Tissue engineering

Tissue engineering is the process by which organs and tissues are grown in the laboratory for therapeutic use. Stem cell researcher Hilary Anderson is studying materials that stimulate stem cell growth and explains how tissue engineering can revolutionise complex surgery

Key words

Stem cells
Materials
Medicine
Immune rejection

Due to the lack of donors, there are unacceptable waiting times for organ transplants. Currently 6000 people are on the UK waiting list, and long waits increase the risk of patient death before surgery. Immune rejection is a problem that further complicates the issue (see Box 1). But what if we could simply build a heart, liver, or kidney? This is the aim of tissue engineers — including chemists, physicists, engineers, and life scientists — who work together to provide novel solutions to the lack of donors.

Although this is the primary goal of tissue engineering, a synthetic organ could also be useful for drug discovery. Pharmaceutical companies rely on animal models to assess the impact of a drug. However, the action of a drug can differ considerably in a mouse in comparison to a human. It would be better to understand how a particular drug could affect human organs before progressing to clinical trials. If it were possible to make a synthetic organ, drugs could be assessed quickly, thus saving money in the research and development phase.

Making artificial organs

So how can you build an artificial heart, liver, or kidney? To begin to construct these organs outside the body (known as in vitro) we must first understand their function and composition in the body (known as in vivo). For example, the trachea is a basic hollow cylindrical shape with few cell types through which air passes in and out of the lungs. In contrast, the heart has a more complex architecture, with specialised valves that separate the heart chambers, as well as electrically synchronised muscle cells that coordinate contraction of other muscle cells. A detailed understanding of the mechanics and natural function of the organ we wish to model helps us determine which materials we need to make an artificial version of it in vitro that can then be implanted into a patient.

Box 1 Immune rejection

Immune rejection is the process by which a transplanted organ is attacked by the body's own immune system. It is also possible to develop a condition called graft versus host disease (GVHD) where some of the donated tissue contains immune cells (white blood cells). The white blood cells prevent infection by killing bacteria and viruses, but with GVHD the cells see the patient tissue as foreign and attack the patient's cells. To prevent this, patients are tissue-typed by compiling a list of proteins on the cell's surface and matched to donors that have similar proteins, reducing the risk of immune rejection.

Scaffolding

Cells have to be added into the body on a support, called a scaffold. In your body, cells are naturally supported by a protein scaffold known as the extracellular matrix (**ECM**) to which all cells (apart from the circulatory cells, such as red blood cells) must attach for survival. It is essential for regenerative medicine to mimic the natural state in vivo by using biocompatible scaffolds.

The ECM is made of proteins including collagen that can control cell behaviour using 'cryptic sites' made of amino acids. Proteins consist of amino acids — and a specific sequence of amino acids (such as the tripeptide sequence arginine, glycine, aspartic acid) acts as a message to the cells. This tripeptide sequence tells the cells to attach to the ECM using receptor proteins expressed on their cell surfaces called **integrins**. Integrins act like sensors and find areas on the ECM to which they stick. When integrins recognise the tripeptide, they send a signal to the cell's nucleus (see Figure 1). The nucleus receives the information and initiates a response, such as making the cells become larger.

Stem cells

Once the scaffold has been made, the appropriate cell type has to coat the construct. But from where do we get the

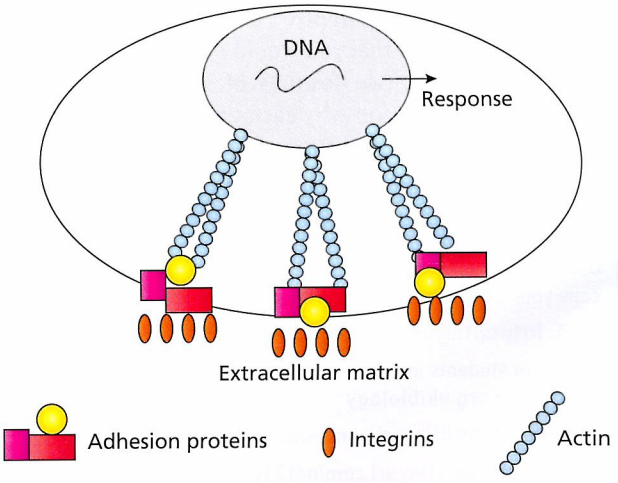


Figure 1 The cell uses integrins to adhere to the ECM proteins. The integrins recruit internal adhesion proteins and actin (the cell skeleton) to send a message to the nucleus. For stem cells, this message could be to differentiate

