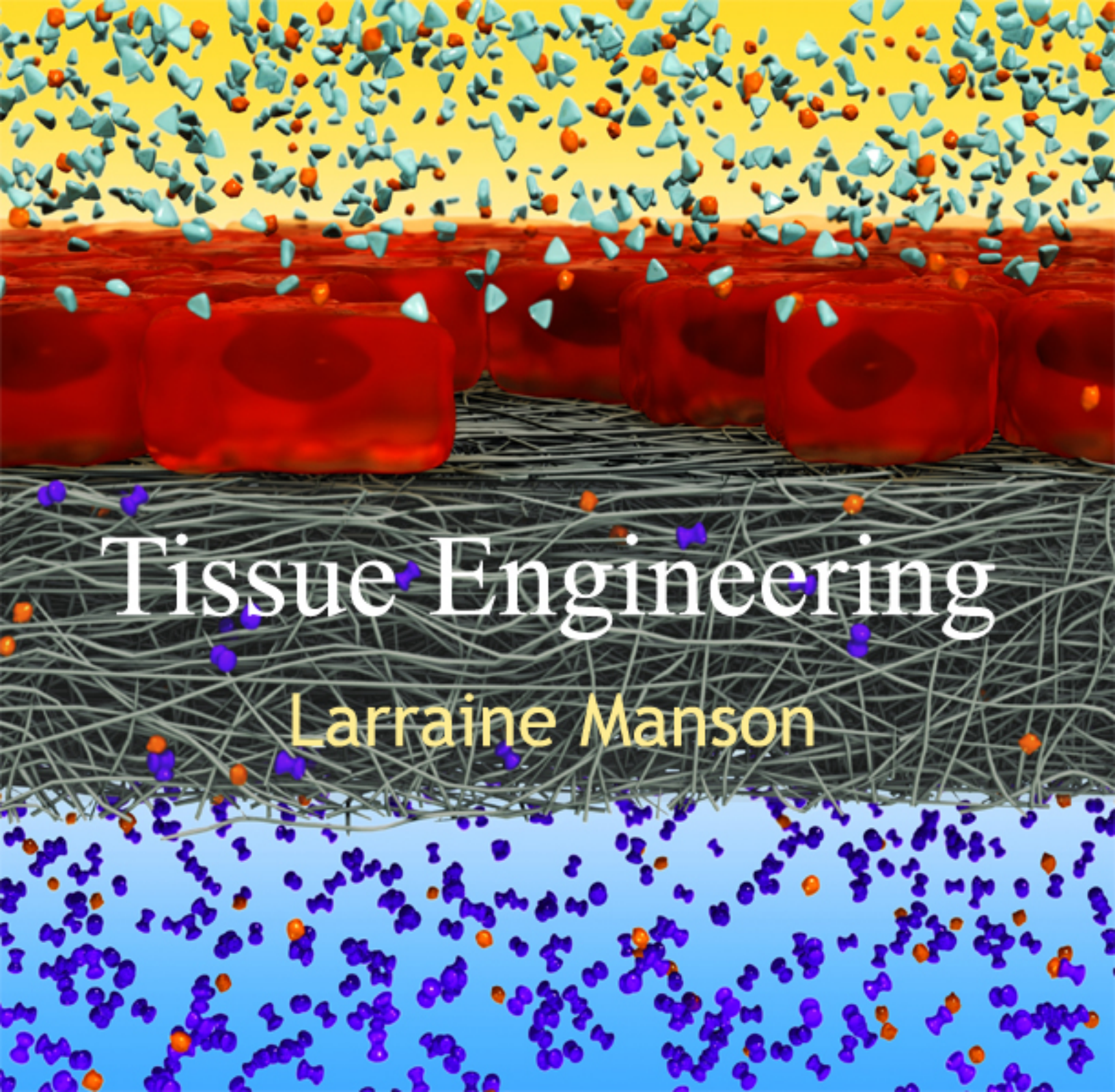
Tissue Engineering
Larraine Manson

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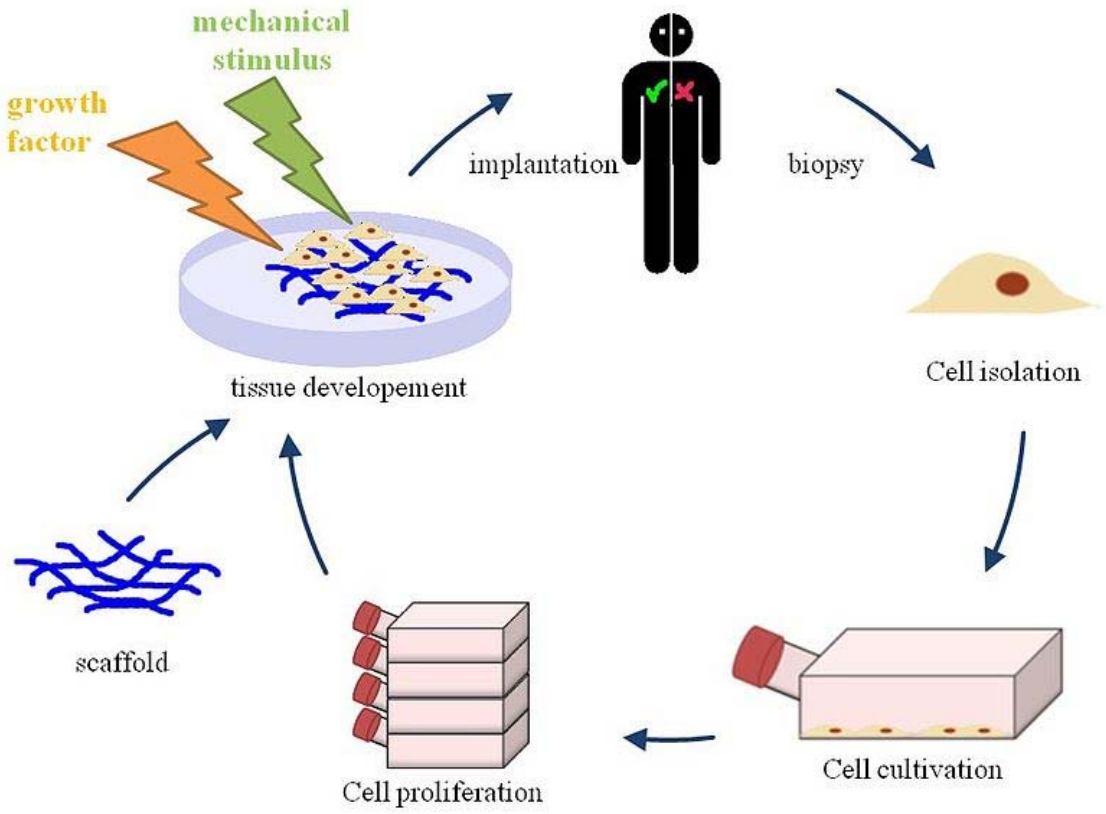
Introduction

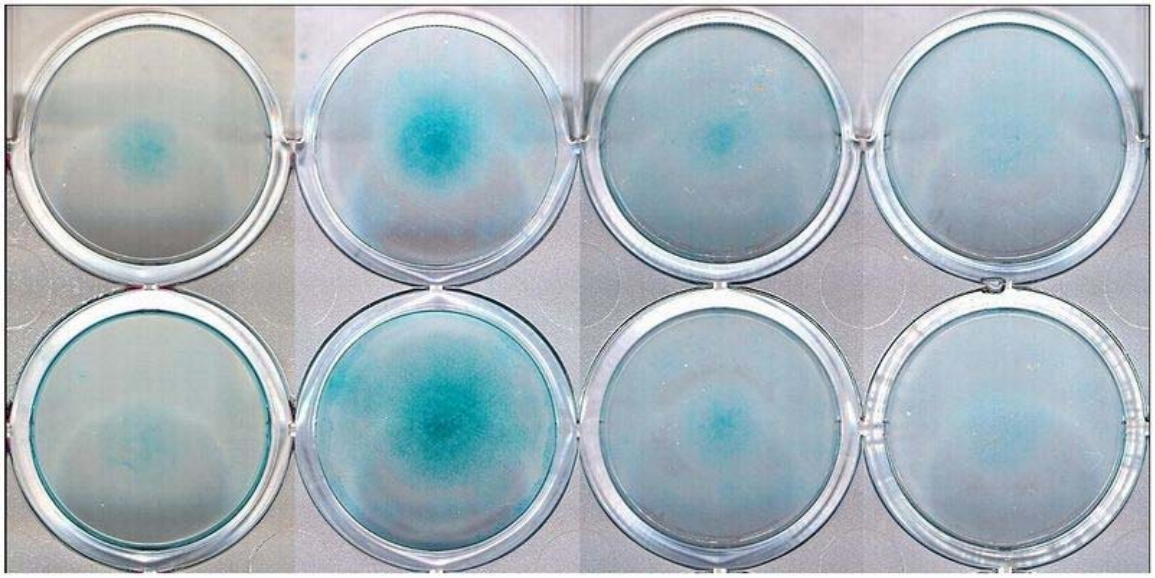


Principle of tissue engineering

Tissue engineering was once categorized as a sub-field of bio materials, but having grown in scope and importance it can be considered as a field in its own right. It is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions. While most definitions of tissue engineering cover a broad range of applications, in practice the term is closely associated with applications that repair or replace portions of or whole tissues (i.e., bone, cartilage, blood vessels, bladder, skin etc.). Often, the tissues involved require certain mechanical and structural properties for proper functioning. The term has also been applied to efforts to perform specific biochemical functions using cells

within an artificially-created support system (e.g. an artificial pancreas, or a bio artificial liver). The term **regenerative medicine** is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of stem cells to produce tissues.

Overview



Micro-mass cultures of C3H-10T1/2 cells at varied oxygen tensions stained with Alcian blue.

A commonly applied definition of tissue engineering, as stated by Langer and Vacanti, is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ". Tissue engineering has also been defined as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use." A further description goes on to say that an "underlying supposition of tissue engineering is that the employment of natural biology of the system will allow for greater success in developing therapeutic strategies aimed at the replacement, repair, maintenance, and/or enhancement of tissue function."

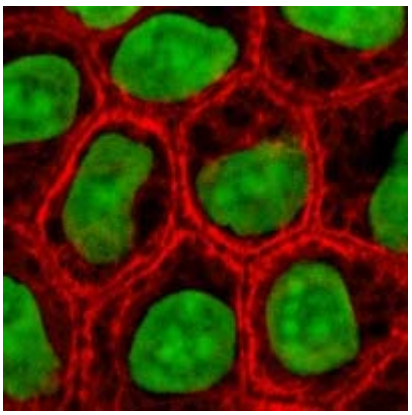
Powerful developments in the multidisciplinary field of tissue engineering have yielded a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. Among the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in laboratory-grown tissues destined for transplantation. The continued success of tissue engineering, and the eventual development of true human replacement parts, will grow from the convergence of engineering and basic research advances in tissue, matrix, growth factor, stem cell, and developmental biology, as well as materials science and bio informatics.

In 2003, the NSF published a report entitled "The Emergence of Tissue Engineering as a Research Field" , which gives a thorough description of the history of this field.

Examples

- Tissue engineered autologous heartvalves and vessels - workgroup of Dr.med.S.Jockenhoevel at the Department of Applied Medical Engineering (RWTH-Aachen University,Germany)
- In vitro meat — Edible artificial animal muscle tissue cultured *in vitro*.
- Bioartificial liver device — several research efforts have produced hepatic assist devices utilizing living hepatocytes.
- Artificial pancreas — research involves using islet cells to produce and regulate insulin, particularly in cases of diabetes.
- Artificial bladders — Anthony Atala (Wake Forest University) has successfully implanted artificially grown bladders into seven out of approximately 20 human test subjects as part of a long-term experiment.
- Cartilage — lab-grown tissue was successfully used to repair knee cartilage.
- Doris Taylor's heart in a jar
- Tissue-engineered airway
- Artificial skin constructed from human skin cells embedded in collagen
- Artificial bone marrow
- Artificial bone
- Artificial penis

Cells as building blocks



Stained cells in culture

Tissue engineering utilizes living cells as engineering materials. Examples include using living fibroblasts in skin replacement or repair, cartilage repaired with living chondrocytes, or other types of cells used in other ways.

Cells became available as engineering materials when scientists at Geron Corp. discovered how to extend telomeres in 1998, producing immortalized cell lines. Before this, laboratory cultures of healthy, noncancerous mammalian cells would only divide a fixed number of times, up to the Hayflick limit.

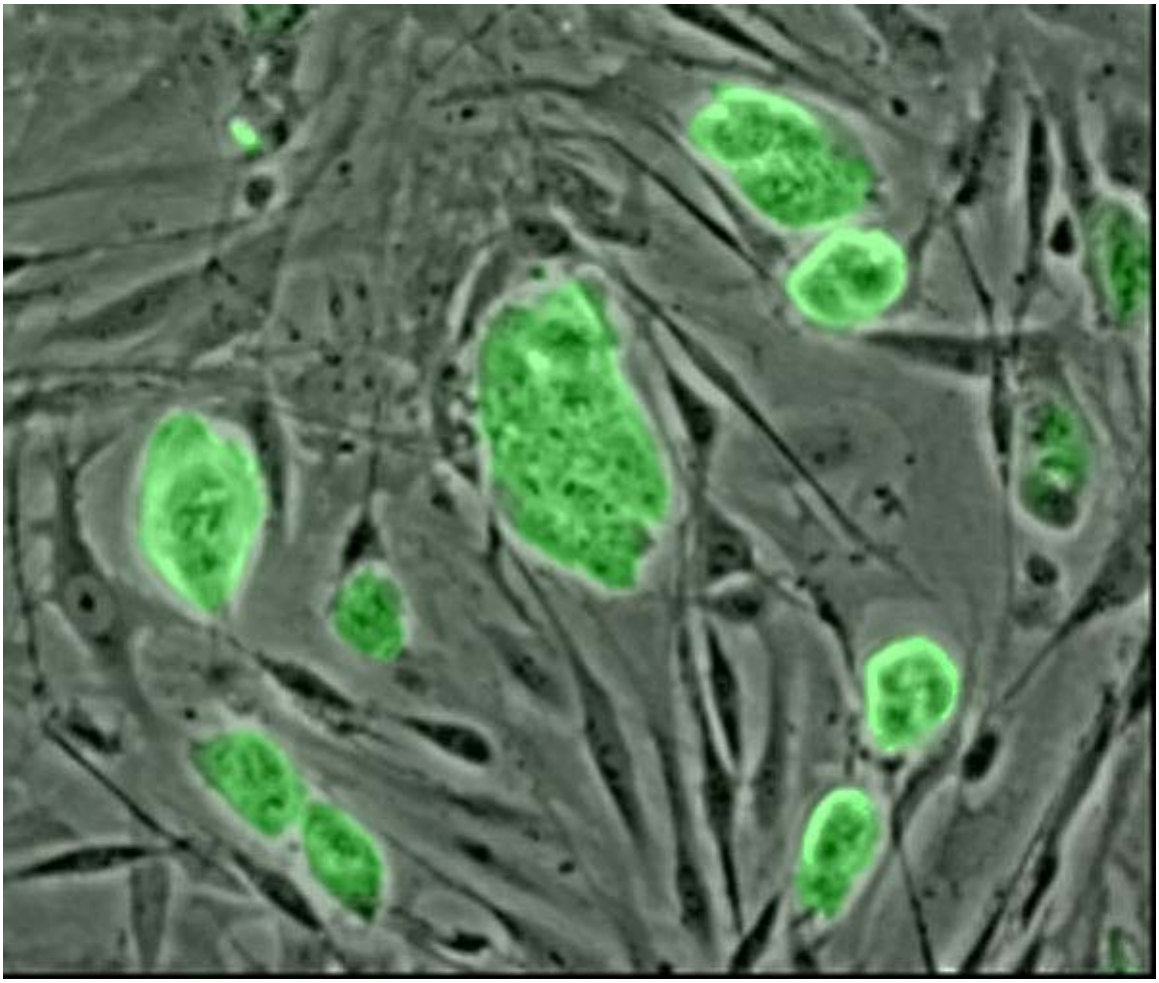
Extraction

From fluid tissues such as blood, cells are extracted by bulk methods, usually centrifugation or apheresis. From solid tissues, extraction is more difficult. Usually the tissue is minced, and then digested with the enzymes trypsin or collagenase to remove the extracellular matrix that holds the cells. After that, the cells are free floating, and extracted using centrifugation or apheresis.
Digestion with trypsin is very dependent on temperature. Higher temperatures digest the matrix faster, but create more damage. Collagenase is less temperature dependent, and damages fewer cells, but takes longer and is a more expensive reagent.

Types of cells

Cells are often categorized by their source:

- **Autologous** cells are obtained from the same individual to which they will be reimplanted. Autologous cells have the fewest problems with rejection and pathogen transmission, however in some cases might not be available. For example in genetic disease suitable autologous cells are not available. Also very ill or elderly persons, as well as patients suffering from severe burns, may not have sufficient quantities of autologous cells to establish useful cell lines. Moreover since this category of cells needs to be harvested from the patient, there are also some concerns related to the necessity of performing such surgical operations that might lead to donor site infection or chronic pain. Autologous cells also must be cultured from samples before they can be used: this takes time, so autologous solutions may not be very quick. Recently there has been a trend towards the use of mesenchymal stem cells from bone marrow and fat. These cells can differentiate into a variety of tissue types, including bone, cartilage, fat, and nerve. A large number of cells can be easily and quickly isolated from fat, thus opening the potential for large numbers of cells to be quickly and easily obtained.



Mouse embryonic stem cells. **More lab photos**

- **Allogeneic** cells come from the body of a donor of the same species. While there are some ethical constraints to the use of human cells for *in vitro* studies, the employment of dermal fibroblasts from human foreskin has been demonstrated to be immunologically safe and thus a viable choice for tissue engineering of skin.

- **Xenogenic** cells are these isolated from individuals of another species. In particular animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular implants.

- **Syngenic** or **isogenic** cells are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models.

- **Primary** cells are from an organism.

- **Secondary** cells are from a cell bank.

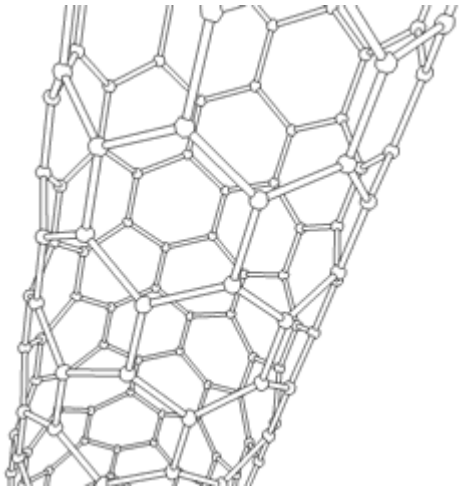
- **Stem cells** are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells. According to their source stem cells are divided into "adult" and "embryonic" stem cells, the first class being multipotent and the latter mostly pluripotent; some cells are totipotent, in the earliest stages of

the embryo. While there is still a large ethical debate related with the use of embryonic stem cells, it is thought that stem cells may be useful for the repair of diseased or damaged tissues, or may be used to grow new organs.

Scaffolds

Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation. These structures, typically called scaffolds, are often critical, both *ex vivo* as well as *in vivo*, to recapitulating the *in vivo* milieu and allowing cells to influence their own microenvironments. Scaffolds usually serve at least one of the following purposes:

- Allow cell attachment and migration
- Deliver and retain cells and biochemical factors
- Enable diffusion of vital cell nutrients and expressed products
- Exert certain mechanical and biological influences to modify the behaviour of the cell phase



Rotating Carbon nanotube shows its 3D structure. Carbon nanotubes are among the numerous candidates for tissue engineering scaffolds since they are biocompatible, resistant to biodegradation and can be functionalized with biomolecules. However, the possibility of toxicity with non-biodegradable nano-materials is not fully understood.

To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. Biodegradability is often an essential factor since scaffolds should preferably be absorbed by the surrounding tissues without the necessity of a surgical removal. The rate at which degradation occurs has to coincide as much as possible with the rate of tissue formation: this means that while cells are fabricating their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body and eventually it will break down leaving the neotissue, newly formed tissue which will take over the mechanical load. Injectability is also important for clinical uses. Recent research on organ printing is showing how crucial a good control of the 3D environment is to insure reproducibility of experiments and offer better results.

Materials

Many different materials (natural and synthetic, biodegradable and permanent) have been investigated. Most of these materials have been known in the medical field before the advent of tissue engineering as a research topic, being already employed as bioresorbable sutures. Examples of these materials are collagen and some polyesters.

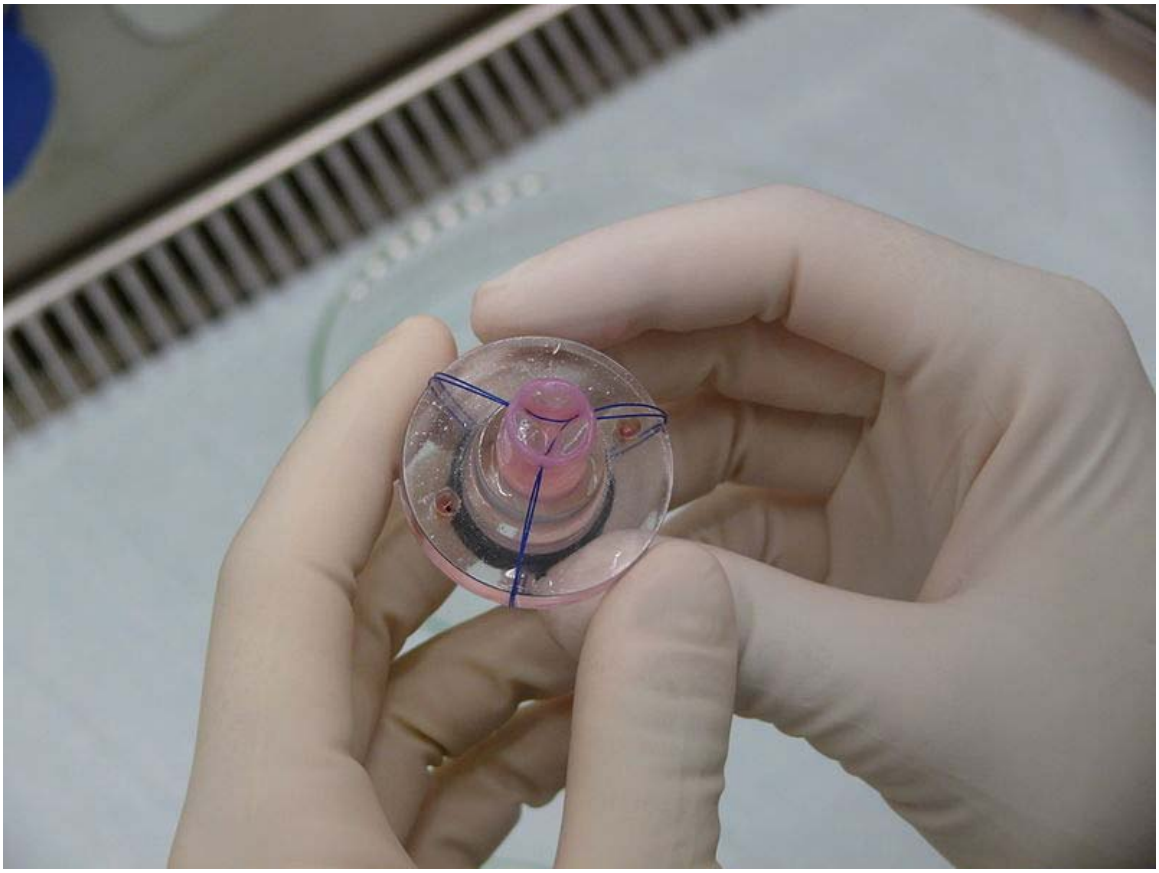
New biomaterials have been engineered to have ideal properties and functional customization: injectability, synthetic manufacture, biocompatibility, non-immunogenicity, transparency, nano-scale fibers, low concentration, resorption rates, etc. PuraMatrix, originating from the MIT labs of Zhang, Rich, Grodzinsky and Langer is one of these new biomimetic scaffold families which has now been commercialized and is impacting clinical tissue engineering.

