented on, especially those which have been fertilised previously during medical treatment.

The final ethical debate surrounding the generation of new body parts using nanotechnology is the question of whether to continue granting treatment to those people who continually and remorselessly abuse their own body. For example, is it ethical to give a chain smoker with throat cancer a new oesophagus if he will continue to smoke and risk getting the disease again? The same principle could apply to an alcoholic with severe cirrhosis who would keep on consuming in excess, even after a new liver was regenerated for him. For many people, the answer would be no as it would be a drain on society and it offers those with selfish habits an excuse to continue at the expense of the NHS. Others, however, would disagree and say that if the chance is available to save someone, it should be taken, regardless of the situation or the cost.

CONCLUSION

After taking all of the above into consideration, I believe that the application of nanotechnology in regenerative medicine is a field with thousands of potential developments. The collagen mimetic peptides that form the nano-scaffolds are not only durable and tensile, they are biodegradable, meaning that they would be able to be broken down in the body after their purpose was fulfilled before being ejected as waste. Their small yet variable nature offers hundreds of possibilities. Scaffolds can be made that mimic the natural composition and disposition of natural biological polymers, encouraging cells to develop in the fashion that we see fit, from constructing whole organs to producing the smallest and most delicate of bodily components. The different methods of manufacturing the artificial framework mean that, not only are the types of material we can obtain varied and all structurally different, it is also economic to produce them, meaning it is cheap and simple to experiment in order to find the most effective material for the job.

At present, these scaffolds can be used to grow basic organs and to construct new tissues. Their potential, though, leads me to believe that they could in the near future, revolutionise medicine. With prospective applications in in-body regeneration, nerve cell reconstruction, bone growth, more complex organ culturing, foetal surgery and repairing damage to the ECM, the nano-scaffolds have the potential to transform and save millions of lives. They offer cheap, genetically suitable and readily available organs to those in need and can offer a

practical and possible solution to some horrific conditions, for example paralysis and motor neurone disease. The potential uses as a detection agent also gives the collagen mimetic polymers additional uses in the medicine of tomorrow.

I believe that the probable benefit that the research offers far outweighs the ethical negatives, especially if any stem cells that are obtained are done so legally and responsibly. The use of nano-scaffolding in regenerative medicine is a promising area that offers new solutions and new possibilities in the treatment of disease by allowing the construction of brand new organs and the potential of readily available, good specimens to conduct research upon.

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<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2426767/> (Figure 2)

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<http://healthfiles.net/disease/cleft-lip/> (Figure 5)