cells? We can use an allogenic transplant, where the donor cells come from a different person. However, this comes with a risk. If the cells from the donor and recipient aren't tissue matched, the body will reject the new organ unless the patient takes immunosuppressant medication. However, it may be possible to use cells taken from the patient's own body, a process known as autologous transplant. An autologous transplant is the preferable option. However, if an organ is needed with urgency, such as the heart, there would not be sufficient time to grow enough cells. To populate a synthetic organ you require a considerable number of cells. This is why researchers have turned to stem cells. Stem cells are specialised cells that can either replicate to form more of themselves (self-renew) or change (differentiate) into other cell types. This is known as symmetric or asymmetric division (see Figure 2a). Some stem cells have the potential to replicate themselves indefinitely, unlike the somatic cells in the rest of the body.

Embryonic stem cells are derived from an embryo. They have the capacity to form any cell type in the body (pluripotent) and can regenerate indefinitely. However, there are ethical issues to consider when using embryos. The use of embryonic stem cells is highly regulated and embryos are only used with appropriate consent.

Adult stem cells, for example mesenchymal stem cells (MSCs) found in the bone marrow, can differentiate into cartilage, bone or fat cells (see Figure 2b). They are multipotent, meaning they can only form certain cell types.

The 2012 Nobel prize for medicine was awarded to Shinya Yamanaka for the creation of induced pluripotent stem cells (iPSC). Skin cells from a mouse were genetically reprogrammed to become uncommitted or 'stem like'.

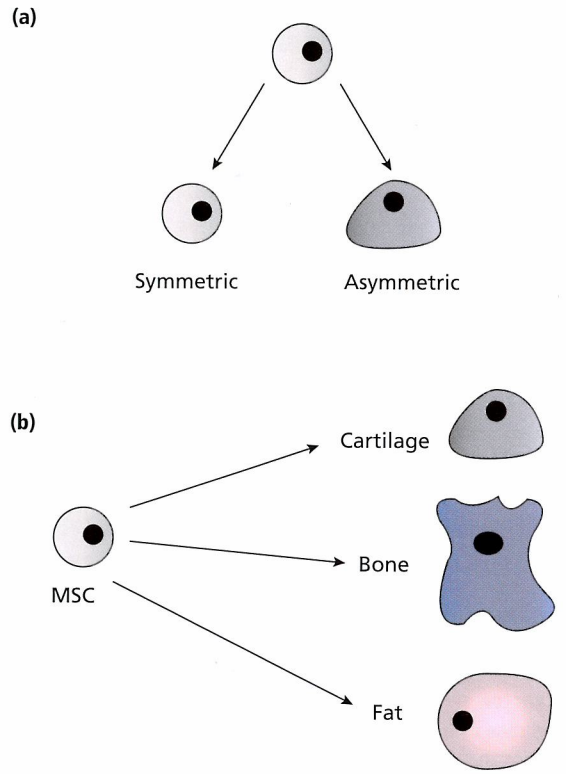


Figure 2 (a) A stem cell can either self renew (symmetric) or differentiate into another cell type (asymmetric division); **(b)** mesenchymal stem cells can differentiate into one of three cell types — cartilage, bone or fat.

Materials as scaffolds

Researchers have tried several ways to make an artificial scaffold support for cells. These include using the ECM proteins from the body that naturally act as a cell scaffold, or synthetic plastics or polymers that are bioinert, meaning that they do not cause a response in the body. The discovery of the bioinert properties of certain plastics was a chance discovery made by ophthalmologist Sir Harold Ridley during the Second World War. Spitfire cockpits were constructed with Perspex, which shattered under fire, spraying into the eyes and skin of airman Gordon Cleaver. However, the Perspex did not cause an immune response in the recipient. Indeed, his body functioned normally even with the plastic perforating his skin.

Natural proteins are rarely used in the laboratory owing to high costs of production, so bioinert artificial polymers are a better choice as they are cheaper and more reproducible, preventing variation between scaffolds. There are several techniques that can be used to make a scaffold:

- decellularised scaffold
- 3D printing
- hydrogels

Decellularised scaffold

Donor organs can have all of their cellular material removed using enzymes or chemical treatments, which leaves the ECM proteins in the exact shape of the organ. The patient's own cells can then be added, often using a **bioreactor** (a culture system containing nutrients and cells). This method avoids immune rejection, but it still requires donor organs.