A commonly used synthetic material is PLA - polylactic acid. This is a polyester which degrades within the human body to form lactic acid, a naturally occurring chemical which is easily removed from the body. Similar materials are polyglycolic acid (PGA) and polycaprolactone (PCL): their degradation mechanism is similar to that of PLA, but they exhibit respectively a faster and a slower rate of degradation compared to PLA.

Scaffolds may also be constructed from natural materials: in particular different derivatives of the extracellular matrix have been studied to evaluate their ability to support cell growth. Proteic materials, such as collagen or fibrin, and polysaccharidic materials, like chitosan or glycosaminoglycans (GAGs), have all proved suitable in terms of cell compatibility, but some issues with potential immunogenicity still remains. Among GAGs hyaluronic acid, possibly in combination with cross linking agents (e.g. glutaraldehyde, water soluble carbodiimide, etc...), is one of the possible choices as scaffold material. Functionalized groups of scaffolds may be useful in the delivery of small molecules (drugs) to specific tissues. Another form of scaffold under investigation is decellularised tissue extracts whereby the remaining cellular remnants/extracellular matrices act as the scaffold .

Synthesis

tissue engineered vascular graft



tissue engineered heart valve

A number of different methods have been described in literature for preparing porous structures to be employed as tissue engineering scaffolds. Each of these techniques presents its own advantages, but none are free of drawbacks.

- **Nanofiber Self-Assembly**: Molecular self-assembly is one of the few methods for creating biomaterials with properties similar in scale and chemistry to that of the natural in vivo extracellular matrix (ECM). Moreover, these hydrogel scaffolds have shown superiority in in vivo toxicology and biocompatibility compared to traditional macroscaffolds and animal-derived materials.

- **Textile technologies**: These techniques include all the approaches that have been successfully employed for the preparation of non-woven meshes of different polymers. In particular, non-woven polyglycolide structures have been tested for tissue engineering applications: such fibrous structures have been found useful to grow different types of cells. The principal drawbacks are related to the difficulties in obtaining high porosity and regular pore size.

- **Solvent Casting & Particulate Leaching (SCPL)**: This approach allows for the preparation of porous structures with regular porosity, but with a limited thickness. First, the polymer is dissolved into a suitable organic solvent (e.g. polylactic acid could be dissolved into dichloromethane), then the solution is cast into a mold filled with porogen particles. Such porogen can be an inorganic salt like sodium chloride, crystals of saccharose, gelatin spheres or paraffin spheres.

The size of the porogen particles will affect the size of the scaffold pores, while the polymer to porogen ratio is directly correlated to the amount of porosity of the final structure. After the polymer solution has been cast the solvent is allowed to fully evaporate, then the composite structure in the mold is immersed in a bath of a liquid suitable for dissolving the porogen: water in the case of sodium chloride, saccharose and gelatin or an aliphatic solvent like hexane for use with paraffin. Once the porogen has been fully dissolved, a porous structure is obtained. Other than the small thickness range that can be obtained, another drawback of SCPL lies in its use of organic solvents which must be fully removed to avoid any possible damage to the cells seeded on the scaffold.

- **Gas Foaming**: To overcome the need to use organic solvents and solid porogens, a technique using gas as a porogen has been developed. First, disc-shaped structures made of the desired polymer are prepared by means of compression molding using a heated mold. The discs are then placed in a chamber where they are exposed to high pressure CO_2 for several days. The pressure inside the chamber is gradually restored to atmospheric levels. During this procedure the pores are formed by the carbon dioxide molecules that abandon the polymer, resulting in a sponge-like structure. The main problems resulting from such a technique are caused by the excessive heat used during compression molding (which prohibits the incorporation of any temperature labile material into the polymer matrix) and by the fact that the pores do not form an interconnected structure.

- **Emulsification/Freeze-drying**: This technique does not require the use of a solid porogen like SCPL. First, a synthetic polymer is dissolved into a suitable solvent (e.g. polylactic acid in dichloromethane) then water is added to the polymeric solution and the two liquids are mixed in order to obtain an emulsion. Before the two phases can separate, the emulsion is cast into a mold and quickly frozen by means of immersion into liquid nitrogen. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure. While emulsification and freeze-drying allow for a faster preparation when compared to SCPL (since it does not require a time consuming leaching step), it still requires the use of solvents. Moreover, pore size is relatively small and porosity is often irregular. Freeze-drying by itself is also a commonly employed technique for the fabrication of scaffolds. In particular, it is used to prepare collagen sponges: collagen is dissolved into acidic solutions of acetic acid or hydrochloric acid that are cast into a mold, frozen with liquid nitrogen and then lyophilized.

- **Thermally Induced Phase Separation (TIPS)**: Similar to the previous technique, this phase separation procedure requires the use of a solvent with a low melting point that is easy to sublime. For example dioxane could be used to dissolve polylactic acid, then phase separation is induced through the addition of a small quantity of water: a polymer-rich and a polymer-poor phase are formed. Following cooling below the solvent melting point and some days of vacuum-drying to sublime the solvent, a porous scaffold is obtained. Liquid-liquid phase separation presents the same drawbacks of emulsification/freeze-drying.

- **Electrospinning**: A highly versatile technique that can be used to produce continuous fibers from submicron to nanometer diameters. In a typical electrospinning set-up, a solution is fed through a spinneret and a high voltage is applied to the tip. The buildup of electrostatic repulsion within the charged solution, causes it to eject a thin fibrous stream. A mounted collector plate or rod with an opposite or grounded charge draws in the continuous fibers, which arrive to form a highly porous network. The primary advantages of this technique are its simplicity and ease of variation. At a laboratory level, a typical electrospinning set-up only requires a high voltage power supply (up to 30 kV), a syringe, a flat tip needle and a conducting collector. By modifying variables such as the distance to collector, magnitude of applied voltage, or solution flow rate—researchers can dramatically change the overall scaffold architecture.

- **CAD/CAM Technologies**: Because most of the above techniques are limited when it comes to the control of porosity and pore size, computer assisted design and manufacturing techniques have been introduced to tissue engineering. First, a three-dimensional structure is designed using CAD software, then the scaffold is realized by using ink-jet printing of polymer powders or through Fused Deposition Modeling of a polymer melt.

Assembly methods

One of the continuing, persistent problems with tissue engineering is mass transport limitations. Engineered tissues generally lack an initial blood supply, thus making it difficult for any implanted cells to obtain sufficient oxygen and nutrients to survive, and/or function properly.

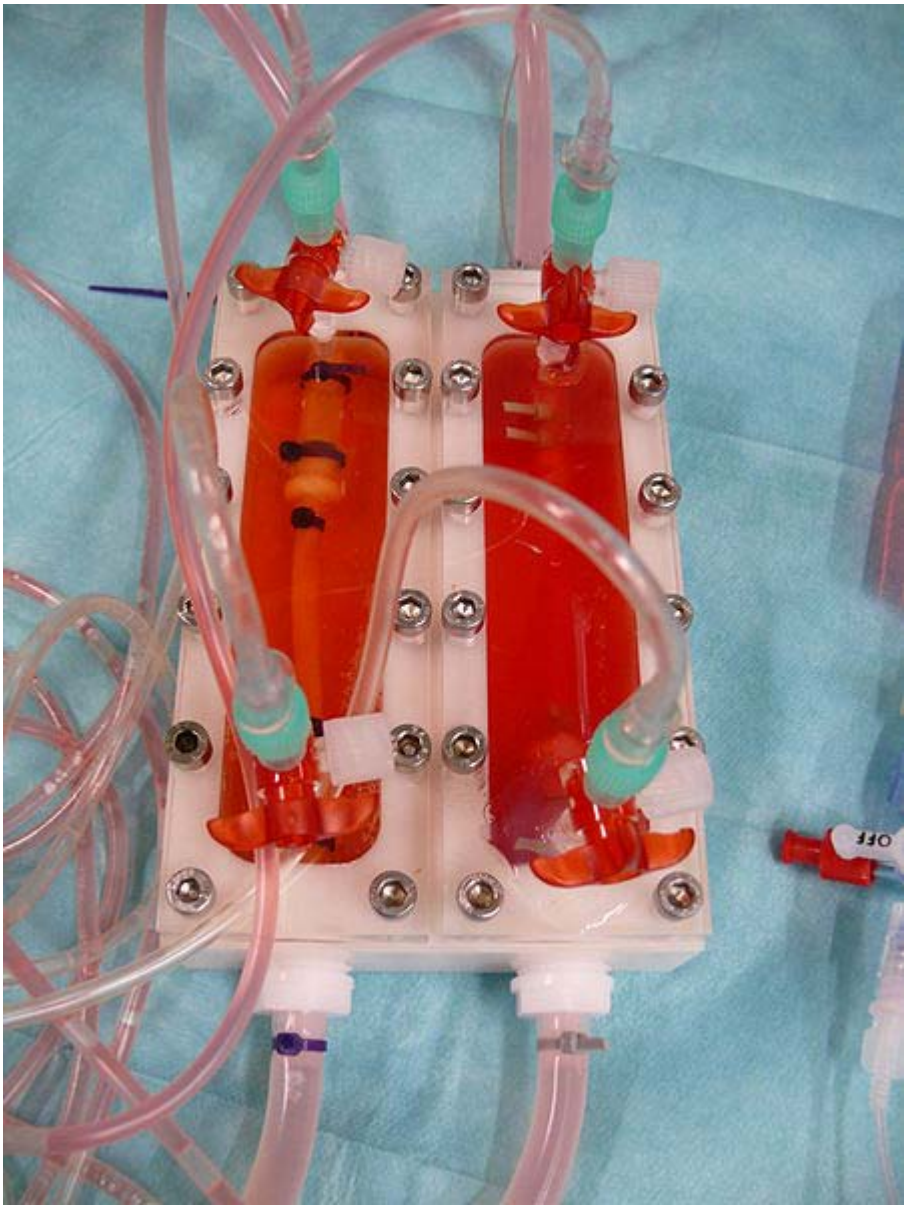
Self-assembly may play an important role here, both from the perspective of encapsulating cells and proteins, as well as creating scaffolds on the right physical scale for engineered tissue constructs and cellular ingrowth.

It might be possible to print organs, or possibly entire organisms. A recent innovative method of construction uses an ink-jet mechanism to print precise layers of cells in a matrix of thermoreversable gel. Endothelial cells, the cells that line blood vessels, have been printed in a set of stacked rings. When incubated, these fused into a tube.

Tissue culture

In many cases, creation of functional tissues and biological structures *in vitro* requires extensive culturing to promote survival, growth and inducement of functionality. In general, the basic requirements of cells must be maintained in culture, which include oxygen, pH, humidity, temperature, nutrients and osmotic pressure maintenance.

Tissue engineered cultures also present additional problems in maintaining culture conditions. In standard cell culture, diffusion is often the sole means of nutrient and metabolite transport. However, as a culture becomes larger and more complex, such as the case with engineered organs and whole tissues, other mechanisms must be employed to maintain the culture, such as the creation of capillary networks within the tissue.



bioreactor for cultivation of vascular grafts

Another issue with tissue culture is introducing the proper factors or stimuli required to induce functionality. In many cases, simple maintenance culture is not sufficient. Growth factors, hormones, specific metabolites or nutrients, chemical and physical stimuli are sometimes required. For example, certain cells respond to changes in oxygen tension as part of their normal development, such as chondrocytes, which must adapt to low oxygen conditions or hypoxia during skeletal development. Others, such as endothelial cells, respond to shear stress from fluid flow, which is encountered in blood vessels. Mechanical stimuli, such as pressure pulses seem to be beneficial to all kind of cardiovascular tissue such as heart valves, blood vessels or pericardium.

Bioreactors

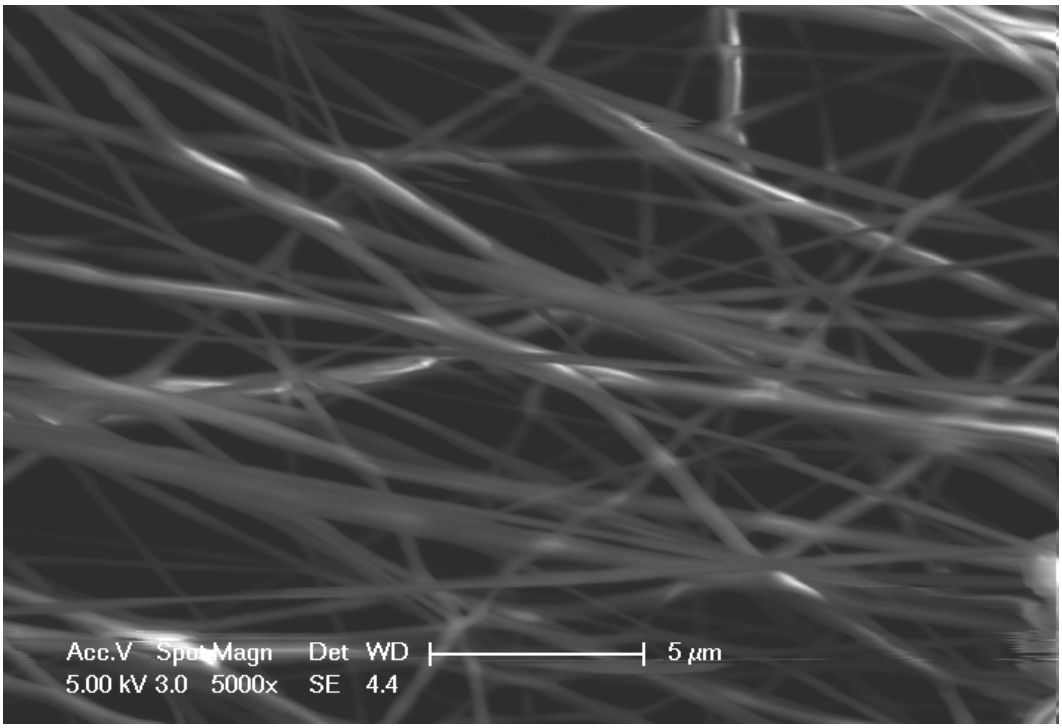
A bioreactor in tissue engineering, as opposed to industrial bioreactors, is a device that attends to simulate a physiological environment in order to promote cell or tissue growth in vivo. A physiological environment can consist of many different parameters such as temperature and oxygen or carbon dioxide concentration, but can extend to all kinds of biological, chemical or mechanical stimuli. Therefore, there are systems that may include the application of forces or stresses to the tissue or even of electrical current in two- or three-dimensional setups.

In academic and industry research facilities, it is typical for bioreactors to be developed to replicate the specific physiological environment of the tissue being grown (e.g., flex and fluid shearing for heart valve growth). Several general-use and application-specific bioreactors are also commercially available, and may provide static chemical stimulation or combination of chemical and mechanical stimulation.

Chapter 1

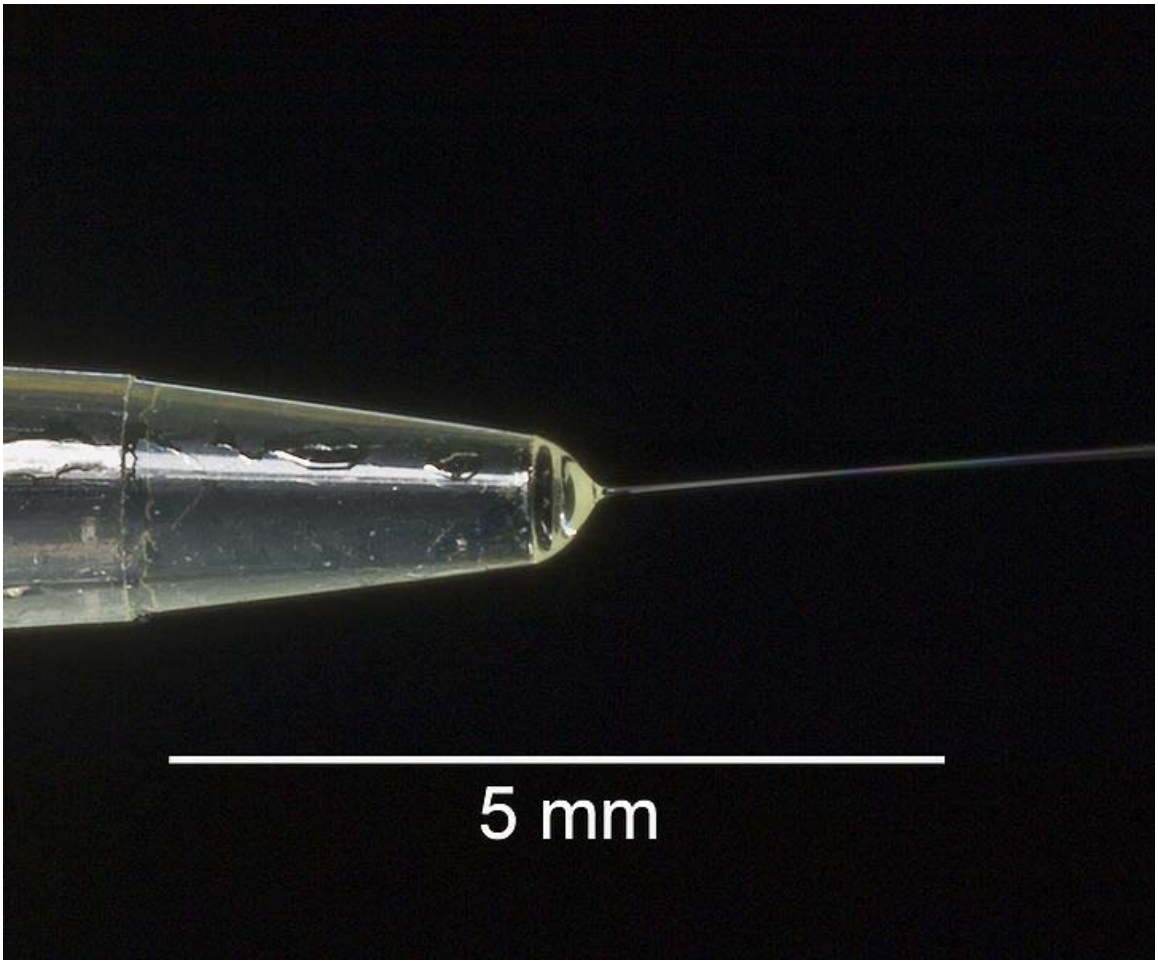
Electrospinning

Electrospinning uses an electrical charge to draw very fine (typically on the micro or nano scale) fibres from a liquid. Electrospinning shares characteristics of both electrospraying and conventional solution dry spinning of fibers. The process is non-invasive and does not require the use of coagulation chemistry or high temperatures to produce solid threads from solution. This makes the process particularly suited to the production of fibres using large and complex molecules. Electrospinning from molten precursors is also practiced; this method ensures that no solvent can be carried over into the final product.



Scanning electron microscope picture of electrospun polycaprolactone fibers.

Process



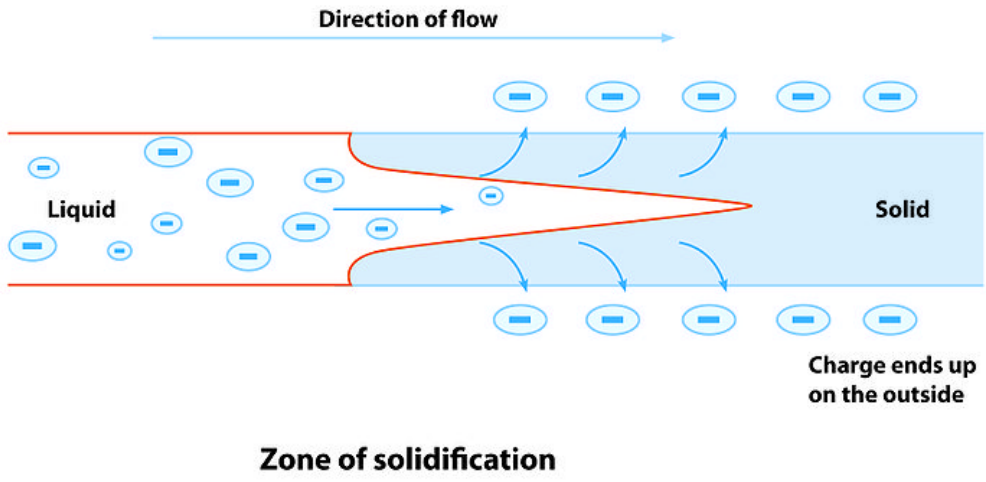
Photograph of a meniscus of polyvinyl alcohol in aqueous solution showing a fibre being electrospun from a Taylor cone.

When a sufficiently high voltage is applied to a liquid droplet, the body of the liquid becomes charged, and electrostatic repulsion counteracts the surface tension and droplet is stretched, at a critical point a stream of liquid erupts from the surface. This point of eruption is known as the Taylor cone (Figure 1).

If the molecular cohesion of the liquid is sufficiently high, stream breakup does not occur (if it does, droplets are electrosprayed) and a charged liquid jet is formed.

As the jet dries in flight (Figure 2), the mode of current flow changes from ohmic to convective as the charge migrates to the surface of the fibre. The jet is then elongated by a whipping process caused by electrostatic repulsion initiated at small bends in the fibre, until it is finally deposited on the grounded collector.

The elongation and thinning of the fibre resulting from this bending instability leads to the formation of uniform fibres with nanometer-scale diameters.