3D printing

Using computer-aided design, a scaffold can be designed in the exact geometry of an organ and can even be tailored to the patient. Natural or synthetic polymers can be used. They are deposited in layers, resulting in a 3D scaffold in a defined shape to which cells can then be added using a bioreactor. It is also possible to print cells in layers, where cells are effectively used as the 'ink'. The technique is very versatile and has so far been used to print bones, an ear and a small section of liver (see Figure 3).

Hydrogels

Hydrogels consist of chains of polymers (natural or artificial) suspended in water. Depending on the type of polymer and the number of bonds it makes with other polymers (called crosslinking) the properties can be tailored to the tissue requirements. In addition, bioactive molecules can be added, including molecules that cause stem cells to differentiate. For example, bone morphogenetic protein (BMP) is a type of molecule that can make bone cells.

Materials to support stem cell growth

Once stem cells are removed from the body, they are no longer under the control of the body. This results in loss of stem cell characteristics. Many strategies have been developed to keep stem cells functioning as stem cells or to force stem cells to differentiate.

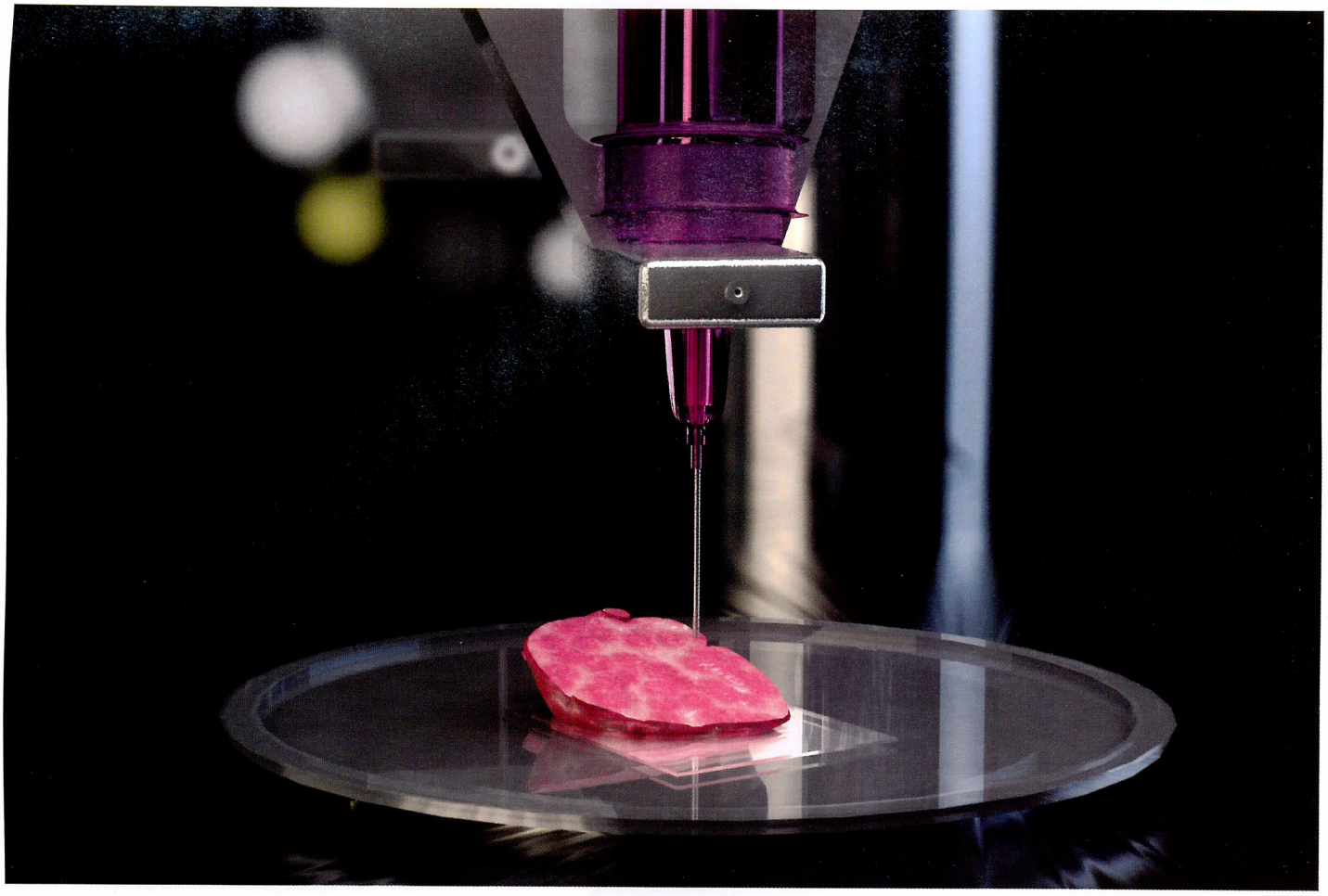


Figure 3 Bioprinting allows the deposition of cells or proteins in a defined manner in three dimensions. The technique is versatile and could be applied to build multiple organs for therapeutical applications

Changes in chemistry

Altering the chemical surface of a material can allow differentiation of stem cells to form defined cell types. For example, a layer of methyl groups ($-CH_3$) can keep MSCs as stem cells, whereas a layer of amino groups ($-NH_2$) can turn MSCs into bone cells. The chemical groups are added to the material by dip pen nanolithography (DPN), where the groups act as the 'ink' and the pen deposits them in a defined arrangement.

Stiffness

We can make soft or hard materials to mimic natural tissues, usually using hydrogels. The greater the number of crosslinks, the stiffer the gel (see Figure 4). MSCs can differentiate to form bone cells on hard surfaces where the cells spread out. Alternatively MSCs can differentiate to fat cells on soft surfaces where the cells round up.

Topography

Topography refers to features such as pits or pillars on the cell surface. They act as 'sticky features' and cells can grab on to them. By varying the spacing of the pits or pillars, we can influence stem cell self-renewal and differentiation.

Stimuli responsive

Alterations to chemistry, stiffness and topography can only provide stem cells with one instruction, either self-renewal or differentiation. Stimuli-responsive materials are a new generation of materials that are being designed to provide both of these instructions, using specific stimuli such as enzymes, temperature or light. The key here is that the material properties differ before and after the stimuli are applied.

Examples of tissue engineered organs

The airway

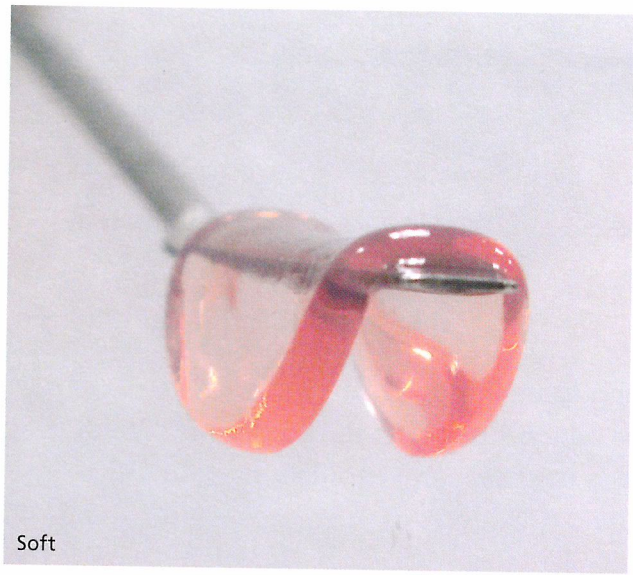
In 2012, a study in the medical journal *The Lancet* described a tracheal implant in a 2-year-old boy. The donor trachea was first decellularised, meaning all the cells were taken away, leaving the protein intact in the shape of a trachea. The patient's own bone marrow cells and epithelial cells were then added in the laboratory and the scaffold was implanted

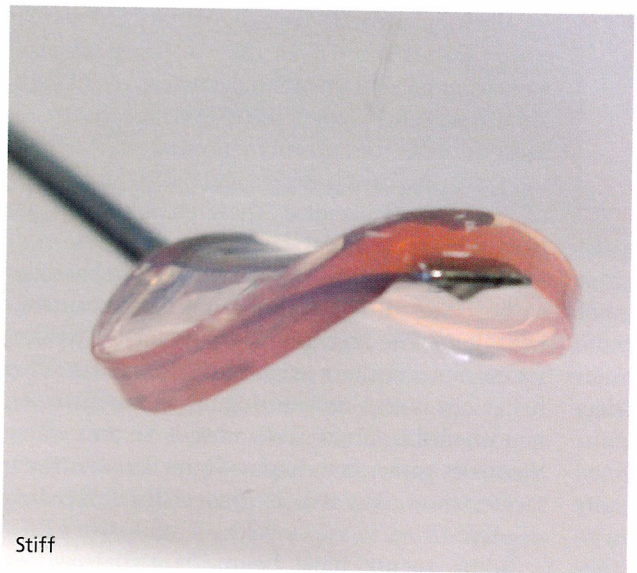
Terms explained

Bioreactor A vessel where a biological process is carried out. In the case of tissue engineering, this contains cells and the nutrients that keep cells alive.