How the distribution of charge in the fibre changes as the fibre dries during flight

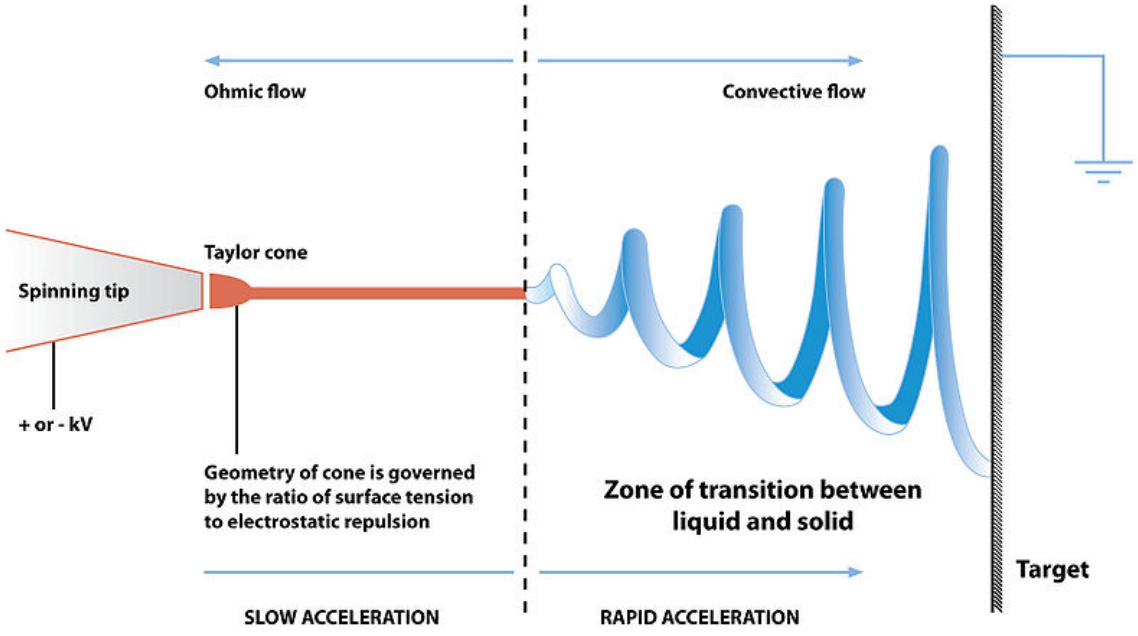


Diagram showing fibre formation by electrospinning

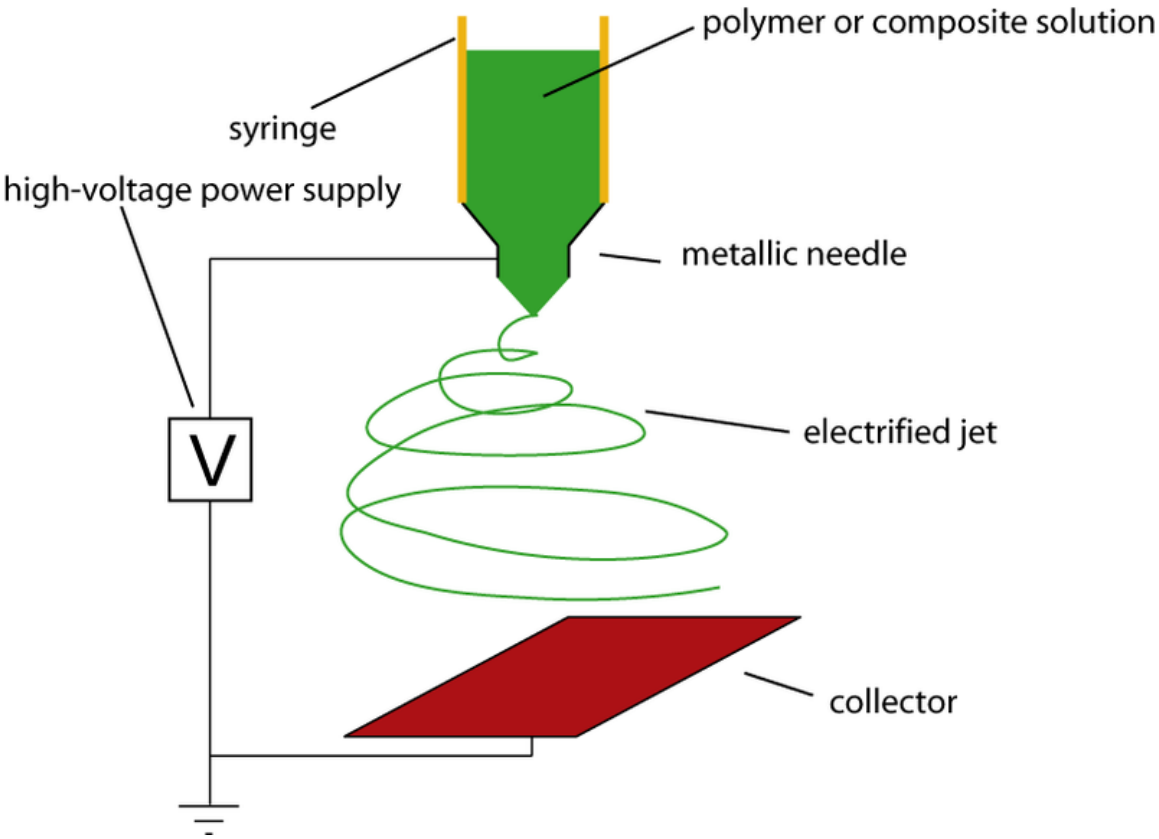
Parameters

1. Molecular Weight, Molecular-Weight Distribution and Architecture (branched, linear etc.) of the polymer
2. Solution properties (viscosity, conductivity & and surface tension)
3. Electric potential, Flow rate & Concentration
4. Distance between the capillary and collection screen

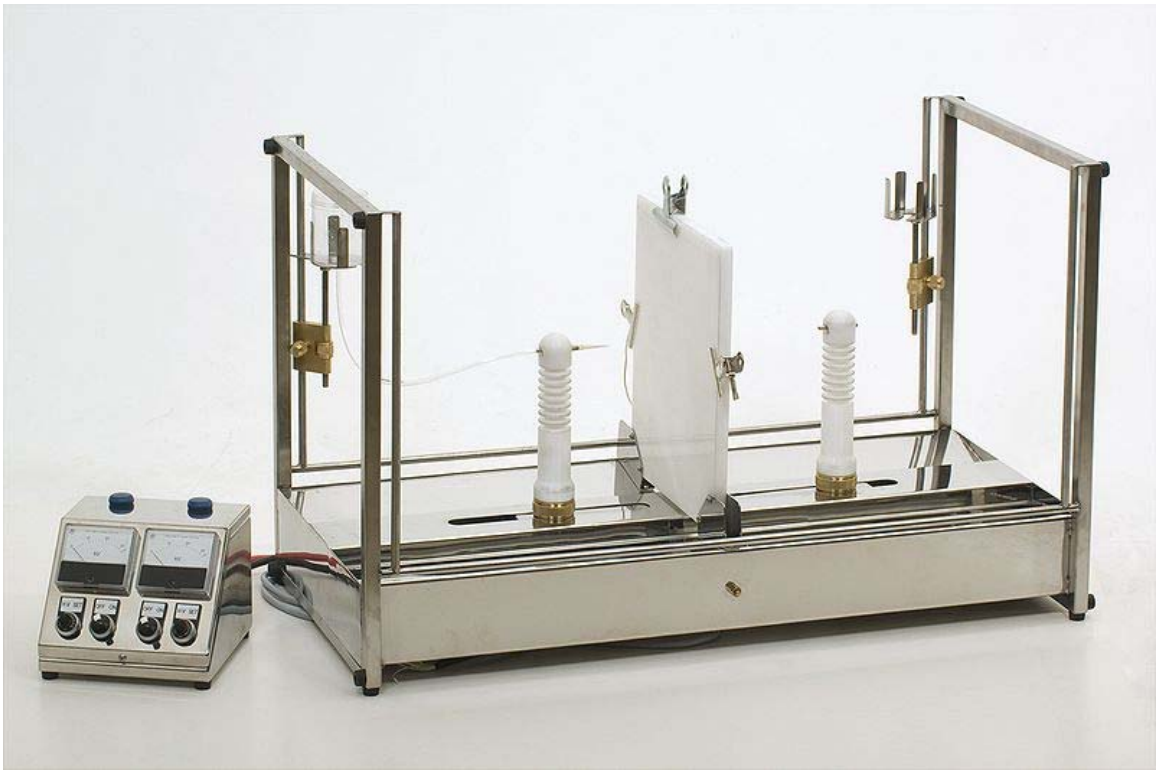
5. Ambient parameters (temperature, humidity and air velocity in the chamber)
6. Motion of target screen (collector)

Apparatus

The standard laboratory setup for electrospinning consists of a spinneret (typically a hypodermic syringe needle) connected to a high-voltage (5 to 50 kV) direct current power supply, a syringe pump, and a grounded collector. A polymer solution, sol-gel, particulate suspension or melt is loaded into the syringe and this liquid is extruded from the needle tip at a constant rate by a syringe pump. Alternatively, the droplet at the tip of the spinneret can be replenished by feeding from a header tank providing a constant feed pressure (figure 3). This constant pressure type feed works better for lower viscosity feedstocks.



Schematic of an electrospinning setup, shown without a syringe pump.



A constant pressure laboratory electrospinning machine (set up for horizontal fibre production)

History

In the late 16th century William Gilbert set out to describe the behaviour of magnetic and electrostatic phenomena. He observed that when a suitably electrically charged piece of amber was brought near a droplet of water it would form a cone shape and small droplets would be ejected from the tip of the cone: this is the first recorded observation of electrospraying.

The process of electrospinning was patented by J.F Cooley in February 1902 (U.S. Patent 692,631) and by W.J. Morton in July 1902 (U.S. Patent 0,705,691.

In 1914 John Zeleny, published work on the behaviour of fluid droplets at the end of metal capillaries. His effort began the attempt to mathematically model the behaviour of fluids under electrostatic forces.

Further developments toward commercialisation were made by Anton Formhals, and described in a sequence of patents from 1934 (U.S. Patent 1,975,504) to 1944 (U.S. Patent 2,349,950) for the fabrication of textile yarns. Electrospinning from a melt rather than a solution was patented by C.L Norton in 1936 (U.S. Patent 2,048,651) using an air-blast to assist fibre formation.

In 1938 N.D Rozenblum and I.V Petryanov-Sokolov, working in Prof. N.A. Fuks' group at the Aerosol Laboratory of the L.Ya Karpov Institute in the USSR, generated electrospun fibres , which they developed into filter materials known as "Petryanov

filters". By 1939, this work had led to the establishment of a factory in Tver' for the manufacture of electrospun smoke filter elements for gas masks. The material, dubbed BF (Battlefield Filter) was spun from cellulose acetate in a solvent mixture of dichloroethane and ethanol. By the 1960s output of spun filtration material was claimed as 20 million m^2 per annum

Between 1964 and 1969 Sir Geoffrey Ingram Taylor produced the theoretical underpinning of electrospinning. Taylor's work contributed to electrospinning by mathematically modelling the shape of the cone formed by the fluid droplet under the effect of an electric field; this characteristic droplet shape is now known as the Taylor cone. He further worked with J. R. Melcher to develop the "leaky dielectric model" for conducting fluids.

In the early 1990s several research groups (notably that of Reneker and Rutledge who popularised the name *electrospinning* for the process) demonstrated that many organic polymers could be electrospun into nanofibers. Since then, the number of publications about electrospinning has been increasing exponentially every year.

Since 1995 there have been further theoretical developments of the driving mechanisms of the electrospinning process. Reznik *et al.* describes extensive work on the shape of the Taylor cone and the subsequent ejection of a fluid jet. Work by Hohman *et al.* investigates the relative growth rates of the numerous proposed instabilities in an electrically forced jet once in flight and endeavours to describe the most important instability to the electrospinning process, the bending (whipping) instability.

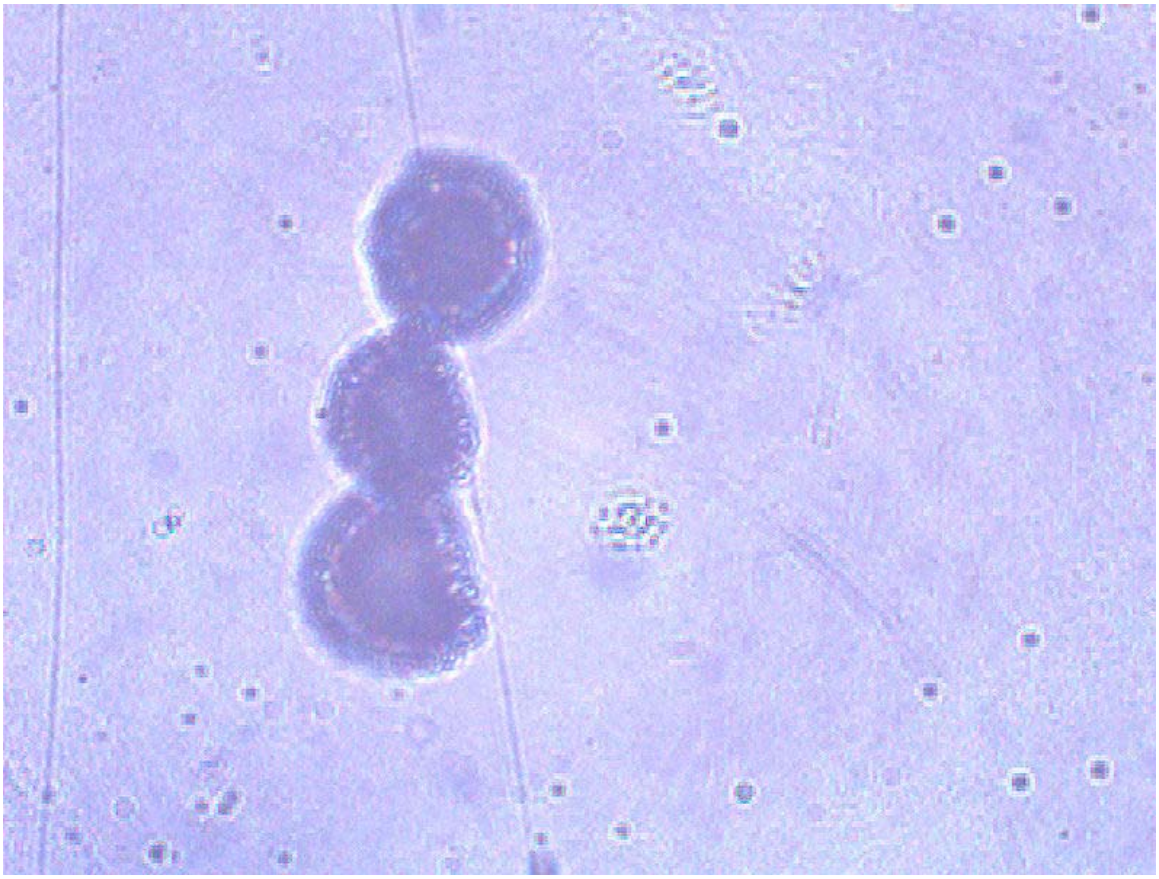
Uses

The size of an electrospun fiber can be in the nano scale and the fibres may possess nanoscale surface texture, leading to different modes of interaction with other materials compared with macroscale materials. In addition to this, the ultrafine fibres produced by electrospinning are expected to have two main properties, a very high surface to volume ratio, and a relatively defect free structure at the molecular level. This first property makes electrospun material suitable for activities requiring a high degree of physical contact, such as providing sites for chemical reactions, or the capture of small sized particulate material by physical entanglement - filtration. The second property should allow electrospun fibres to approach the theoretical maximum strength of the spun material, opening up the possibility of making high mechanical performance composite materials.

Filtration

The use of nanofiber webs as a filtering medium is well established. Due to the small size of the fibres London-Van Der Waals forces are an important method of adhesion between the fibres and the captured materials. Polymeric nanofibers have been used in air filtration applications for more than seven decades. Due to poor bulk mechanical properties of thin nanowebs, they are laid over a filtration medium substrate. The small fiber diameters cause slip flows at fiber surfaces, causing an increase in the interception and inertial impaction efficiencies of these composite filter media. The enhanced filtration efficiency at the same pressure drop is possible with fibers having diameters

less than 0.5 micrometre. Since the essential properties of protective clothing are high moisture vapor transport, increased fabric breathability, and enhanced toxic chemical resistance, electrospun nanofiber membranes are good candidates for these applications.



Lycopodium club moss spores (diameter about 60 micrometres) captured on an electrospun polyvinyl alcohol fibre

Textile manufacturing

The majority of early patents for electrospinning were for textile applications, however little woven fabric was actually produced, perhaps due to difficulties in handling the barely visible fibres. However, electrospinning has the potential to produce seamless non-woven garments by integrating advanced manufacturing with fibre electrospinning. This would introduce multi-functionality (flame, chemical, environmental protection) by blending fibers into electrospinlaced (using electrospinning to combine different fibers and coatings to form three dimensional shapes, such as clothing) layers in combination with polymer coatings.

Medical

1. Artificial organ components
2. Tissue engineering
3. Implant materials
4. Drug delivery

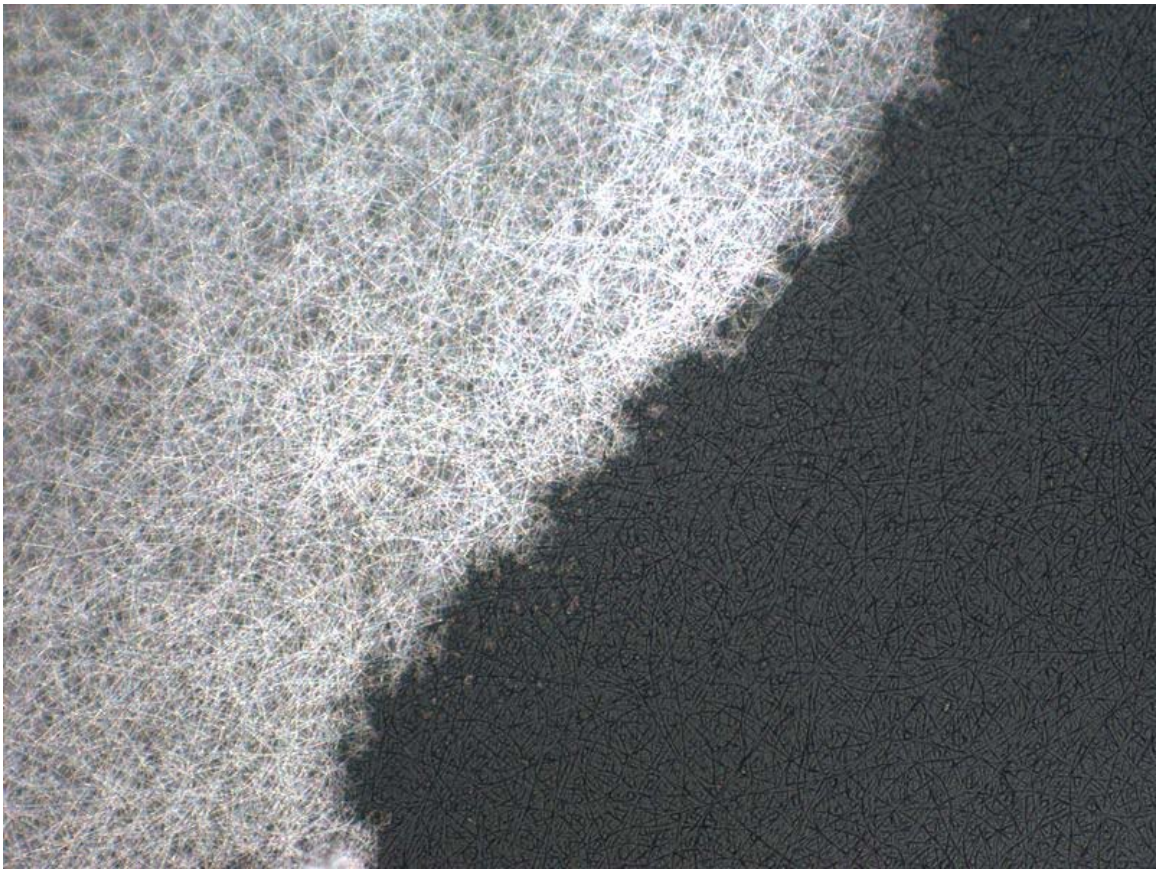
5. Wound dressing
6. Medical textile materials

Composites

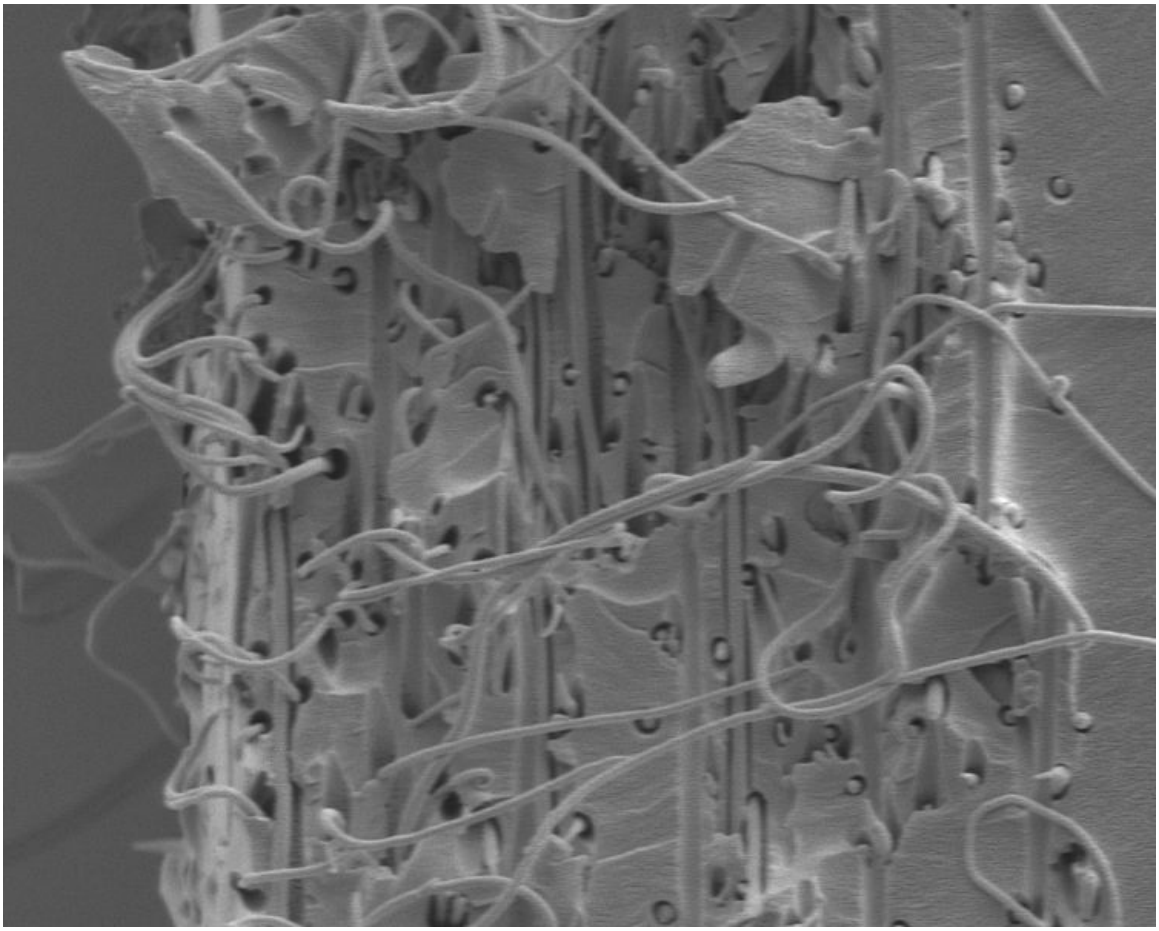
Ultrafine electrospun fibres show clear potential for the manufacture of long fibre composite materials.

Application is limited by difficulties in making sufficient quantities of fibre to make substantial large scale articles in a reasonable time scale. For this reason medical applications requiring relatively small amounts of fibre are a popular area of application for electrospun fibre reinforced materials.

Electrospinning is hence being investigated as a source of cost-effective, easy to manufacture wound dressings, medical implants, and scaffolds for the production of artificial human tissues. These scaffolds fulfil a similar purpose as the extracellular matrix in natural tissue. Biodegradable polymers, such as polycaprolactone, are typically used for this purpose. These fibers may then be coated with collagen to promote cell attachment, although collagen has successfully been spun directly into membranes.



Light microscope picture of epoxy resin impregnating an electrospun polyvinyl alcohol reinforcing fiber mat



SEM picture of the fracture surface of a Polyvinyl alcohol long fibre - epoxy matrix composite - the section thickness is about 12 micrometres

Catalysts

Electrospun fibers may have potential as a surface for enzymes to be immobilized on. These enzymes could be used to break down toxic chemicals in the environment, among other things.

Chapter 2

Soft Tissue

In anatomy, the term **soft tissue** refers to tissues that connect, support, or surround other structures and organs of the body, not being bone. Soft tissue includes tendons, ligaments, fascia, skin, fibrous tissues, fat, and synovial membranes (which are connective tissue), and muscles, nerves and blood vessels (which are not connective tissue).

It is sometimes defined by what it is not. For example, soft tissue has been defined as "nonepithelial, extraskeletal mesenchyme exclusive of the reticuloendothelial system and glia".

Composition

The characteristic substances inside the extracellular matrix of this kind of tissue are the collagen, elastin and ground substance. Normally the soft tissue is very hydrated because of the ground substance. The fibroblasts are the most common cell responsible for the production of soft tissues' fibers and ground substance. Variations of fibroblasts, like chondroblasts, may also produce these substances .

Mechanical Characteristics

At small strains, elastin confers stiffness to the tissue and stores most of the strain energy. The collagen fibers are comparatively inextensible and are usually loose (wavy,crimped). With increasing tissue deformation the collagen is gradually stretched in the direction of deformation. When taut, these fibers produce a strong growth in tissue stiffness. The composite behavior is analogous to a nylon stocking, whose rubber band does the role of elastin as the nylon does the role of collagen. In soft tissues the collagen limits the deformation and protects the tissues from injury.

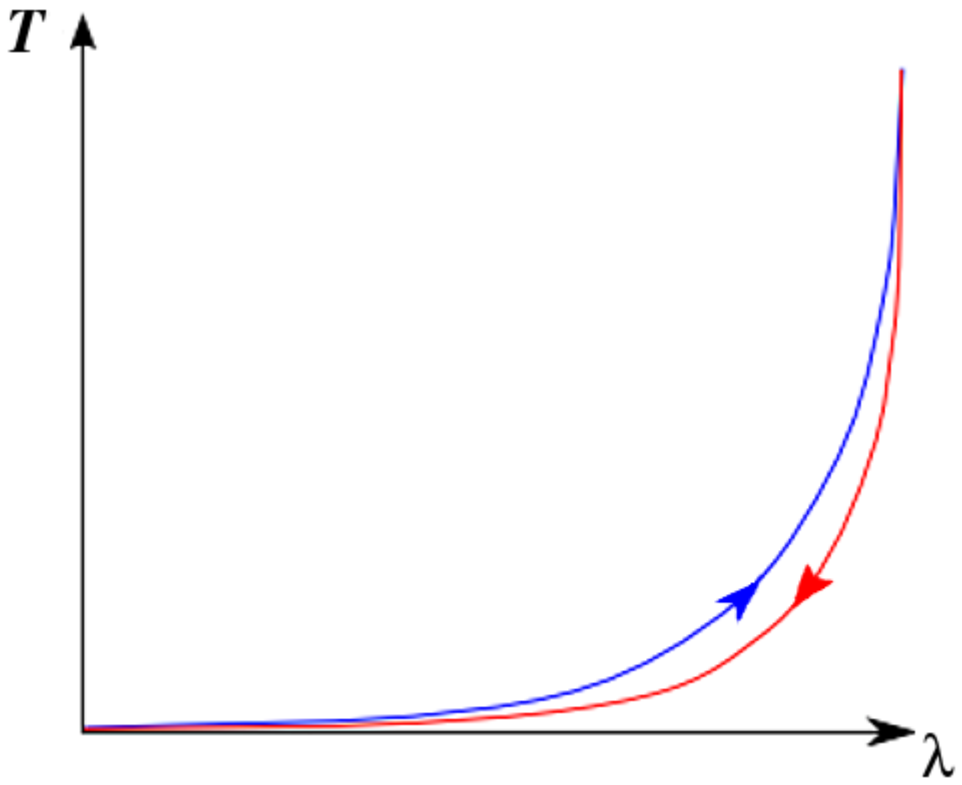


Figure 1: Graph of lagrangian stress (T) versus stretch ratio (λ) of a preconditioned soft tissue.

Soft tissues have the potential to undergo big deformations and still come back to the initial configuration when unloaded. The stress-strain curve is nonlinear, as can be seen in Figure 1. The soft tissues are also viscoelastic, incompressible and usually anisotropic. Some viscoelastic properties observable in soft tissues are: relaxation, creep and hysteresis .

Pseudoelasticity

Even though soft tissues have viscoelastic properties, i.e. stress as function of strain rate, it can be approximated by a hyperelastic model after **precondition** to a load pattern. After some cycles of loading and unloading the material, the mechanical response becomes independent of strain rate.

$$\mathbf{S} = \mathbf{S}(\mathbf{E}, \dot{\mathbf{E}}) \quad \rightarrow \quad \mathbf{S} = \mathbf{S}(\mathbf{E})$$

Despite the independence of strain rate, preconditioned soft tissues still present hysteresis, so the mechanical response can be modeled as hyperelastic with different material constants at loading and unloading. By this method the elasticity theory is used to model an inelastic material. Fung has called this model as **pseudoelastic** to point out that the material is not truly elastic .

Residual stress

In physiological state soft tissues usually present residual stress that may be released when the tissue is excised. Physiologists and histologists must be aware of this fact to avoid mistakes when analyzing excised tissues. This retraction usually causes a visual artifact .

Fung-elastic material

Fung developed a constitutive equation for preconditioned soft tissues which is

$$W = \frac{1}{2}\left[q + c\left(e^{Q} - 1\right)\right]$$

with

$$q = a_{ijkl}E_{ij}E_{kl} \qquad Q = b_{ijkl}E_{ij}E_{kl}$$

quadratic forms of Green-Lagrange strains E_{ij} and a_{ijkl}, b_{ijkl} and c material constants . w is the strain energy function per volume unit, which is the mechanical strain energy for a given temperature.

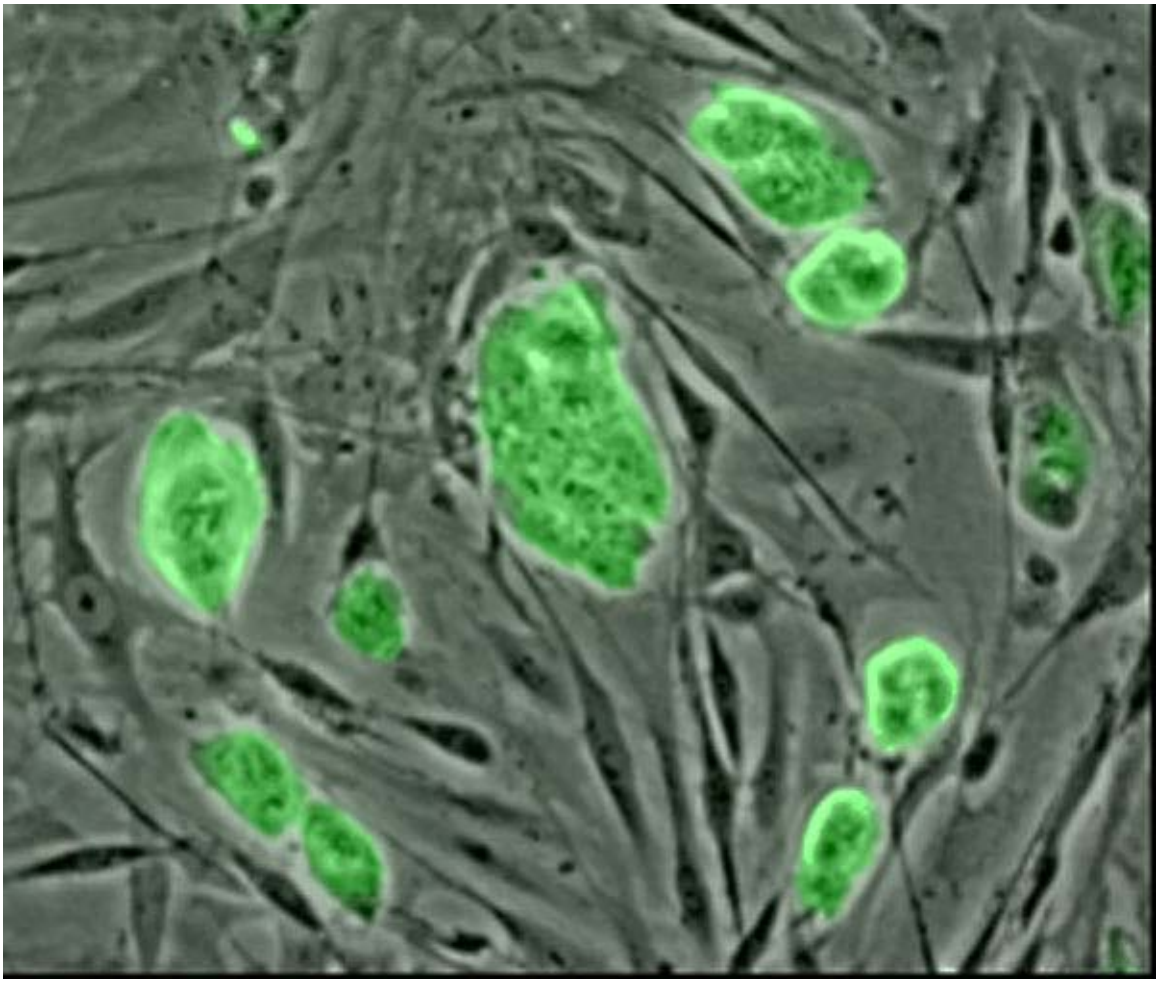
Remodeling and Growth

Soft tissues have the potential to grow and remodel reacting to chemical and mechanical long term changes. The rate the fibroblasts produce tropocollagen is proportional to these stimuli. Diseases, injuries and changes in the level of mechanical load may induce remodeling. An example of this phenomenon is the thickening of farmer's hands. The remodeling of connective tissues is very know in bones by the Wolff's law (bone remodeling). Mechanobiology is the science that study the relation between stress and growth at cellular level .

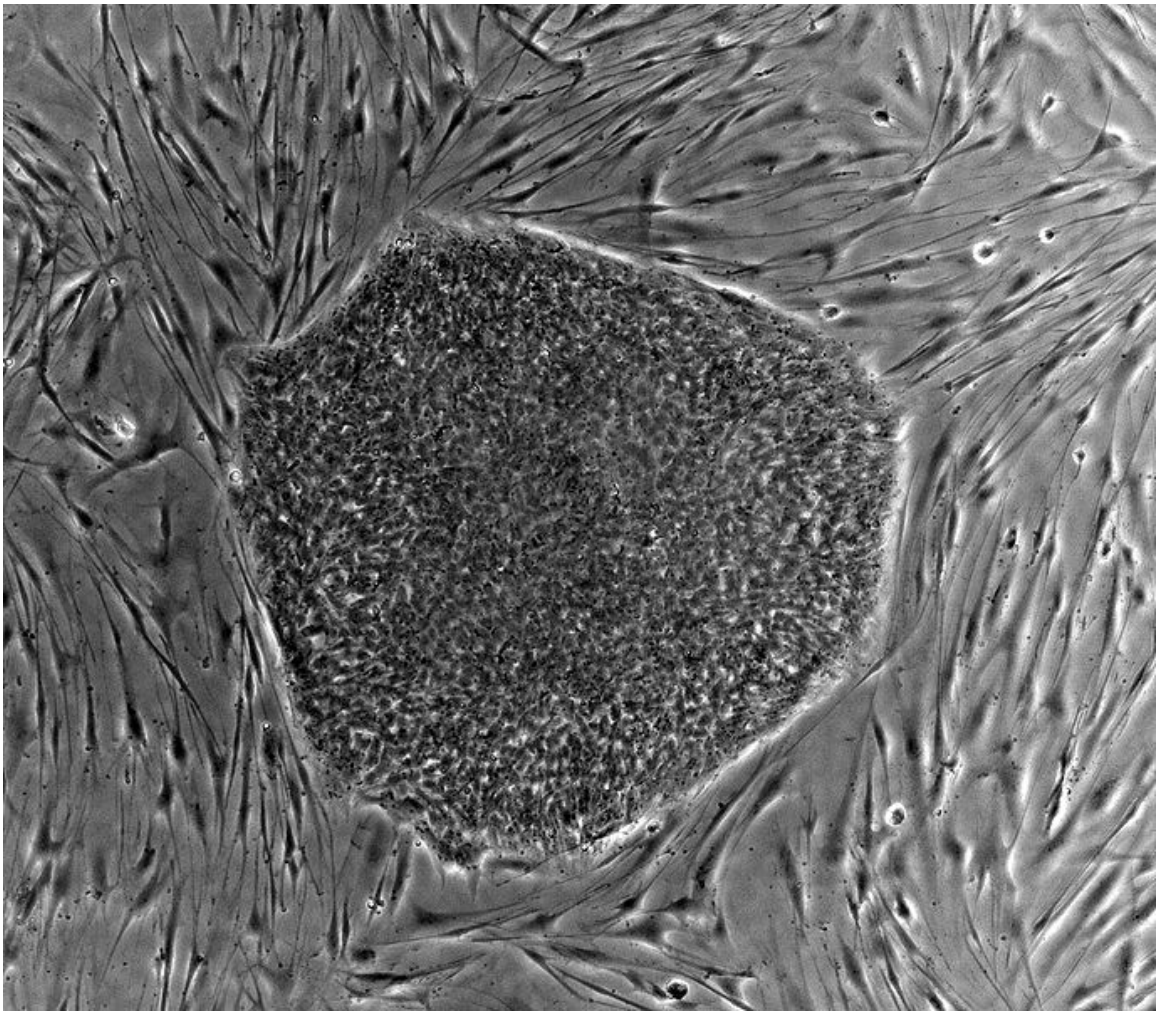
Growth and remodeling have a major role in the etiology of some common soft tissue diseases, like arterial stenosis and aneurisms and any soft tissue fibrosis.

Chapter 3

Stem Cell



Mouse embryonic stem cells with fluorescent marker



Human embryonic stem cell colony on mouse embryonic fibroblast feeder layer

Stem cells are biological cells found in all multicellular organisms, that can divide through mitosis and differentiate into diverse specialized cell types. In mammals, there are two broad types of stem cells: embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenished in adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

Stem cells can now be artificially grown and transformed into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells are routinely used in medical therapies. Stem cells can be taken from a variety of sources, including umbilical cord blood and bone marrow. Embryonic cell lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies. Research of stem cells grew out of findings by Ernest A. McCulloch and James E. Till at the University of Toronto in the 1960s.

Properties

The classical definition of a stem cell requires that it possess two properties:

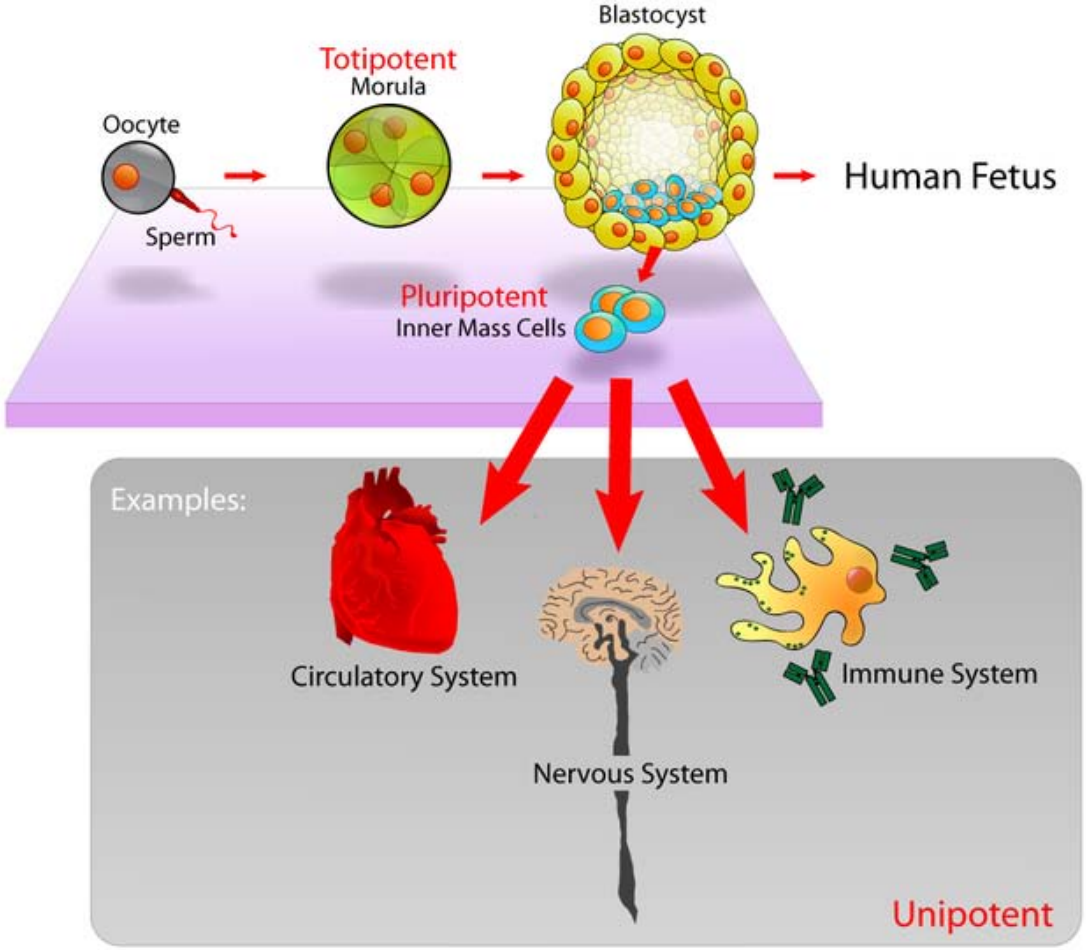
- *Self-renewal* - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state.
- *Potency* - the capacity to differentiate into specialized cell types. In the strictest sense, this requires stem cells to be either totipotent or pluripotent - to be able to give rise to any mature cell type, although multipotent or unipotent progenitor cells are sometimes referred to as stem cells.

Self-renewal

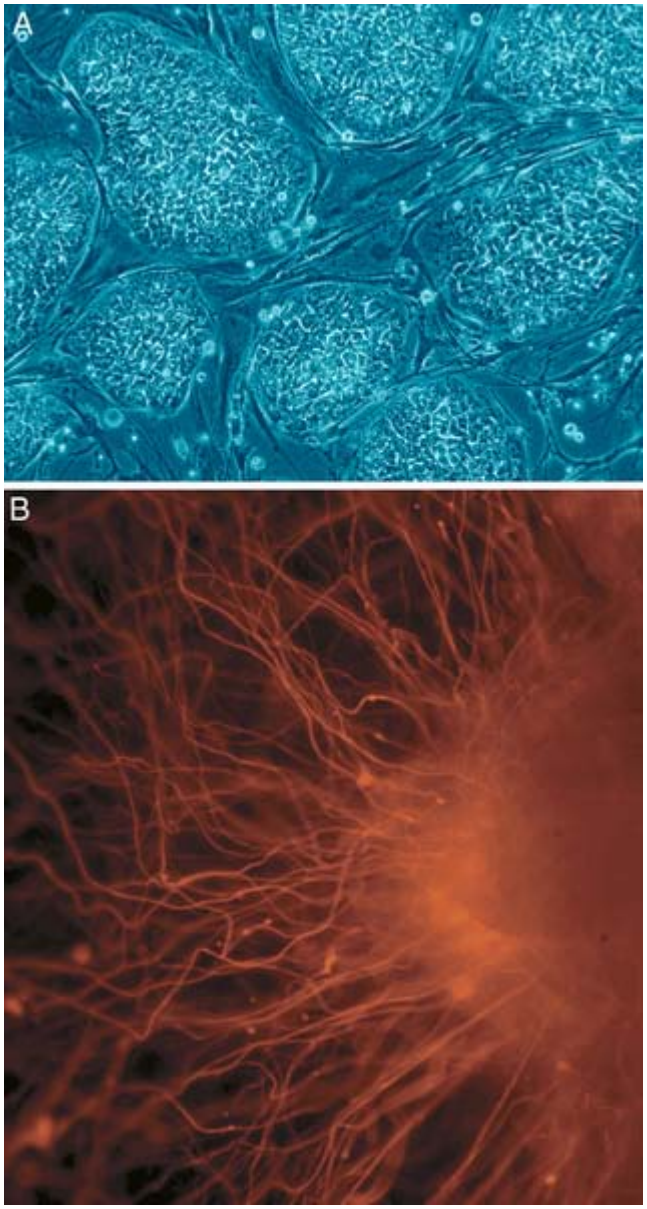
Two mechanisms exist to ensure that the stem cell population is maintained:

1. Obligatory asymmetric replication - a stem cell divides into one father cell that is identical to the original stem cell, and another daughter cell that is differentiated
2. Stochastic differentiation - when one stem cell develops into two differentiated daughter cells, another stem cell undergoes mitosis and produces two stem cells identical to the original.

Potency definitions



Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta.



Human embryonic stem cells
A: Cell colonies that are not yet differentiated.
B: Nerve cell

Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell.

- Totipotent (a.k.a omnipotent) stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable, organism. These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.
- Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers.
- Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells.

- Oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells.
- Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

Identification

The practical definition of a stem cell is the functional definition - a cell that has the potential to regenerate tissue over a lifetime. For example, the gold standard test for a bone marrow or hematopoietic stem cell (HSC) is the ability to transplant one cell and save an individual without HSCs. In this case, a stem cell must be able to produce new blood cells and immune cells over a long term, demonstrating potency. It should also be possible to isolate stem cells from the transplanted individual, which can themselves be transplanted into another individual without HSCs, demonstrating that the stem cell was able to self-renew.

Properties of stem cells can be illustrated *in vitro*, using methods such as clonogenic assays, where single cells are characterized by their ability to differentiate and self-renew. As well, stem cells can be isolated based on a distinctive set of cell surface markers. However, *in vitro* culture conditions can alter the behavior of cells, making it unclear whether the cells will behave in a similar manner *in vivo*. Considerable debate exists whether some proposed adult cell populations are truly stem cells.

Embryonic

Embryonic stem cell lines (ES cell lines) are cultures of cells derived from the epiblast tissue of the inner cell mass (ICM) of a blastocyst or earlier morula stage embryos. A blastocyst is an early stage embryo—approximately four to five days old in humans and consisting of 50–150 cells. ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. In other words, they can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type. They do not contribute to the extra-embryonic membranes or the placenta.

Nearly all research to date has taken place using mouse embryonic stem cells (mES) or human embryonic stem cells (hES). Both have the essential stem cell characteristics, yet they require very different environments in order to maintain an undifferentiated state. Mouse ES cells are grown on a layer of gelatin and require the presence of Leukemia Inhibitory Factor (LIF). Human ES cells are grown on a feeder layer of mouse embryonic fibroblasts (MEFs) and require the presence of basic Fibroblast Growth Factor (bFGF or FGF-2). Without optimal culture conditions or genetic manipulation, embryonic stem cells will rapidly differentiate.

A human embryonic stem cell is also defined by the presence of several transcription factors and cell surface proteins. The transcription factors Oct-4, Nanog, and Sox2 form the core regulatory network that ensures the suppression of genes that lead to differentiation and the maintenance of pluripotency. The cell surface antigens most commonly used to identify hES cells are the glycolipids SSEA3 and SSEA4 and the

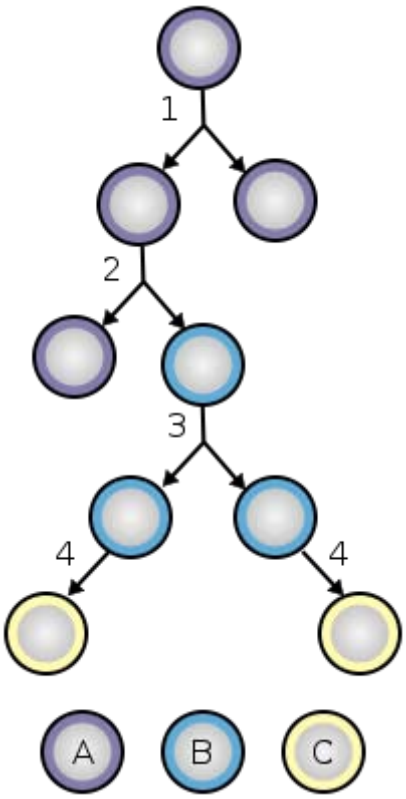
keratan sulfate antigens Tra-1-60 and Tra-1-81. The molecular definition of a stem cell includes many more proteins and continues to be a topic of research.