ECM The extracellular matrix is made of proteins and provides a platform for cells to sit on in the body.

Integrins The part of the cell that grabs on to the ECM. Through integrin signalling, the cell can process the conditions in its environment.





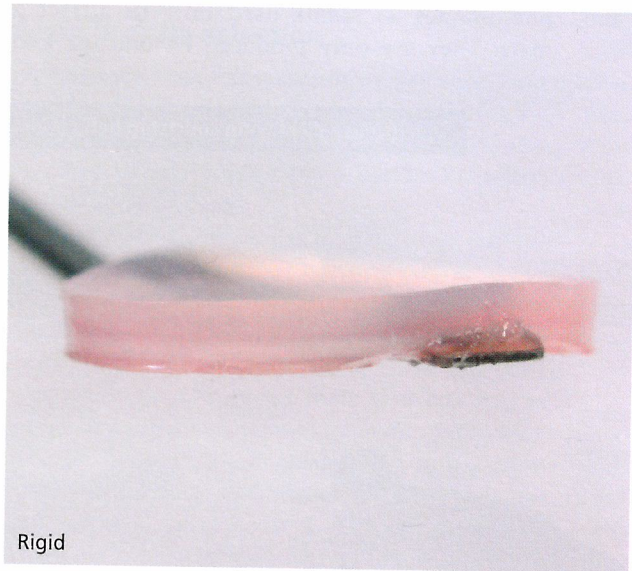


Figure 4 Hydrogels can be used to create scaffolds of different stiffness by the amount of crosslinking. Soft surfaces have fewer crosslinked proteins and can be used to differentiate mesenchymal stem cells to fat cells

Further reading

Sir Harold Ridley obituary: **https://tinyurl.com/mlnd52p**

'Can organs be purpose-grown using stem cells? Trust me, I'm a doctor, BBC 2: **https://tinyurl.com/m3o3jqe**

'The ABCs of organ engineering', Wake Forest School of Medicine: **https://tinyurl.com/kjowlqy**

Shinya Yamanaka — Facts: **https://tinyurl.com/zhlmba2**

Video — 3D printed organs for transplant: **www.youtube.com/watch?v=JpBy_T94TWw**

The Ott Lab: **http://ottlab.mgh.harvard.edu** and **http://ottlab.mgh.harvard.edu/?page_id=48**

into the patient. Over the course of 2 years, the cells colonised the trachea and became specialised trachea cells.

The bladder

The current method to replace a damaged or diseased bladder uses a section of the gastrointestinal tract (gut). This treatment is not a perfect solution and often leads to a series of complications such as infection and diseases including cancer. To address this problem researchers in North Carolina, USA, suggested using an artificial polymer scaffold to replace the damaged organ. The treatment was successful in that bladder function was improved.

The heart

Bioartificial hearts pose a real challenge to tissue engineers. There are multiple components involved in making a beating heart, and the architecture alone is complex (four chambers separated by valves). One research group in Boston, USA, using a rat model, decellularised the heart and then added cells to it using a bioreactor. The bioreactor had the added benefit that it simulated the natural pressure and flow of blood in the heart. This is called 'biomimicry' where the laboratory process mimics the natural process in the body. The results showed an increase in contraction of cells and cell survival.

The future

The field of tissue engineering is constantly evolving. Improvements are made at each stage, from the support of cells in vitro to the techniques to make scaffolds. Effective collaborations across all scientific fields result in innovative technologies that will hopefully one day be channelled into better therapeutics.

Points for discussion

- What are the advantages of tissue engineering?
- What are the benefits of 3D printing over using a decellularised organ?
- Is it ethically acceptable to use embryonic stem cells?

Hilary Anderson is a final year PhD student at the Centre for Cell Engineering at the University of Glasgow, @UofGCCE. Her project aims to use cell-secreted enzymes to reorganise a stimuli-responsive material.