After nearly ten years of research, there are no approved treatments using embryonic stem cells. The first human trial was approved by the US Food & Drug Administration in January 2009. However, as of August 2010, the first human trial had not yet been initiated. The first human medical trial for embryonic stem cells started in Atlanta on October 13, 2010 for spinal injury victims. ES cells, being pluripotent cells, require specific signals for correct differentiation - if injected directly into another body, ES cells will differentiate into many different types of cells, causing a teratoma. Differentiating ES cells into usable cells while avoiding transplant rejection are just a few of the hurdles that embryonic stem cell researchers still face. Many nations currently have moratoria on either ES cell research or the production of new ES cell lines. Because of their combined abilities of unlimited expansion and pluripotency, embryonic stem cells remain a theoretically potential source for regenerative medicine and tissue replacement after injury or disease.

Fetal

Fetal stem cells are primitive cell types found in the organs of fetuses.

Adult



Stem cell division and differentiation. A - stem cell; B - progenitor cell; C - differentiated cell; 1 - symmetric stem cell division; 2 - asymmetric stem cell division; 3 - progenitor division; 4 - terminal differentiation

Also known as somatic (from Greek Σωματικός, "of the body") stem cells and germline (giving rise to gametes) stem cells, they can be found in children, as well as adults.

Pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including umbilical cord blood. A great deal of adult stem cell research has focused on clarifying their capacity to divide or self-renew indefinitely and their differentiation potential. In mice, pluripotent stem cells are directly generated from adult fibroblast cultures. Unfortunately, many mice don't live long with stem cell organs.

Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, etc.).

Adult stem cell treatments have been successfully used for many years to treat leukemia and related bone/blood cancers through bone marrow transplants. Adult stem cells are also used in veterinary medicine to treat tendon and ligament injuries in horses.

The use of adult stem cells in research and therapy is not as controversial as embryonic stem cells, because the production of adult stem cells does not require the destruction of an embryo. Additionally, because in some instances adult stem cells can be obtained from the intended recipient, (an autograft) the risk of rejection is essentially non-existent in these situations. Consequently, more US government funding is being provided for adult stem cell research.

An extremely rich source for adult mesenchymal stem cells is the developing tooth bud of the mandibular third molar. While considered multipotent they may prove to be pluripotent. The stem cells eventually form enamel (ectodrm), dentin,periodontal ligament, blood vessels, dental pulp, nervous tissues, including a minimum of 29 different unique end organs. Because of extreme ease in collection at 8–10 years of age before calcification and minimal to no morbidity will probably constitute a major source for personal banking, research and multiple therapies. These stem cells have been shown capable of producing hepatocytes.

Amniotic

Multipotent stem cells are also found in amniotic fluid. These stem cells are very active, expand extensively without feeders and are not tumorigenic. Amniotic stem cells are multipotent and can differentiate in cells of adipogenic, osteogenic, myogenic, endothelial, hepatic and also neuronal lines. All over the world, universities and research institutes are studying amniotic fluid to discover all the qualities of amniotic stem cells, and scientists such as Anthony Atala and Giuseppe Simoni have discovered important results.

From an ethical point of view, stem cells from amniotic fluid can solve a lot of problems, because it's possible to catch amniotic stem cells without destroying embryos. For example, the Vatican newspaper "Osservatore Romano" called amniotic stem cell "the future of medicine".

It's possible to collect amniotic stem cells for donors or for autologuous use: the first US amniotic stem cells bank opened in 2009 in Medford, MA, by Biocell Center Corporation and collaborates with various hospitals and universities all over the world.

Induced pluripotent

These are not adult stem cells, but rather reprogrammed cells (e.g. epithelial cells) given pluripotent capabilities. Using genetic reprogramming with protein transcription factors, pluripotent stem cells equivalent to embryonic stem cells have been derived from human adult skin tissue. Shinya Yamanaka and his colleagues at Kyoto University used the transcription factors Oct3/4, Sox2, c-Myc, and Klf4 in their experiments on cells from human faces. Junying Yu, James Thomson, and their colleagues at the University of Wisconsin–Madison used a different set of factors, Oct4, Sox2, Nanog and Lin28, and carried out their experiments using cells from human foreskin.

As a result of the success of these experiments, Ian Wilmut, who helped create the first cloned animal Dolly the Sheep, has announced that he will abandon nuclear transfer as an avenue of research.

Frozen blood samples can be used as a source of induced pluripotent stem cells, opening a new avenue for obtaining the valued cells.

Lineage

To ensure self-renewal, stem cells undergo two types of cell division. Symmetric division gives rise to two identical daughter cells both endowed with stem cell properties. Asymmetric division, on the other hand, produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell. It is possible that the molecular distinction between symmetric and asymmetric divisions lies in differential segregation of cell membrane proteins (such as receptors) between the daughter cells.

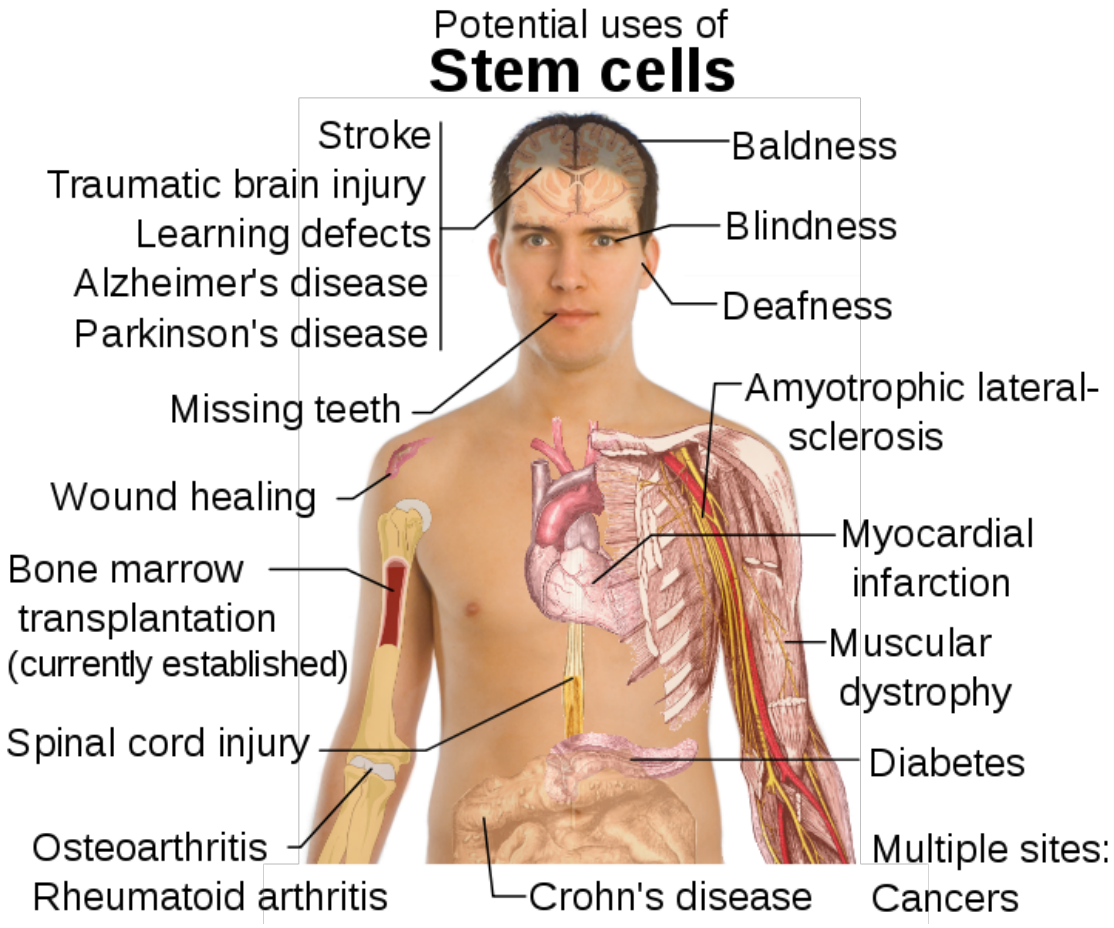
An alternative theory is that stem cells remain undifferentiated due to environmental cues in their particular niche. Stem cells differentiate when they leave that niche or no longer receive those signals. Studies in *Drosophila* germarium have identified the signals dpp and adherens junctions that prevent germarium stem cells from differentiating.

The signals that lead to reprogramming of cells to an embryonic-like state are also being investigated. These signal pathways include several transcription factors including the oncogene c-Myc. Initial studies indicate that transformation of mice cells with a combination of these anti-differentiation signals can reverse differentiation and may allow adult cells to become pluripotent. However, the need to transform these cells with an oncogene may prevent the use of this approach in therapy.

Challenging the terminal nature of cellular differentiation and the integrity of lineage commitment, it was recently determined that the somatic expression of combined transcription factors can directly induce other defined somatic cell fates; researchers identified three neural-lineage-specific transcription factors that could directly convert mouse fibroblasts (skin cells) into fully functional neurons. This "induced neurons" (iN)

cell research inspires the researchers to induce other cell types implies that *all* cells are totipotent: with the proper tools, all cells may form all kinds of tissue.

Treatments



Diseases and conditions where stem cell treatment is promising or emerging. Bone marrow transplantation is, as of 2009, the only established use of stem cells.

Medical researchers believe that stem cell therapy has the potential to dramatically change the treatment of human disease. A number of adult stem cell therapies already exist, particularly bone marrow transplants that are used to treat leukemia. In the future, medical researchers anticipate being able to use technologies derived from stem cell research to treat a wider variety of diseases including cancer, Parkinson's disease, spinal cord injuries, Amyotrophic lateral sclerosis, multiple sclerosis, and muscle damage, amongst a number of other impairments and conditions. However, there still exists a great deal of social and scientific uncertainty surrounding stem cell research, which could possibly be overcome through public debate and future research, and further education of the public.

One concern of treatment is the possible risk that transplanted stem cells could form tumors and have the possibility of becoming cancerous if cell division continues uncontrollably.

Stem cells, however, are already studied extensively. While some scientists are hesitant to associate the therapeutic potential of stem cells as the first goal of the research, they find the investigation of stem cells as a goal worthy in itself.

Contrarily, supporters of embryonic stem cell research argue that such research should be pursued because the resultant treatments could have significant medical potential. It is also noted that excess embryos created for in vitro fertilization could be donated with consent and used for the research.

The recent development of iPS cells has been called a bypass of the legal controversy. Laws limiting the destruction of human embryos have been credited for being the reason for development of iPS cells, but it is still not completely clear whether hiPS cells are equivalent to hES cells. Recent work demonstrates hotspots of aberrant epigenomic reprogramming in hiPS cells (Lister, R., et al., 2011).

Research patents

The patents covering a lot of work on human embryonic stem cells are owned by the Wisconsin Alumni Research Foundation (WARF). WARF does not charge academics to study human stem cells but does charge commercial users. WARF sold Geron Corp. exclusive rights to work on human stem cells but later sued Geron Corp. to recover some of the previously sold rights. The two sides agreed that Geron Corp. would keep the rights to only three cell types. In 2001, WARF came under public pressure to widen access to human stem-cell technology.

A request for reviewing the WARF patents 5,843,780; 6,200,806; 7,029,913 US Patent and Trademark Office were filed by non-profit patent-watchdogs The Foundation for Taxpayer & Consumer Rights, and the Public Patent Foundation as well as molecular biologist Jeanne Loring of the Burnham Institute. According to them, two of the patents granted to WARF are invalid because they cover a technique published in 1993 for which a patent had already been granted to an Australian researcher. Another part of the challenge states that these techniques, developed by James A. Thomson, are rendered obvious by a 1990 paper and two textbooks. Based on this challenge, patent 7,029,913 has been rejected in 2010. The two remaining hES WARF patents are due to expire in 2015.

The outcome of this legal challenge is particularly relevant to the Geron Corp. as it can only license patents that are upheld.

Key research events

- 1908 - The term "stem cell" was proposed for scientific use by the Russian histologist Alexander Maksimov (1874–1928) at congress of hematologic society in Berlin. It postulated existence of haematopoietic stem cells.
- 1960s - Joseph Altman and Gopal Das present scientific evidence of adult neurogenesis, ongoing stem cell activity in the brain; like André Gernez, their reports contradict Cajal's "no new neurons" dogma and are largely ignored.
- 1963 - McCulloch and Till illustrate the presence of self-renewing cells in mouse bone marrow.

- 1968 - Bone marrow transplant between two siblings successfully treats SCID.
- 1978 - Haematopoietic stem cells are discovered in human cord blood.
- 1981 - Mouse embryonic stem cells are derived from the inner cell mass by scientists Martin Evans, Matthew Kaufman, and Gail R. Martin. Gail Martin is attributed for coining the term "Embryonic Stem Cell".
- 1992 - Neural stem cells are cultured *in vitro* as neurospheres.
- 1997 - Leukemia is shown to originate from a haematopoietic stem cell, the first direct evidence for cancer stem cells.
- 1998 - James Thomson and coworkers derive the first human embryonic stem cell line at the University of Wisconsin–Madison.
- 1998 - John Gearhart (Johns Hopkins University) extracted germ cells from fetal gonadal tissue (primordial germ cells) before developing pluripotent stem cell lines from the original extract.
- 2000s - Several reports of adult stem cell plasticity are published.
- 2001 - Scientists at Advanced Cell Technology clone first early (four- to six-cell stage) human embryos for the purpose of generating embryonic stem cells.
- 2003 - Dr. Songtao Shi of NIH discovers new source of adult stem cells in children's primary teeth.
- 2004–2005 - Korean researcher Hwang Woo-Suk claims to have created several human embryonic stem cell lines from unfertilised human oocytes. The lines were later shown to be fabricated.
- 2005 - Researchers at Kingston University in England claim to have discovered a third category of stem cell, dubbed cord-blood-derived embryonic-like stem cells (CBEs), derived from umbilical cord blood. The group claims these cells are able to differentiate into more types of tissue than adult stem cells.
- 2005 - Researchers at UC Irvine's Reeve-Irvine Research Center are able to partially restore the ability of mice with paralyzed spines to walk through the injection of human neural stem cells.
- August 2006 - Mouse Induced pluripotent stem cells: the journal *Cell* publishes Kazutoshi Takahashi and Shinya Yamanaka.
- October 2006 - Scientists at Newcastle University in England create the first ever artificial liver cells using umbilical cord blood stem cells.
- January 2007 - Scientists at Wake Forest University led by Dr. Anthony Atala and Harvard University report discovery of a new type of stem cell in amniotic fluid. This may potentially provide an alternative to embryonic stem cells for use in research and therapy.
- June 2007 - Research reported by three different groups shows that normal skin cells can be reprogrammed to an embryonic state in mice. In the same month, scientist Shoukhrat Mitalipov reports the first successful creation of a primate stem cell line through somatic cell nuclear transfer
- October 2007 - Mario Capecchi, Martin Evans, and Oliver Smithies win the 2007 Nobel Prize for Physiology or Medicine for their work on embryonic stem cells from mice using gene targeting strategies producing genetically engineered mice (known as knockout mice) for gene research.
- November 2007 - Human induced pluripotent stem cells: Two similar papers released by their respective journals prior to formal publication: in *Cell* by Kazutoshi Takahashi and Shinya Yamanaka, "Induction of pluripotent stem cells from adult human fibroblasts by defined factors", and in *Science* by Junying Yu, et al., from the research group of James Thomson, "Induced pluripotent stem cell

lines derived from human somatic cells": pluripotent stem cells generated from mature human fibroblasts. It is possible now to produce a stem cell from almost any other human cell instead of using embryos as needed previously, albeit the risk of tumorigenesis due to c-myc and retroviral gene transfer remains to be determined.

- January 2008 - Robert Lanza and colleagues at Advanced Cell Technology and UCSF create the first human embryonic stem cells without destruction of the embryo
- January 2008 - Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts
- February 2008 - Generation of pluripotent stem cells from adult mouse liver and stomach: these iPS cells seem to be more similar to embryonic stem cells than the previously developed iPS cells and not tumorigenic, moreover genes that are required for iPS cells do not need to be inserted into specific sites, which encourages the development of non-viral reprogramming techniques.
- March 2008-The first published study of successful cartilage regeneration in the human knee using autologous adult mesenchymal stem cells is published by clinicians from Regenerative Sciences
- October 2008 - Sabine Conrad and colleagues at Tübingen, Germany generate pluripotent stem cells from spermatogonial cells of adult human testis by culturing the cells in vitro under leukemia inhibitory factor (LIF) supplementation.
- 30 October 2008 - Embryonic-like stem cells from a single human hair.
- 1 March 2009 - Andras Nagy, Keisuke Kaji, *et al.* discover a way to produce embryonic-like stem cells from normal adult cells by using a novel "wrapping" procedure to deliver specific genes to adult cells to reprogram them into stem cells without the risks of using a virus to make the change. The use of electroporation is said to allow for the temporary insertion of genes into the cell.
- 28 May 2009 Kim *et al.* announced that they had devised a way to manipulate skin cells to create patient specific "induced pluripotent stem cells" (iPS), claiming it to be the 'ultimate stem cell solution'.
- 11 October 2010 First trial of embryonic stem cells in humans.
- 25 October 2010 - Ishikawa et *al.* write in the Journal of Experimental Medicine that research shows that transplanted cells which contain their new host's nuclear DNA could still be rejected by the invidual's immune system due to foreign mitochondrial DNA. Tissues made from a person's stem cells could therefore be rejected, because mitochondrial genomes tend to accumulate mutations.

Chapter 4

Stem Cell Treatments

Stem cell treatments are a type of intervention strategy that introduces new cells into damaged tissue in order to treat disease or injury. Many medical researchers believe that stem cell treatments have the potential to change the face of human disease and alleviate suffering. The ability of stem cells to self-renew and give rise to subsequent generations with variable degrees of differentiation capacities, offers significant potential for generation of tissues that can potentially replace diseased and damaged areas in the body, with minimal risk of rejection and side effects.

A number of stem cell therapeutics exist, but most are at experimental stages and/or costly, with the notable exception of bone marrow transplantation. Medical researchers anticipate that adult and embryonic stem cells will soon be able to treat cancer, Type 1 diabetes mellitus, Parkinson's disease, Huntington's disease, Celiac Disease, cardiac failure, muscle damage and neurological disorders, and many others. Nevertheless, before stem cell therapeutics can be applied in the clinical setting, more research is necessary to understand stem cell behavior upon transplantation as well as the mechanisms of stem cell interaction with the diseased/injured microenvironment.

Current treatments

For over 30 years, bone marrow, and more recently, umbilical cord blood stem cells, have been used to treat cancer patients with conditions such as leukemia and lymphoma. During chemotherapy, most growing cells are killed by the cytotoxic agents. These agents, however, cannot discriminate between the leukemia or neoplastic cells, and the hematopoietic stem cells within the bone marrow. It is this side effect of conventional chemotherapy strategies that the stem cell transplant attempts to reverse; a donor's healthy bone marrow reintroduces functional stem cells to replace the cells lost in the host's body during treatment.

Potential treatments

Brain damage

Stroke and traumatic brain injury lead to cell death, characterized by a loss of neurons and oligodendrocytes within the brain. Healthy adult brains contain neural stem cells which divide to maintain general stem cell numbers, or become progenitor cells. In healthy adult animals, progenitor cells migrate within the brain and function primarily to maintain neuron populations for olfaction (the sense of smell). Interestingly, in pregnancy and after injury, this system appears to be regulated by growth factors and can increase the rate at which new brain matter is formed. Although the reparative process appears to initiate following trauma to the brain, substantial recovery is rarely observed in adults, suggesting a lack of robustness.

Stem cells may also be used to treat brain degeneration, such as in Parkinson's and Alzheimer's disease.

Cancer

Research injecting neural (adult) stem cells into the brains of dogs has shown to be very successful in treating cancerous tumors. Using conventional techniques, brain cancer is difficult to treat because it spreads so rapidly. Researchers at the Harvard Medical School transplanted human neural stem cells into the brain of rodents that received intracranial tumours. Within days, the cells migrated into the cancerous area and produced cytosine deaminase, an enzyme that converts a non-toxic pro-drug into a chemotheraputic agent. As a result, the injected substance was able to reduce the tumor mass by 81 percent. The stem cells neither differentiated nor turned tumorigenic. Some researchers believe that the key to finding a cure for cancer is to inhibit proliferation of cancer stem cells. Accordingly, current cancer treatments are designed to kill cancer cells. However, conventional chemotherapy treatments cannot discriminate between cancerous cells and others. Stem cell therapies may serve as potential treatments for cancer. Research on treating Lymphoma using adult stem cells is underway and has had human trials. Essentially, chemotherapy is used to completely destroy the patients own lymphocytes, and stem cells injected, eventually replacing the immune system of the patient with that of the healthy donor.

Spinal cord injury

A team of Korean researchers reported on November 25, 2003, that they had transplanted multipotent adult stem cells from umbilical cord blood to a patient suffering from a spinal cord injury and that following the procedure, she could walk on her own, without difficulty. The patient had not been able to stand up for roughly 19 years. For the unprecedented clinical test, the scientists isolated adult stem cells from umbilical cord blood and then injected them into the damaged part of the spinal cord.

According to the October 7, 2005 issue of *The Week*, University of California, Irvine researchers transplanted multipotent human fetal-derived neural stem cells into paralyzed mice, resulting in locomotor improvements four months later. The observed recovery was associated with differentiation of transplanted cells into new neurons and

oligodendrocytes- the latter of which forms the myelin sheath around axons of the central nervous system, thus insulating neural impulses and facilitating communication with the brain.

In January 2005, researchers at the University of Wisconsin–Madison differentiated human blastocyst stem cells into neural stem cells, then into pre-mature motor neurons, and finally into spinal motor neurons, the cell type that, in the human body, transmits messages from the brain to the spinal cord and subsequently mediates motor function in the periphery. The newly generated motor neurons exhibited electrical activity, the signature action of neurons. Lead researcher Su-Chun Zhang described the process as "[teaching] the blastocyst stem cells to change step by step, where each step has different conditions and a strict window of time."

Transformation of blastocyst stem cells into motor neurons had eluded researchers for decades. While Zhang's findings were a significant contribution to the field, the ability of transplanted neural cells to establish communication with neighboring cells remains unclear. Accordingly, studies using chicken embryos as a model organism can be an effective proof-of-concept experiment. If functional, the new cells could be used to treat diseases like Lou Gehrig's disease, muscular dystrophy, and spinal cord injuries.

Heart damage

Several clinical trials targeting heart disease have shown that adult stem cell therapy is safe, effective, and equally efficient in treating old and recent infarcts. Adult stem cell therapy for treating heart disease was commercially available in at least five continents at the last count (2007).

Possible mechanisms of recovery include:

- Generation of heart muscle cells
- Stimulation of growth of new blood vessels to repopulate damaged heart tissue
- Secretion of growth factors
- Assistance via some other mechanism

It may be possible to have adult bone marrow cells differentiate into heart muscle cells.

Haematopoiesis (blood cell formation)

The specificity of the human immune cell repertoire is what allows the human body to defend itself from rapidly adapting antigens. However, the immune system is vulnerable to degradation upon the pathogenesis of disease, and because of the critical role that it plays in overall defense, its degradation is often fatal to the organism as a whole. Diseases of hematopoietic cells are called hematopathology. The specificity of the immune cells is what allows recognition of foreign antigens, causing further challenges in the treatment of immune disease. Identical matches between donor and recipient must be made for successful transplantation treatments, but matches are uncommon, even between first-degree relatives. Research using both hematopoietic adult stem cells and embryonic stem cells has provided insight into the possible mechanisms and methods of treatment for many of these ailments.

Fully mature human red blood cells may be generated *ex vivo* by hematopoietic stem cells (HSCs), which are precursors of red blood cells. In this process, HSCs are grown together with stromal cells, creating an environment that mimics the conditions of bone marrow, the natural site of red blood cell growth. Erythropoietin, a growth factor, is added, coaxing the stem cells to complete terminal differentiation into red blood cells. Further research into this technique should have potential benefits to gene therapy, blood transfusion, and topical medicine.

Baldness

Hair follicles also contain stem cells, and some researchers predict research on these follicle stem cells may lead to successes in treating baldness through an activation of the stem cells progenitor cells. This treatment is expected to work by activating already existing stem cells on the scalp. Later treatments may be able to simply signal follicle stem cells to give off chemical signals to nearby follicle cells which have shrunk during the aging process, which in turn respond to these signals by regenerating and once again making healthy hair.

Missing teeth

In 2004, scientists at King's College London discovered a way to cultivate a complete tooth in mice and were able to grow them stand-alone in the laboratory. Researchers are confident that this technology can be used to grow live teeth in human patients.

In theory, stem cells taken from the patient could be coaxed in the lab into turning into a tooth bud which, when implanted in the gums, will give rise to a new tooth, and would be expected to grow within two months. It will fuse with the jawbone and release chemicals that encourage nerves and blood vessels to connect with it. The process is similar to what happens when humans grow their original adult teeth.

Many challenges remain, however, before stem cells could be a choice for the replacement of missing teeth in the future.

Deafness

Heller has reported success in re-growing cochlea hair cells with the use of embryonic stem cells.

Blindness and vision impairment

Since 2003, researchers have successfully transplanted corneal stem cells into damaged eyes to restore vision. Using embryonic stem cells, scientists are able to grow a thin sheet of totipotent stem cells in the laboratory. When these sheets are transplanted over the damaged cornea, the stem cells stimulate renewed repair, eventually restore vision. The latest such development was in June 2005, when researchers at the Queen Victoria Hospital of Sussex, England were able to restore the sight of forty patients using the same technique. The group, led by Dr. Sheraz Daya, was able to successfully use adult stem cells obtained from the patient, a relative, or even a cadaver. Further rounds of trials are ongoing.

In April 2005, doctors in the UK transplanted corneal stem cells from an organ donor to the cornea of Deborah Catlyn, a woman who was blinded in one eye when acid was thrown in her eye at a nightclub. The cornea, which is the transparent window of the eye, is a particularly suitable site for transplants. In fact, the first successful human transplant was a cornea transplant. The absence of blood vessels within the cornea makes this area a relatively easy target for transplantation. The majority of corneal transplants carried out today are due to a degenerative disease called keratoconus.

The University Hospital of New Jersey reports that the success rate for growth of new cells from transplanted stem cells varies from 25 percent to 70 percent.

In 2009, researchers at the University of Pittsburgh Medical center demonstrated that stem cells collected from human corneas can restore transparency without provoking a rejection response in mice with corneal damage.

Amyotrophic lateral sclerosis

Stem cells have resulted in significant locomotor improvements in rats with an Amyotrophic lateral sclerosis-like disease. In a rodent model that closely mimics the human form of ALS, animals were injected with a virus to kill the spinal cord motor nerves which mediate movement. Animals subsequently received stem cells in the spinal cord. Transplanted cells migrated to the sites of injury, contributed to regeneration of the ablated nerve cells, and restored locomotor function.

Graft vs. host disease and Crohn's disease

Phase III clinical trials expected to end in second-quarter 2008 were conducted by Osiris Therapeutics using their in-development product Prochymal, derived from adult bone marrow. The target disorders of this therapeutic are graft-versus-host disease and Crohn's disease.

Neural and behavioral birth defects

A team of researchers led by Prof. Joseph Yanai were able to reverse learning deficits in the offspring of pregnant mice who were exposed to heroin and the pesticide organophosphate. This was done by direct neural stem cell transplantation into the brains of the offspring. The recovery was almost 100 percent, as shown in behavioral tests that suggested improved to normal behavior and learning scores in animals receiving cell transplantation. On the molecular level, brain chemistry of the treated animals was also restored to normal. Through the work, which was supported by the US National Institutes of Health, the US-Israel Binational Science Foundation and the Israel anti-drug authorities, the researchers discovered that the stem cells worked even in cases where most of the cells died out in the host brain.

The scientists found that before they die the neural stem cells succeed in inducing the host brain to produce large numbers of stem cells which repair the damage. These findings, which answered a major question in the stem cell research community, were published earlier this year in the leading journal, Molecular Psychiatry. Scientists are now developing procedures to administer the neural stem cells in the least invasive way

possible - probably via blood vessels, making therapy practical and clinically feasible. Researchers also plan to work on developing methods to take cells from the patient's own body, turn them into stem cells, and then transplant them back into the patient's blood via the blood stream. Aside from decreasing the chances of immunological rejection, the approach will also eliminate the controversial ethical issues involved in the use of stem cells from human embryos.

Diabetes

Diabetes patients lose the function of insulin-producing beta cells within the pancreas. Human embryonic stem cells may be grown in cell culture and stimulated to form insulin-producing cells that can be transplanted into the patient.

However, clinical success is highly dependent on the development of the following procedures:

- Transplanted cells should proliferate
- Transplanted cells should differentiate in a site-specific manner
- Transplanted cells should survive in the recipient (prevention of transplant rejection)
- Transplanted cells should integrate within the targeted tissue
- Transplanted cells should integrate into the host circuitry and restore function

Orthopaedics

Clinical case reports in the treatment of orthopaedic conditions have been reported. To date, the focus in the literature for musculoskeletal care appears to be on mesenchymal stem cells. Centeno et al. have published MRI evidence of increased cartilage and meniscus volume in individual human subjects. The results of trials that include a large number of subjects, are yet to be published. However, a published safety study conducted in a group of 227 patients over a 3-4 year period shows adequate safety and minimal complications associated with mesenchymal cell transplantation.

Wakitani has also published a small case series of nine defects in five knees involving surgical transplantation of mesenchymal stem cells with coverage of the treated chondral defects.

Wound healing

Stem cells can also be used to stimulate the growth of human tissues. In an adult, wounded tissue is most often replaced by scar tissue, which is characterized in the skin by disorganized collagen structure, loss of hair follicles and irregular vascular structure. In the case of wounded fetal tissue, however, wounded tissue is replaced with normal tissue through the activity of stem cells. A possible method for tissue regeneration in adults is to place adult stem cell "seeds" inside a tissue bed "soil" in a wound bed and allow the stem cells to stimulate differentiation in the tissue bed cells. This method elicits a regenerative response more similar to fetal wound-healing than adult scar tissue formation. Researchers are still investigating different aspects of the "soil" tissue that are conducive to regeneration.

Infertility

Culture of human embryonic stem cells in mitotically inactivated porcine ovarian fibroblasts (POF) causes differentiation into germ cells (precursor cells of oocytes and spermatozoa), as evidenced by gene expression analysis.

Human embryonic stem cells have been stimulated to form Spermatozoon-like cells, yet still slightly damaged or malformed. It could potentially treat azoospermia.

Clinical Trials

On January 23, 2009, the US Food and Drug Administration gave clearance to Geron Corporation for the initiation of the first clinical trial of an embryonic stem cell-based therapy on humans. The trial will evaluate the drug GRNOPC1, embryonic stem cell-derived oligodendrocyte progenitor cells, on patients with acute spinal cord injury.

As of mid 2010 hundreds of phase III clinical trials involving stem cells have been registered.

Stem cell use in animals

Veterinary applications

Potential contributions to veterinary medicine

Research currently conducted on horses, dogs, and cats can benefit the development of stem-cell treatments in veterinary medicine and can target a wide range of injuries and diseases such as myocardial infarction, stroke, tendon and ligament damage, osteoarthritis, osteochondrosis and muscular dystrophy both in large animals, as well as humans. While investigation of cell-based therapeutics generally reflects human medical needs, the high degree of frequency and severity of certain injuries in racehorses has put veterinary medicine at the forefront of this novel regenerative approach. Companion animals can serve as clinically relevant models that closely mimic human disease.

Development of regenerative treatment models

Veterinary applications of stem cell therapy as a means of tissue regeneration have been largely shaped by research that began with the use of adult-derived mesenchymal stem cells to treat animals with injuries or defects affecting bone, cartilage, ligaments and/or tendons. Because mesenchymal stem cells can differentiate into the cells that make up bone, cartilage, tendons, and ligaments (as well as muscle, fat, and possibly other tissues), they have been the main type of stem cells studied in the treatment of diseases affecting these tissues. Mesenchymal stem cells are primarily derived from adipose tissue or bone marrow. Since an elevated immune response following cell transplantation may result in rejection of exogenous cells (except in the case of cells derived from a very closely genetically related individual), mesenchymal stem cells are often derived from the patient prior to injection in a process known as autologous transplantation. Surgical repair of bone fractures in dogs and sheep has demonstrated that engraftment of mesenchymal stem cells derived from a genetically different donor within the same species, termed

allogeneic transplantation, does not elicit an immunological response in the recipient animal and can mediate regeneration of bone tissue in major bony fractures and defects. Stem cells can speed up bone repair in fractures/defects that would normally require extensive grafting, suggesting that mesenchymal stem cell use may provide a useful alternative to conventional grafting techniques. Treating tendon and ligament injuries in horses using stem cells, whether derived from adipose tissue or bone-marrow, has support in the veterinary literature. While further studies are necessary to fully characterize the use of cell-based therapeutics for treatment of bone fractures, stem cells are thought to mediate repair via five primary mechanisms: 1) providing an antiinflammatory effect, 2) homing to damaged tissues and recruiting other cells, such as endothelial progenitor cells, that are necessary for tissue growth, 3) supporting tissue remodeling over scar formation, 4) inhibiting apoptosis, and 5) differentiating into bone, cartilage, tendon, and ligament tissue.

Significance of stem cell microenvironments

The microenvironment into which stem cells are transplanted significantly alters the capacity of engrafted cells for recovery and repair. The microenviroment provides growth factors and other chemical signals that guide appropriate differentiation of transplanted cell populations and direct transplanted cells to sites of trauma or disease. Repair and recovery can then be mediated via three primary mechanisms: 1) formation and/or recruitment of new blood cells to the damaged region; 2) prevention of programed cell death or apoptosis; and 3) suppression of inflammation. To further enrich blood supply to the damaged areas, and consequently promote tissue regeneration, platelet-rich plasma could be used in conjunction with stem cell transplantation. The efficacy of some stem cell populations may also be affected by the method of delivery; for instance, to regenerate bone, stem cells are often introduced in a scaffold where they produce the minerals necessary for generation of functional bone.

Sources of autologous (patient-derived) stem cells

Autologous stem cells intended for regenerative therapy are generally isolated either from the patient's bone marrow or from adipose tissue. The number of stem cells transplanted into damaged tissue may alter efficacy of treatment. Accordingly, stem cells derived from bone marrow aspirates, for instance, are cultured in specialized laboratories for expansion to millions of cells. Although adipose-derived tissue also requires processing prior to use, the culturing methodology for adipose-derived stem cells is not as extensive as that for bone marrow-derived cells. While it is thought that bone-marrow derived stem cells are preferred for bone, cartilage, ligament, and tendon repair, others believe that the less challenging collection techniques and the multi-cellular microenvironment already present in adipose-derived stem cell fractions make the latter the preferred source for autologous transplantation.

Currently Available Treatments for Horses and Dogs Suffering from Orthopedic Conditions

Autologous or allogeneic stem cells are currently used as an adjunctive therapy in the surgical repair of some types of fractures in dogs and horses. Autologous stem cell-based treatments for ligament injury, tendon injury, osteoarthritis, osteochondrosis, and sub-

chondral bone cysts have been commercially available to practicing veterinarians to treat horses since 2003 in the United States and since 2006 in the United Kingdom. Autologous stem-cell based treatments for tendon injury, ligament injury, and osteoarthritis in dogs have been available to veterinarians in the United States since 2005. Over 3000 privately-owned horses and dogs have been treated with autologous adipose-derived stem cells. The efficacy of these treatments has been shown in double-blind clinical trials for dogs with osteoarthritis of the hip and elbow and horses with tendon damage. The efficacy of using stem cells, whether adipose-derived or bone-marrow derived, for treating tendon and ligament injuries in horses has support in the veterinary literature.

Developments in Stem Cell Treatments in Veterinary Internal Medicine

Currently, research is being conducted to develop stem cell treatments for: 1) horses suffering from COPD, neurologic disease, and laminitis; and 2) dogs and cats suffering from heart disease, liver disease, kidney disease, neurologic disease, and immune-mediated disorders.

Embryonic stem cell controversy

There is widespread controversy over the use of human embryonic stem cells. This controversy primarily targets the techniques used to derive new embryonic stem cell lines, which often requires the destruction of the blastocyst. Opposition to the use of human embryonic stem cells in research is often based on philosophical, moral or religious objections.

There have been tens of thousands of successful adult stem cell treatments just in China over the last decade, while the research community has yet to produce even one positive patient result using embryonic stem cells. The alternatives do not require the destruction of an embryo, such as the use of umbilical cord blood, milk teeth stem cells, bone marrow stem cells or using induced pluripotent stem cells. The stem cells from these alternative sources also lack the severe side effects which are universally seen in embryonic stem cell therapies and most often result in fatal rejection by the subject; the most common being mutations of the stem cells into tumors.

Stem cell treatments around the world

China

Stem cell research and treatment is currently being practiced at a clinical level in the People's Republic of China. The Ministry of Health of the People's Republic of China has permitted the use of stem cell therapy for conditions beyond those approved of in Western countries such as the United States, United Kingdom, and Australia. The Western World has scrutinized China for its failed attempts to meet international documentation standards of these trials and procedures, despite the overwhelmingly positive anecdotal results.

Stem cell therapies provided in China utilize a variety of cell types including umbilical cord stem cells and olfactory ensheathing cells. The stem cells are then expanded in

centralized blood banks before being used in stem cell treatments. State-funded companies based in the Shenzhen Hi-Tech Industrial Zone treat the symptoms of numerous disorders with adult stem cell therapy. Hospitals throughout eastern China provide numerous therapies to patients in coordination with the stem cell providers. These companies' therapies are currently focused on the treatment of neurodegenerative and cardiovascular disorders. The most radical successes of Chinese adult stem cell therapy have been in treating the brain. These therapies administer stem cells directly to the brain to promote greater motor and brain function in patients with Cerebral Palsy, Alzheimer's, and brain injuries. However, retrospective studies have shown that Chinese use of fetal-derived brain tissue in spinal cord injured human subjects were not as promising as once thought: the phenotype and the fate of the transplanted cells, described as olfactory ensheathing cells, were unknown. As well, perioperative morbidity and lack of functional benefit were identified as the most serious clinical shortcomings. Furthermore, the extent of regulatory policy in the use of stem cell therapies in China is unclear. In the absence of a valid clinical trials protocol, and more regulatory oversight, Western regulatory agencies advise patients and physicians to be cautious when selecting Chinese stem cell therapeutic centers.

Mexico

Stem cell treatment is currently being practiced at a clinical level in Mexico. An International Health Department Permit (COFEPRIS) is required. This permit allows the use of stem cell types beyond those approved of in Western countries such as the United States or Europe. Stem cell therapies provided in Mexico utilize patient Adipose, Bone Marrow, or Donor Placenta sources.

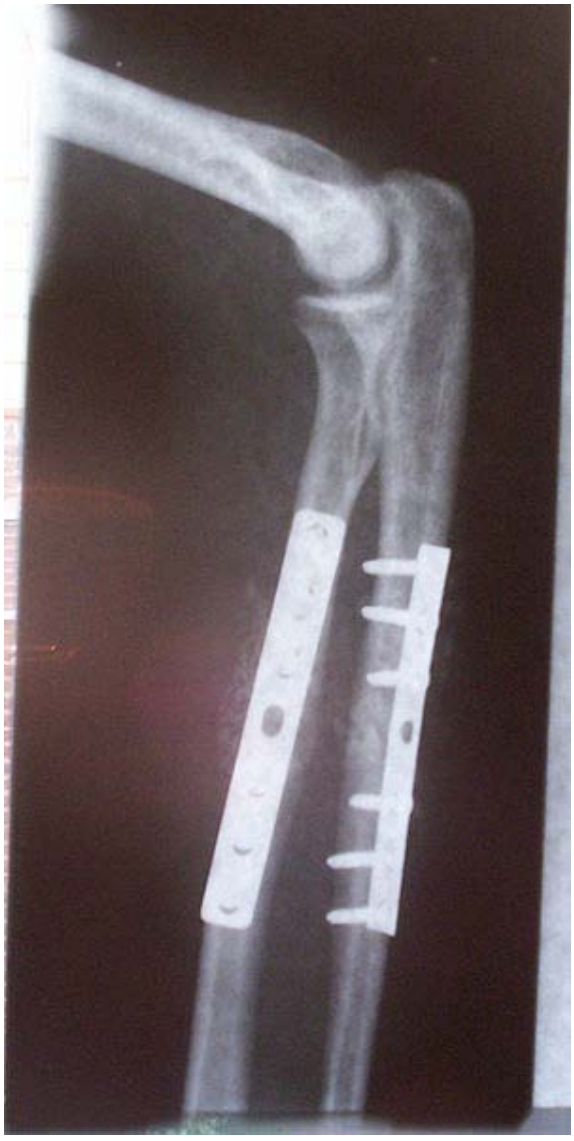
South Korea

In 2005, South Korean scientists claimed to have generated stem cells that were tailored to match the recipient. Each of the 11 new stem cell lines was developed using somatic cell nuclear transfer (SCNT) technology. The resultant cells were thought to match the genetic material of the recipient, thus suggesting minimal to no cell rejection.

This study, however, was eventually discredited as the primary researcher,Dr. Woo Suk Hwang, admitted to using cells obtained from his research staff. In Dec 2005, claims were put forward that his research had been manipulated to wrongfully indicate positive results. Later that month, these claims were confirmed by an academic panel.

Chapter 5

Implant (Medicine)



Orthopedic implants to repair fractures to the radius and ulna. Note the visible break in the ulna. (right forearm)

An **implant** is a medical device manufactured to replace a missing biological structure, support a damaged biological structure, or enhance an existing biological structure. Medical implants are man-made devices, in contrast to a transplant, which is a transplanted biomedical tissue. The surface of implants that contact the body might be made of a biomedical material such as titanium, silicone or apatite depending on what is the most functional. In some cases implants contain electronics e.g. artificial pacemaker and cochlear implants. Some implants are bioactive, such as subcutaneous drug delivery devices in the form of implantable pills or drug-eluting stents.

Applications

Among the most common types of medical implants are the pins, rods, screws and plates used to anchor fractured bones while they heal.

Electrically-powered implants

Active implants require electricity for their operation. Artificial pacemaker is an example of such devices that is used for treatment of Bradycardia in which the heart beats too slowly. Pacemakers can help raise the heart beat to a more normal rate through electrical stimulation of heart. Active implants could be powered up using batteries, transcutaneous energy transmission, or scavenging energy from the environment .

Bio-implants

A **bio-implant** may be defined as a biomaterial surgically implanted in a person's body to replace damaged tissue. Common areas of application include orthopedic (especially maxillofacial) re-constructive prosthesis, cardiac prostheses (artificial heart valves like the Chitra heart valve), skin and cornea.

Dental implants

Dental implants are one of the few medical devices which permanently cross the boundary between the inside and the outside of the body, since the base of the implant is osseointegrated in the bone of the mandible or maxilla and the top of the implant is in the mouth, where it can be crowned with an artificial tooth.

Orthopedic implants

In orthopedic surgery, *implants* may refer to devices that are placed over or within bones to hold a fracture reduction while *prosthesis* would be the more appropriate term for devices that replace a part or whole of a defunct joint. (In this context *implants* may be placed within or outside the body.)

Types of orthopedic implants

There are many types of orthopedic implants and each orthopedic implant is designed to correct the affected joint so that it withstands the associated movement and stress and to

enhance mobility and decrease pain. Broadly speaking, Orthopedic implants are available for the hip, knee, shoulder and elbow. Safety Locking Plates

- Interlocking Nail
- Nails, Wires & Pins
- Cranio Maxillofacial Implants
- Mini Fragment Implants
- Small Fragment Implants
- Large Fragment Implants
- Cannulated Screws
- DHS/DCS & Angled Blade Plates
- Hip Prosthesis
- ACL/PCL Reconstruction System
- Spine Surgery
- External Fixators...

Complications

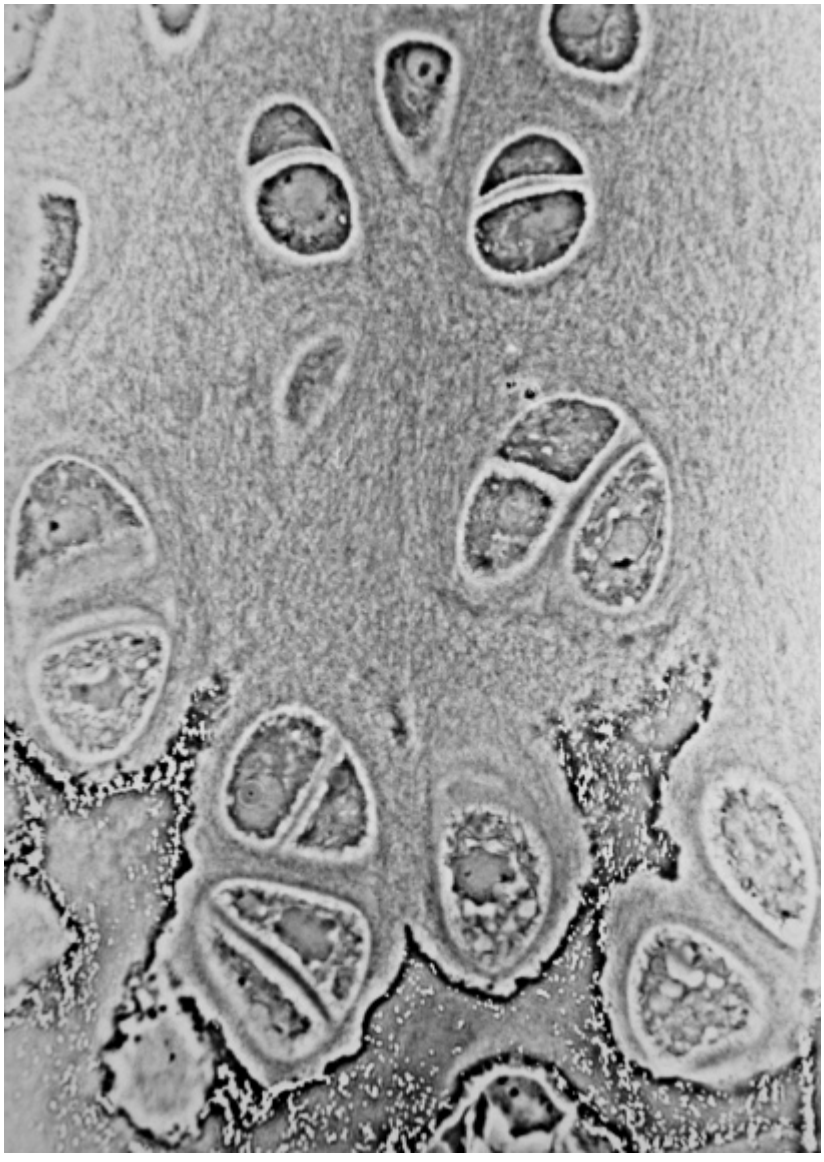
The process of implantation of medical devices is subject to the same complications as any other invasive medical procedure, including infection, inflammation, and pain. Implants also run the risk of rejection if they ellicit a reaction from the host immune system.

Failures

There have been many examples of implant failures, including rupture of silicone breast implants, hip replacement joints and artificial heart valves, such as the Bjork–Shiley valve, all of which have caused FDA intervention. The consequences of implant failure depend on the critical nature of the implant, and its position in the body. Thus heart valve failure is likely to threaten the life of the individual, while breast implant or hip joint failure is less likely to be life-threatening.

Chapter 6

Cartilage



Hyaline cartilage showing chondrocytes and organelles, lacunae and matrix

Cartilage is a flexible connective tissue found in many areas in the bodies of humans and other animals, including the joints between bones, the rib cage, the ear, the nose, the elbow, the knee, the ankle, the bronchial tubes and the intervertebral discs. It is not as hard and rigid as bone but is stiffer and less flexible than muscle.

Cartilage is composed of specialized cells called chondroblasts that produce a large amount of extracellular matrix composed of Type II collagen (except fibrocartilage which also contains type I collagen) fibers, abundant ground substance rich in proteoglycan, and elastin fibers. Chondroblasts that get caught in the matrix are called chondrocytes. They lie in spaces, called lacunae, up to eight chondrocytes per lacunae. Cartilage is classified in three types, **elastic cartilage**, **hyaline cartilage** and **fibrocartilage**, which differ in the relative amounts of these three main components.

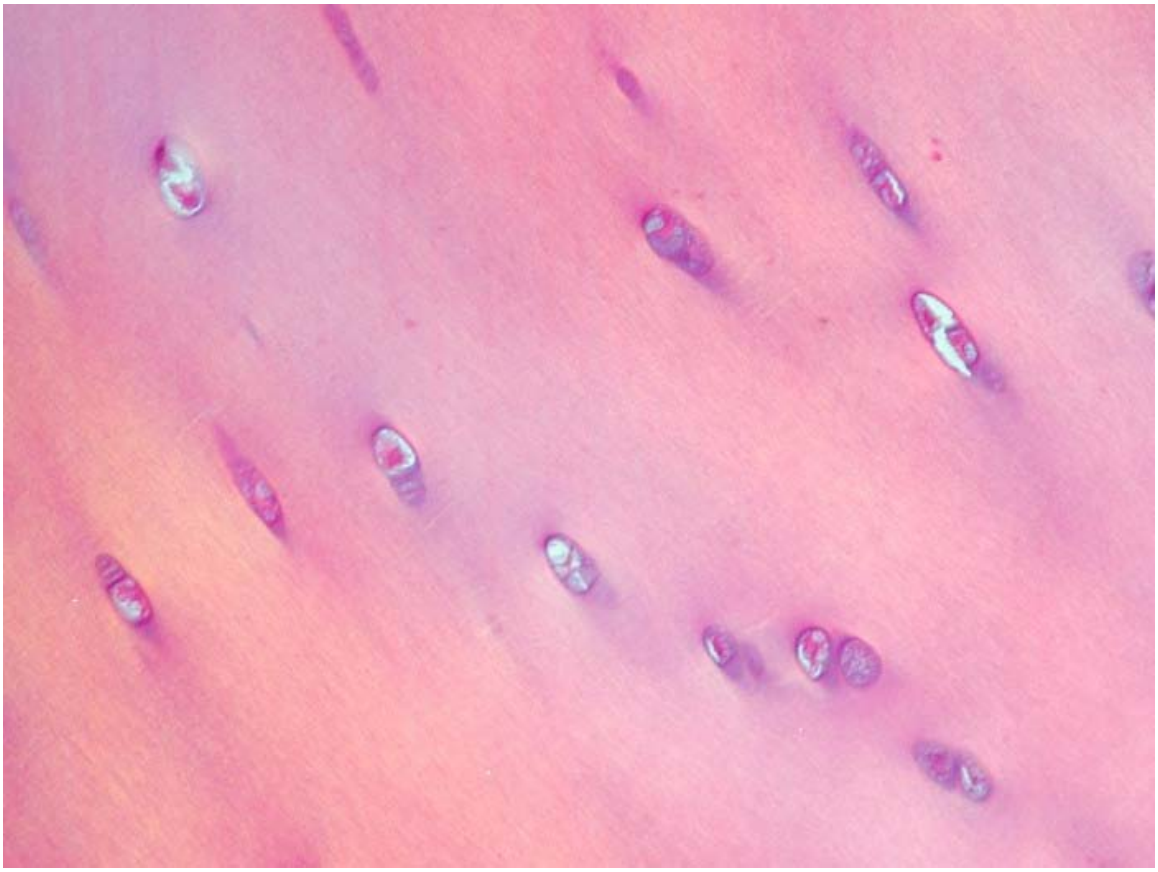
Unlike other connective tissues, cartilage does not contain blood vessels. Because of this, it heals very slowly. The chondrocytes are supplied by diffusion, helped by the pumping action generated by compression of the articular cartilage or flexion of the elastic cartilage. Thus, compared to other connective tissues, cartilage grows and repairs more slowly.

Growth and development

In embryogenesis, the skeletal system is derived from the mesoderm germ layer. Chondrification (also known as chondrogenesis) is the process by which cartilage is formed from condensed mesenchyme tissue, which differentiates into chondroblasts and begins secreting the molecules that form the extracellular matrix.

Imaging

Cartilage does not absorb x-rays under normal In vivo conditions, but a dye can be injected into the synovial membrane so the x-rays will be absorbed by the dye. The resulting void on the radiographic film between the bone and meniscus represents the cartilage. For In vitro x-ray scans the outer soft tissue is most likely removed so the cartilage and air boundary is enough to contrast the presence of cartilage due to refraction of the x-ray.



Histological image of hyaline cartilage stained with haematoxylin & eosin, under polarized light

Diseases and treatment

There are several diseases which can affect the cartilage. Chondrodystrophies are a group of diseases characterized by disturbance of growth and subsequent ossification of cartilage. Some common diseases affecting/involving the cartilage are listed below.

- Osteoarthritis: The cartilage covering bones (articular cartilage - a subset of hyaline cartilage) is thinned, eventually completely worn out, resulting in a "bone against bone" joint, reduced motion and pain. Osteoarthritis affects the joints exposed to high stress and is therefore considered the result of "wear and tear" rather than a true disease. It is treated by Arthroplasty, the replacement of the joint by a synthetic joint often made of a Stainless Steel alloy (cobalt chromoly) and High Molecular Weight Polyethylene (HMWPE). Chondroitin sulfate, a monomer of the polysaccharide portion of proteoglycan, has been claimed to reduce the symptoms of osteoarthritis, possibly by increasing the synthesis of the extracellular matrix, but recent research has not produced evidence to support this claim
- Traumatic rupture or detachment: The cartilage in the knee is frequently damaged, and can be partially repaired through knee cartilage replacement therapy
- Achondroplasia: Reduced proliferation of chondrocytes in the epiphyseal plate of long bones during infancy and childhood, resulting in dwarfism.
- Costochondritis: Inflammation of cartilage in the ribs, causing chest pain.

- Spinal disc herniation : Asymmetrical compression of an intervertebral disc ruptures the sac-like disc, causing a herniation of its soft content. The hernia often compresses the adjacent nerves and causes back pain.
- Relapsing polychondritis: a destruction, probably autoimmune, of cartilage, especially of the nose and ears, causing disfiguration. Death occurs by suffocation as the larynx loses its rigidity and collapses.

Tumors made up of cartilage tissue, either benign or malignant, can occur. They usually appear in bone, rarely in pre-existing cartilage. The benign tumors are called chondroma, the malignant ones chondrosarcoma. Tumors arising from other tissues may also produce a cartilage-like matrix, the best known being pleomorphic adenoma of the salivary glands. Conversely, chondrostatin, an ingredient of cartilage, is being investigated by Washington University researchers for its potential ability to shrink breast and musculoskeletal tumors.

The matrix of cartilage acts as a barrier, preventing the entry of lymphocytes or diffusion of immunoglobulins. This property allows for the transplantation of cartilage from one individual to another without fear of tissue rejection.

Repair

Cartilage has limited repair capabilities: Because chondrocytes are bound in lacunae, they cannot migrate to damaged areas. Therefore if damaged, it is difficult to heal. Also, because hyaline cartilage does not have a blood supply, the deposition of new matrix is slow. Damaged hyaline cartilage is usually replaced by fibrocartilage scar tissue. Over the last years, surgeons and scientists have elaborated a series of cartilage repair procedures that help to postpone the need for joint replacement.

Bioengineering techniques are being developed to generate new cartilage, using a cellular "scaffolding" material and cultured cells to grow artificial cartilage.

Cartilage in animals

Cartilaginous fish

Cartilaginous fish (chondrichthyes) like sharks, rays and skates have a skeleton composed entirely of cartilage. Shark cartilage is a popular but unproven dietary supplement.

Invertebrate cartilage

Cartilage tissue can also be found among invertebrates such as horseshoe crabs, marine snails, and cephalopods.

Chapter 7

Artificial Pancreas

The **artificial pancreas** is a technology in development to help people with diabetes automatically control their blood glucose level by providing the substitute endocrine functionality of a healthy pancreas.

There are several important exocrine (digestive) and endocrine (hormonal) functions of the pancreas, but it is the lack of insulin production which is the motivation to develop a substitute. While the current state of insulin replacement therapy is appreciated for its life-saving capability, the task of manually managing the blood sugar level with insulin alone is arduous and inadequate.

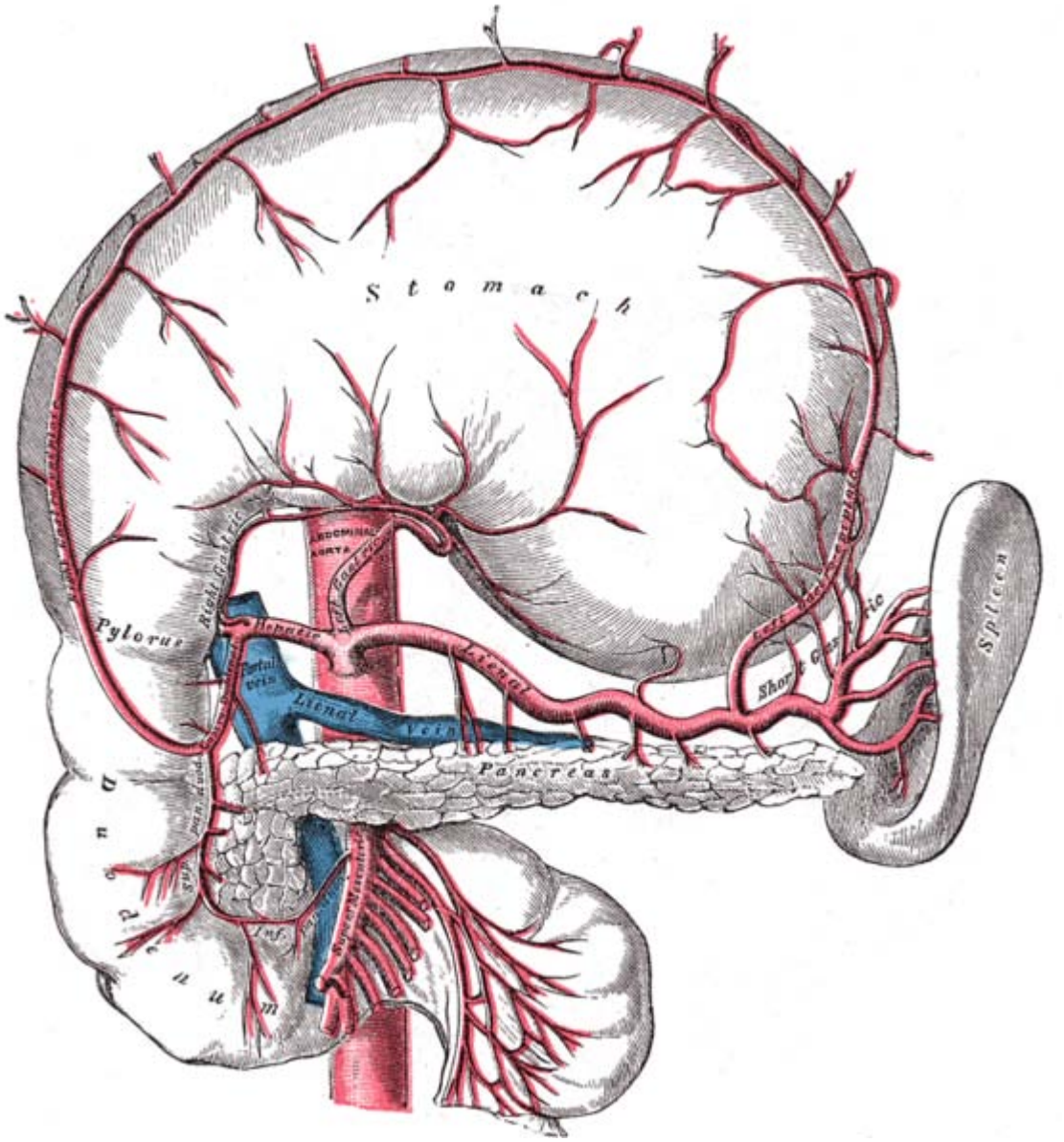
The goal of the artificial pancreas is threefold:

1. to improve insulin replacement therapy until glycemic control is practically normal as evident by the avoidance of the complications of hyperglycemia, and
2. to ease the burden of therapy for the insulin-dependent.
3. to mimic normal stimulation of the liver by the pancreas.

Different approaches under consideration include:

- the medical equipment approach -- using an insulin pump under closed loop control using real-time data from a continuous blood glucose sensor.
- the bioengineering approach -- the development of a bio-artificial pancreas consisting of a biocompatible sheet of encapsulated beta cells. When surgically implanted, the islet sheet will behave as the endocrine pancreas and will be viable for years.
- the stimulation of the liver through delivery of pulses of insulin as seen in normal pancreatic insulin stimulation of hepatic tissues.
- the gene therapy approach -- the therapeutic infection of a diabetic person by a genetically engineered virus which causes a DNA change of intestinal cells to become insulin-producing cells.

Background in endocrine physiology



The pancreas (below the stomach and above the duodenum) releases endocrine hormones (insulin, amylin, and glucagon) into the portal vein, where it flows directly to the liver.

The pancreas produces three hormones that are important to glycemic control:

- insulin, which lowers blood glucose;
- amylin, which slows digestion and slows the rate of glucose entering the bloodstream, and temporarily suppresses release of glucagon;
- and glucagon, which raises the blood glucose.

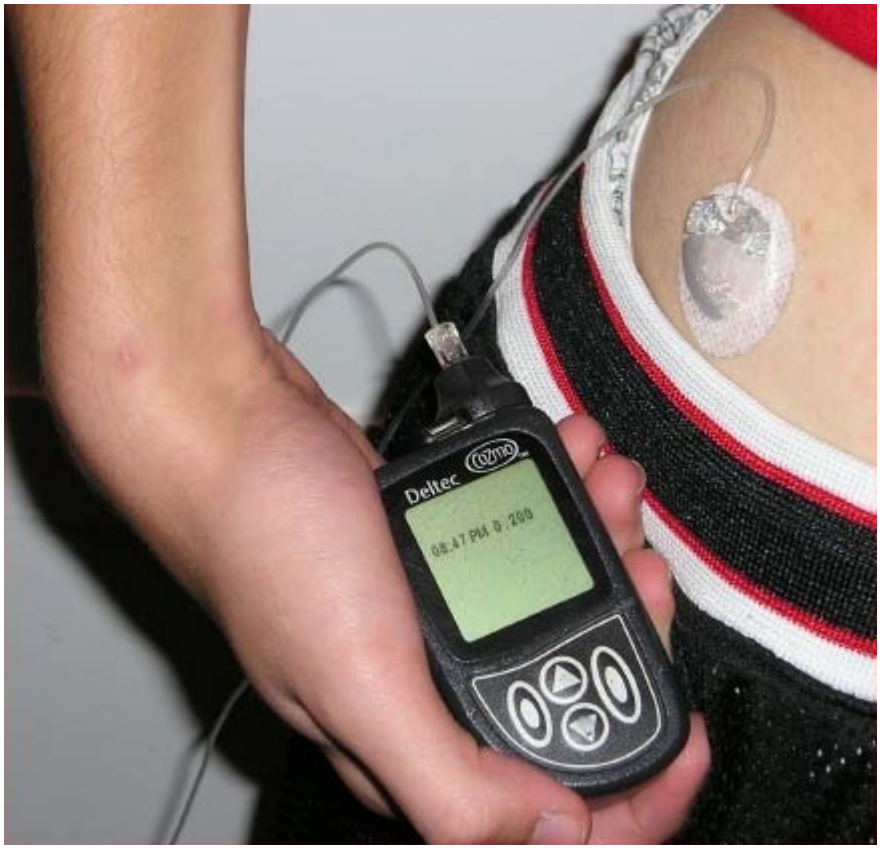
Upon digestion of carbohydrates, glucose levels in the blood will begin to rise. As the blood and glucose flow into the pancreas, insulin and amylin are cosecreted by the pancreatic beta cells directly into the bloodstream in response to elevated blood glucose levels. In the presence of glucose these insulin responses are almost exclusively delivered

in boluses every 4 to 6 minutes. Insulin causes blood glucose to be removed from the bloodstream and stored in the liver and muscle cells. Notice as the blood sugar goes higher, additional insulin will bring the blood sugar back down in a classic negative feedback loop. As insulin is released from the beta cells, amylin is also released into the bloodstream. Amylin slows gastric emptying, and also inhibits the release of glucagon from the pancreatic alpha cells. The effect of amylin is to spread out the blood glucose peak after eating, reducing the quantity of insulin needed. As the blood sugar level comes back toward normal, the beta cells will stop spurting insulin and amylin. As the glucose level approaches a low mark, the pancreatic alpha cells will release glucagon directly into the bloodstream. Glucagon causes the liver to release stored glucose back into the bloodstream. Notice that increased glucagon will increase blood glucose levels to produce a positive error in the negative feedback loop. Together, the three endocrine hormones work as a system to maintain the blood glucose level between high and low boundaries. By delivering the insulin in boluses as presented by a non-diabetic pancreas, the goal of an artificial pancreas can be achieved.

When the beta cell produces insulin from proinsulin, a connecting peptide (or C-peptide) is also manufactured and released into the bloodstream. Absence of C-peptide in the blood indicates that insulin has not been released from the pancreas, and this fact confirms the diagnosis of diabetes type 1. C-peptide was believed to be only a by-product of natural insulin production, however recent studies suggest that C-peptide exerts beneficial therapeutic effects on diabetic nociceptive neuropathy.

Ideally, to replicate the natural function of the pancreas as closely as possible, an artificial pancreas might someday replace all of the beneficial endocrine functions lost, including the delivery of insulin, amylin, glucagon, and C-peptide.

Background in insulin therapy



The Insulin pump is used to automatically deliver basal insulin continuously, and bolus insulin at meal times by pressing the buttons. Before meals, a blood glucose value is entered into the pump to calculate the correction bolus to bring the blood glucose level back to the target value.

In insulin-dependent persons, blood glucose levels have been roughly controlled using insulin alone. The number of grams of carbohydrate is estimated by measuring foods, and the measurement is used to determine the amount of insulin necessary to *cover* the meal. The calculation is based on a simple *open-loop model*: an insulin to carbohydrate ratio (adjusted based on past success) is multiplied by the grams of carbohydrate to calculate the units of insulin needed. That quantity of insulin is then adjusted based on a pre-meal blood glucose measurement (insulin bolus increased for a high blood sugar or insulin bolus delayed and reduced for a low blood sugar). Insulin is injected or infused under the skin, and enters the bloodstream in approximately 15 minutes. After the insulin has acted in the bloodstream, the blood glucose level can be tested again and then adjusted with injection of more insulin, or eating more carbohydrates, until balance is restored. Assuming the design requirement is to truly mimic normal pancreatic delivery of insulin to the liver in order to achieve proper hepatic stimulation, and to cause normal insulin induced functions, until another system is available to deliver portal vein concentrations of insulin, a intravenous infusion device will be needed.

There are notable differences with insulin replacement compared to the function of pancreatic insulin delivery:

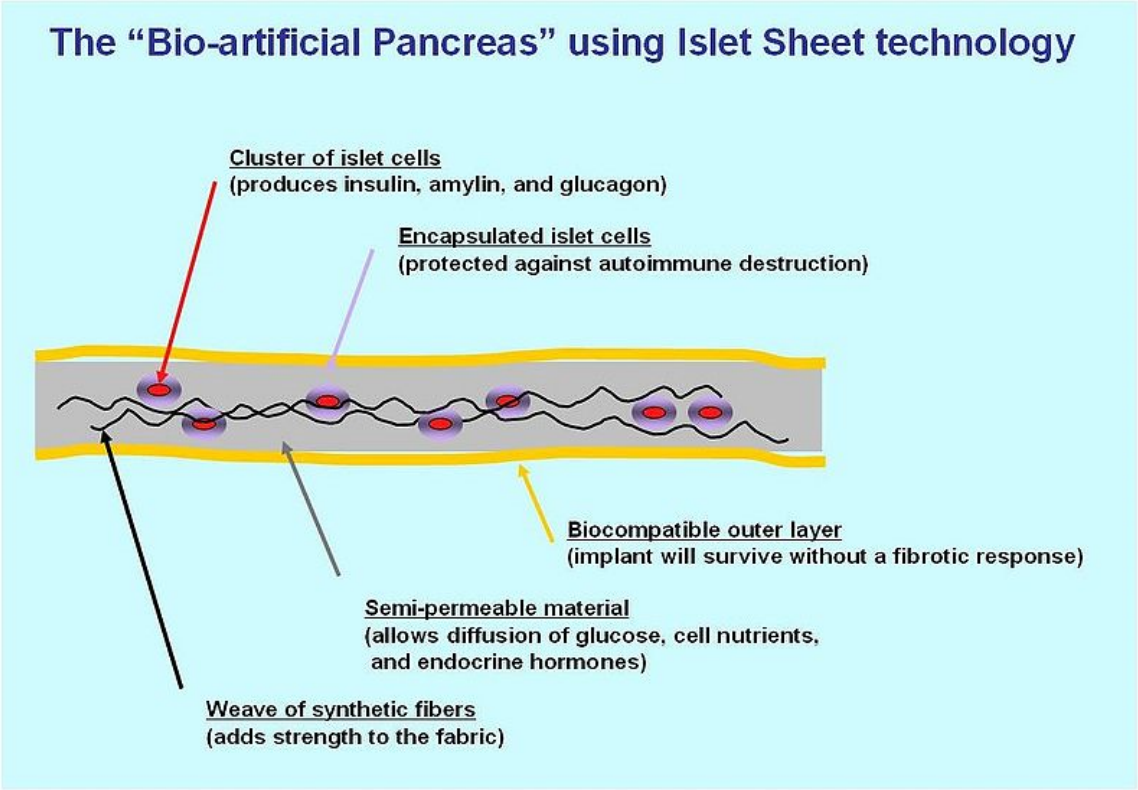
1. the insulin dose is predicted based on measured food (where accuracy of measured carbohydrate is difficult) whereas pancreatic insulin is released in proportional response to actual blood glucose levels;
2. pancreatic insulin is released into to the portal vein, where it flows almost directly to the liver, which is the major organ for storing glycogen (50% of insulin produced is used by the liver);
3. pancreatic insulin is pulsatile which helps maintain the insulin sensitivity of hepatic tissues;
4. injected insulin is delivered subcutaneously (under the skin) but not directly to the bloodstream, so there is a delay before injected insulin begins to reduce blood glucose (although this can be compensated by injecting insulin 15 minutes before eating);
5. insulin which is not delivered intravenously cannot achieve normal momentary concentrations in the portal vein which connects the pancreas to the liver;
6. replacement insulin therapy does not include amylin (although Symlin is now available for use), which can reduce the insulin need by 50%;
7. replacement insulin is dosed as a best compromise between aggressive use for lowering the blood sugar when eating but also conservative use to avoid a post-prandial low blood sugar due to excess insulin, whereas pancreatic function releases insulin aggressively and later includes automatic release of glucagon at the end of an insulin cycle to manage the blood sugar level and avoid hypoglycemia.

An insulin pump to infuse a rapid-acting insulin is the first step in simulating the function of the pancreas. The pump can accurately deliver small increments of insulin compared to an injection, and its electronic controls permit shaping a bolus over time to match the insulin profile required for a given situation. The insulin pump is controlled by the pump user to bolus manually based on a recent blood glucose measurement and an estimate of the grams of carbohydrate consumed. This predictive approach is said to be *open-loop*. Once a bolus has been calculated and delivered, the pump continues to deliver its basal rate insulin in the manner that has been programmed into the pump controls based on the predicted insulin requirements of its user.

While insulin replacement is appreciated as a life saving therapy, its practical use in controlling blood glucose levels sufficiently to avoid the long term complications associated with hyperglycemia is not ideal. Also, it is generally agreed that even with very tight glucose control, there are a significant number of patients who go on to develop all of the life impacting complications of diabetes. Thus, the goal of the Artificial Pancreas should be to normalize carbohydrate and lipid metabolism at a minimum.

Approaches to an Artificial Pancreas

Bioengineering approach



The Bio-artificial pancreas: this diagram shows a cross section of bio-engineered tissue with encapsulated islet cells which deliver endocrine hormones in response to glucose.

A biological approach to the artificial pancreas is to implant bioengineered tissue containing islet cells, which would secrete the amount of insulin, amylin, and glucagon needed in response to sensed glucose.

When islet cells have been transplanted via the Edmonton protocol, insulin production (and glycemic control) was restored at the expense of immunosuppression. Encapsulation of the islet cells in a protective coating has been developed to block the immune response to transplanted cells, which relieves the burden of immunosuppression and benefits the longevity of the transplant.

One concept of the bio-artificial pancreas uses encapsulated islet cells to build an *islet sheet* which can be surgically implanted to function as an artificial pancreas.

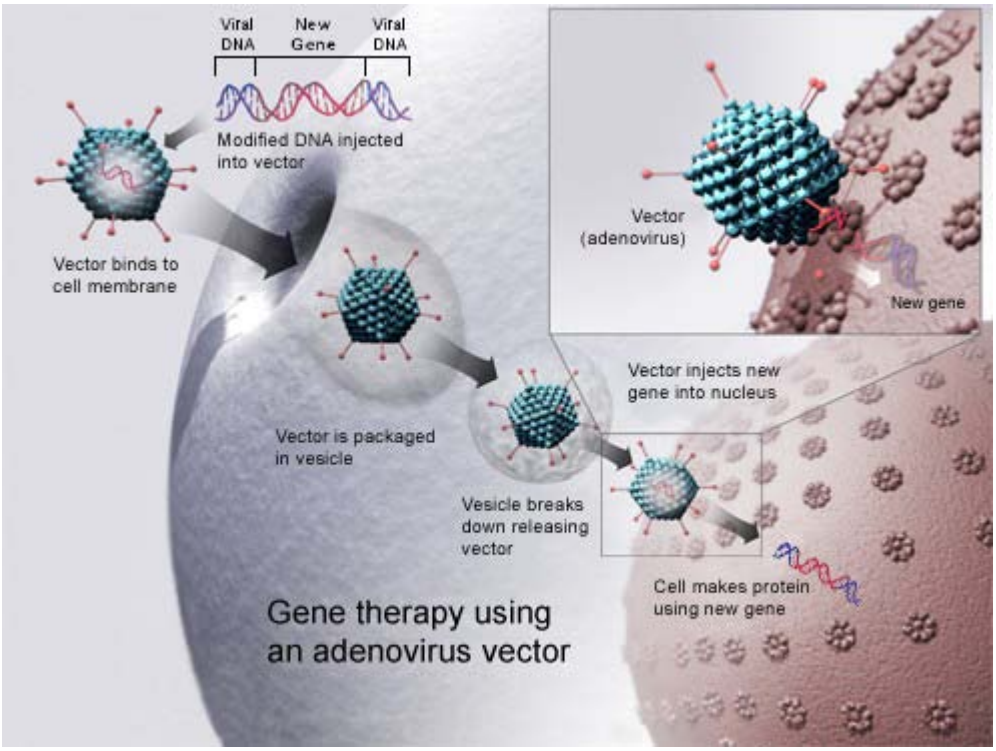
This islet sheet design consists of:

- an inner mesh of fibers to provide strength for the islet sheet;
- islet cells, encapsulated to avoid triggering a proliferating immune response, adhered to the mesh fibers;
- a semi-permeable protective layer around the sheet, to allow the diffusion of nutrients and secreted hormones;

- a protective coating, to prevent a foreign body response resulting in a fibrotic reaction which walls off the sheet and causes failure of the islet cells.

Islet sheet research is pressing forward with large animal studies at the present, with plans for human clinical trials within a few years.

Gene therapy approach



Gene therapy: Designing a viral vector to deliberately infect cells with DNA to carry on the viral production of insulin in response to the blood sugar level.

Technology for gene therapy is advancing rapidly such that there are multiple pathways possible to support endocrine function, with potential to practically cure diabetes.

- Gene therapy can be used to **manufacture insulin directly**: an oral medication, consisting of viral vectors containing the insulin sequence, is digested and delivers its genes to the upper intestines. Those intestinal cells will then behave like any viral infected cell, and will reproduce the insulin protein. The virus can be controlled to infect only the cells which respond to the presence of glucose, such that insulin is produced only in the presence of high glucose levels. Due to the limited numbers of vectors delivered, very few intestinal cells would actually be impacted and would die off naturally in a few days. Therefore by varying the amount of oral medication used, the amount of insulin created by gene therapy can be increased or decreased as needed. As the insulin producing intestinal cells die off, they are boosted by additional oral medications.
- Gene therapy might eventually be used to **cure the cause of beta cell destruction**, thereby curing the new diabetes patient before the beta cell destruction is complete and irreversible.

- Gene therapy can be used to **turn duodenum cells and duodenum adult stem cells into beta cells** which produce insulin and amylin naturally. By delivering beta cell DNA to the intestine cells in the duodenum, a few intestine cells will turn into beta cells, and subsequently adult stem cells will develop into beta cells. This makes the supply of beta cells in the duodenum self replenishing, and the beta cells will produce insulin in proportional response to carbohydrates consumed.

Medical equipment approach

Development of Continuous Blood Glucose Monitoring

Technology for continuous blood glucose monitoring supports the mission of the artificial pancreas by:

1. automatically providing a blood glucose reading every few minutes without finger sticks from the user,
2. monitoring trends pertaining to rising and falling blood sugars, which is helpful in the prediction of blood glucose levels in the immediate future,
3. comparing blood sugar levels and predictions against a high blood sugar threshold, and then prompting the user that a correction bolus from an insulin pump is needed immediately,
4. comparing blood sugar levels and predictions against a low blood sugar threshold, and then prompting the user to reduce the basal insulin from the pump or to eat something.

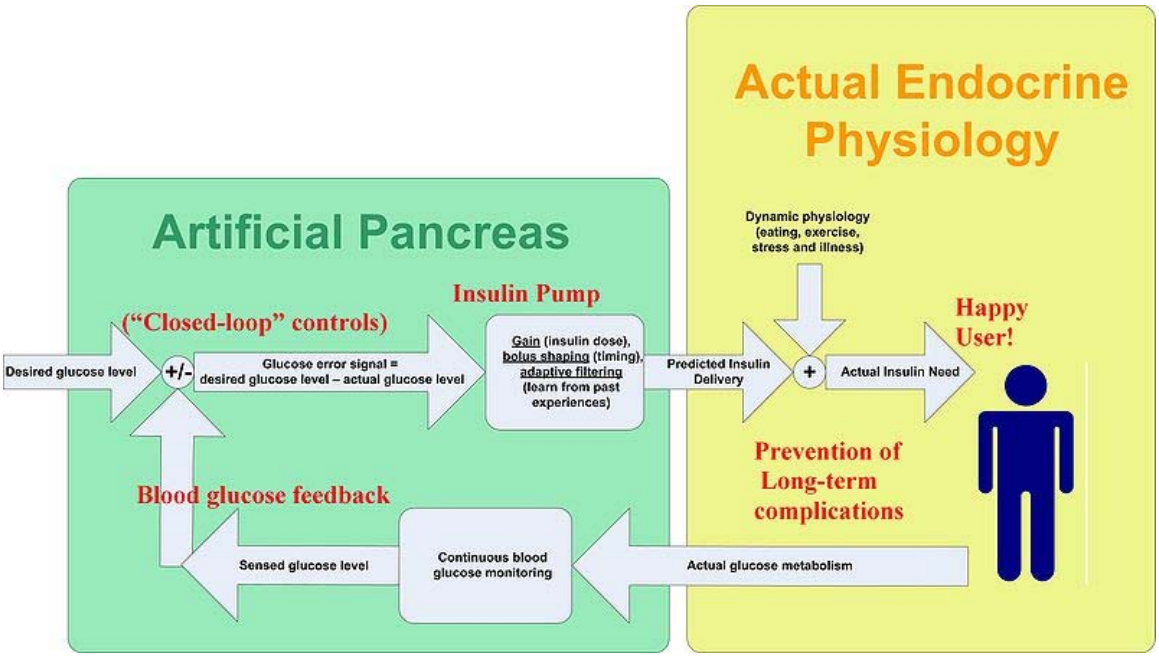
These capabilities suggest that a stream of real-time data can be used to "close the loop" and control the insulin pump directly.

Some issues with the present performance of continuous sensing technology suggest that additional study is needed for application to the artificial pancreas:

1. continuous sensors require calibration a few times a day, by performing a manual blood glucose test with a finger stick, and then entering the blood glucose data into the continuous system for a sensor correction,
2. continuous sensors are measuring interstitial glucose, so there is a time delay between the sensor data and the true blood glucose,
3. automatic control removes the intellect of the user, which can be an additional safeguard when the data is subject to error and must be verified before taking action.

As the state of the art for blood glucose monitoring continues to advance, so does the promise of the artificial pancreas.

Feedback of real-time blood glucose data to an insulin pump for basal control



The medical equipment approach to an artificial pancreas: automatic control of an insulin pump with feedback from a continuous blood glucose sensor.

The first step in controlling an insulin pump based on continuous blood glucose data is to automatically control the basal rate of the insulin pump. When a bolus has not recently been performed, the pump can manage the blood glucose level by adjusting the basal rate as needed:

- when the blood sugar is increasing, a small correction bolus can be automatically delivered and a higher basal rate can be set;
- when the blood sugar is decreasing, the basal rate can be halted to deny the quantity of insulin needed to bring the blood glucose level back up until the basal rate can be continued at a new lower rate;
- and with adaptive filtering techniques, the pump can "learn" the unique basal rates for the person as a function of the time of day.

When controlling the basal rate alone, the closed loop can still correct a meal bolus error that was too large or small for the food consumed by:

- recognizing an imbalance between the bolus "insulin on board" and the level of blood glucose,
- automatically bolusing to correct a shortage of insulin,
- automatically reducing or interrupting the basal rate to correct an abundance of insulin,
- and using adaptive filtering techniques to "learn" the carbohydrate to insulin ratios for each meal bolus.

First clinical tests: implantable insulin pumps and continuous glucose sensors

In France, a human clinical trial of an artificial pancreas is underway. The system is fully automated by combining Medtronic MiniMed's long-term glucose sensor and its implantable insulin pump. A summary of the project shows promise as well as some present limitations:

- The implantable sensor is inserted into a neck vein leading to the heart.
- The sensor is connected, via an electrical-type wire under the skin, to the implantable insulin pump: as blood sugar levels fluctuate, a signal tells the pump how much insulin to deliver.
- The sensor accurately measured glucose in 95% of cases when compared with values obtained by fingersticks.
- The blood glucose levels were maintained in the normal range more than 50% of the time in the patients using the pump connected to the sensor.
- Events of hypoglycemia dropped to less than 5%.
- While implantable insulin pumps work for an average of eight years before they have to be changed, the sensors stop working after an average of nine months,
- The mathematical programs that calculate just how much insulin should be delivered at different parts of the day also needs to be refined.

Insulin and amylin combination

When **pramlintide** (brand name Symlin or synthetic amylin) is used in combination with insulin, the benefits for post-prandial glycemic control are substantial.

Pramlintide is a relatively new treatment for diabetes. The treatment involves:

- a separate injection of pramlintide before a meal,
- a reduction in insulin bolus by 50% for that meal.

Pramlintide can be infused using an insulin pump. At the present time, the mixing of pramlintide and insulin in the same cartridge is not an approved practice, so two infusion pumps are used simultaneously. Since insulin and amylin are co-secreted by the pancreatic beta cells in response to raising blood glucose levels, using pramlintide and insulin together more closely duplicates the function of the pancreas.

Symlin has potential to support the artificial pancreas project because:

- Insulin and pramlintide may in the future be automatically infused together
  - at a mixture from a single automatic insulin pump, or
  - two infusion pumps could be used automatically with the insulin pump acting as master and the simlin pump acting as slave, or
  - a dual system in one pump machine (two cartridges, a dual infusion set tube, and two subcutaneous insertions);
- because it improves post prandial glycemic excursions relative to insulin alone, this supports the possible use of an automatic bolus with less impact due to the delay of the insulin bolus;
- and because it simply duplicates the natural pancreas function, the full benefits of which are not fully understood.

Feedback of real-time blood glucose data to an insulin pump for bolus control

The ability of the electronic controls of the infusion pump, particularly in the bolus shaping capability, suggests that the control algorithm may replicate the function of the healthy pancreas in a more copycat fashion. At present, the insulin bolus is a predictive dose based on what is about to be eaten, and then infused completely. Even with the benefit of the closed-loop control of the basal insulin, the standard bolus is still a "guess and then fix it later" approach. Compare to the pancreatic physiology, where insulin and amylin are released from the beta cells in pulses almost directly to the liver in response to the immediate blood glucose level. The natural release from the beta cells is a closed loop response to sensed glucose, and the shape of the insulin delivery is adaptable and appropriate to the food eaten and the body's present metabolic capability.

As technology for continuous blood glucose monitoring improves, the integrated components will support a typical application of control theory by employing the proportional, integral, and derivative control algorithm. This will make it feasible to infuse an *adaptive bolus* that changes its shape and integral dose based on the measured performance of the bolus in progress, depending on:

- **the rate of glucose increase** (i.e the derivative function would deliver more insulin for a rapid increase in blood sugar);
- **the peak of the glucose curve** (i.e. the proportional function would deliver more insulin for a higher peak in the blood sugar); and
- **the duration of elevated glucose** (i.e. the integral function would deliver more insulin for a long duration of high blood sugar).

The adaptive bolus could start with an assumption of a typical proportions and a bolus shape like the *combination bolus*. This could include:

1. a prebolus of pramlintide (optional perhaps, but resolves issue with insulin timing)
2. initiation of a combination bolus with the initial spike sized in proportion to the present blood glucose level and trends in the change of blood glucose level,
3. modification to the square wave portion of the bolus, increasing or extending if blood sugar is increasing, and decreasing or limiting in duration when blood sugar is decreasing.

The benefits of an automatic bolus delivery might include:

- increased accuracy in the total insulin delivered relative to what was needed,
- freedom to the user of the artificial pancreas,
- elimination of glycemic excursions due to user error (such as forgetting to bolus in conventional pump therapy),
- adaptability to changes in digestion of carbohydrates based on food choices,
- adaptability to variable metabolic needs due to stress, illness, or exercise.

Glucagon combination

The purpose of glucagon is to raise blood sugar, primarily by promoting release of stored glucose in the liver. Human glucagon has been synthesized by recombinant DNA technology and is available in a dry powder form in the glucagon rescue kit. Glucagon injection pens are also sometimes provided to diabetics in the UK along with insulin. This is useful for rescue of unconscious diabetics from a severe state of hypoglycemia.

In healthy pancreatic function, glucagon production is initially suppressed by beta cell production of insulin and amylin when blood sugar is high, and then is later produced by low or falling blood sugar. The natural pancreatic function uses glucagon at the end of an insulin cycle to release glucose from the liver, with two advantages:

1. to prevent low blood sugar, and
2. to speed the overall insulin action by cancelling the insulin tail.

If an artificial pancreas was to simulate the natural endocrine pancreas to the maximum extent, then insulin and amylin would be used at the beginning of an insulin cycle and glucagon would be used at the end of the insulin cycle. Research with diabetic pigs given insulin-glucagon combination via separate subcutaneous infusion pumps demonstrated closed loop control without incidence of hypoglycemia. While the copycat endocrine function including glucagon seems desirable, the benefits relative to the cost and complexity of an artificial pancreas without glucagon are not yet known.

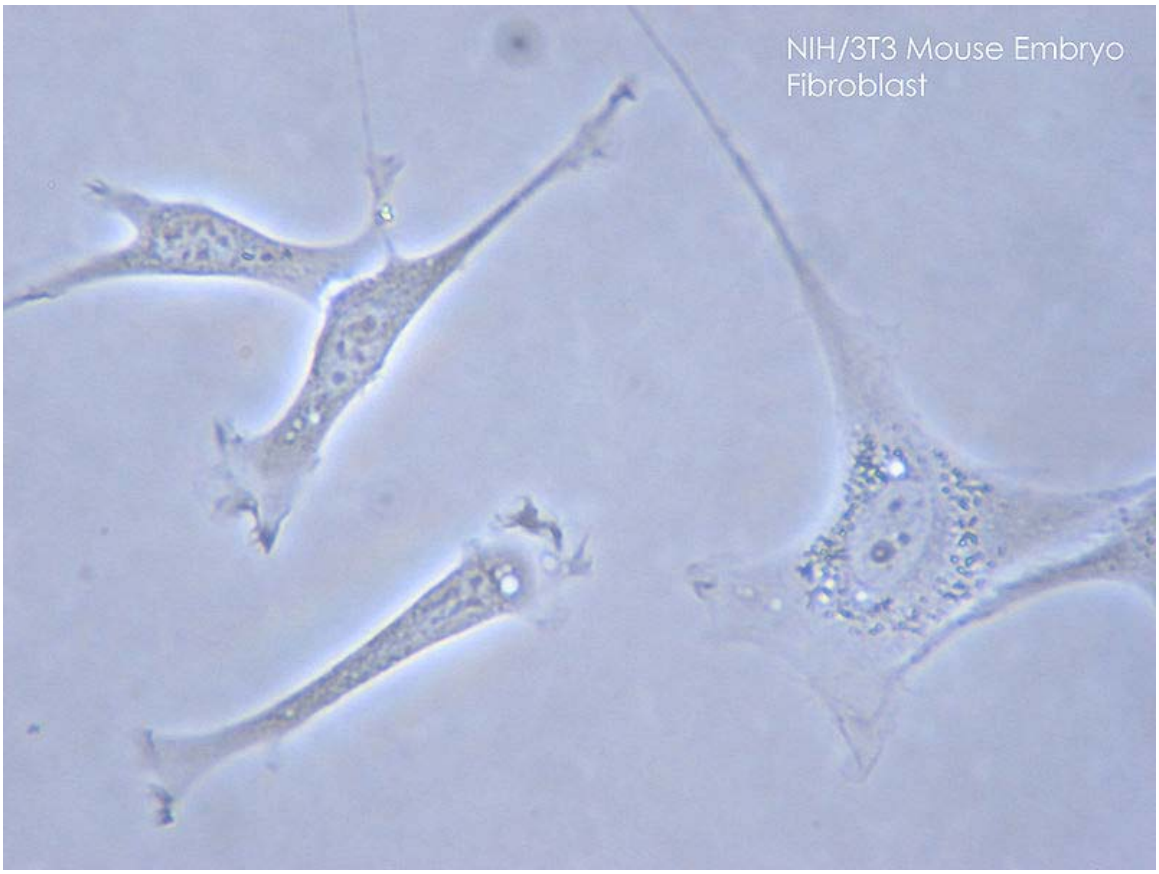
Initiatives around the globe

In the United States in 2006, the Juvenile Diabetes Research Foundation (JDRF) launched a multi-year initiative to help accelerate the availability of an artificial pancreas to people with diabetes. The overall goal of the Artificial Pancreas Project is to accelerate the development, regulatory approval, and acceptance of continuous glucose monitoring and artificial pancreas technology in the shortest possible timeframe. The long term goal is for broad patient access and a thriving competitive market for these devices and products.

JDRF's role in quickening the development and availability of the Artificial Pancreas consists of funding research in order to look over the outcomes of patients using the Artificial Pancreas, keeping close contact with the Food and Drug Administration so that the standards of the patient are met, advocating for health care coverage of technologies such as the Artificial Pancreas and working to ensure clinical acceptance of technologies such as the Artificial Pancreas.

Chapter 8

Fibroblast



NIH/3T3 Fibroblasts in cell culture

A **fibroblast** is a type of cell that synthesizes the extracellular matrix and collagen, the structural framework (stroma) for animal tissues, and plays a critical role in wound healing. Fibroblasts are the most common cells of connective tissue in animals.

Background information

Fibroblasts and fibrocytes are two states of the same cells, the former being the activated state, the latter the less active state, concerned with maintenance and tissue metabolism. Currently, there is a tendency to call both forms fibroblasts. The suffix "blast" is used in cellular biology to denote a stem cell or a cell in an activated state of metabolism.

Fibroblasts are morphologically heterogeneous with diverse appearances depending on their location and activity. Though morphologically inconspicuous, ectopically transplanted fibroblasts can often retain positional memory of the location and tissue context where they had previously resided, at least over a few generations. This remarkable behavior may lead to discomfort in the rare event that they stagnate there excessively.

Embryologic origin

The main function of fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix. Fibroblasts secrete the precursors of all the components of the extracellular matrix, primarily the ground substance and a variety of fibers. The composition of the extracellular matrix determines the physical properties of connective tissues.

Like other cells of connective tissue, fibroblasts are derived from primitive mesenchyme. Thus they express the intermediate filament protein vimentin, a feature used as a marker to distinguish their mesodermal origin. However, this test is not specific as epithelial cells cultured in vitro on adherent substratum may also express vimentin after some time.

In certain situations epithelial cells can give rise to fibroblasts, a process called epithelial-mesenchymal transition (EMT).

Conversely, fibroblasts in some situations may give rise to epithelia by undergoing a mesenchymal to epithelial transition (MET) and organizing into a condensed, polarized, laterally connected true epithelial sheet. This process is seen in many developmental situations (e.g. nephron and notocord development).

Structure and function

Fibroblasts have a branched cytoplasm surrounding an elliptical, speckled nucleus having one or two nucleoli. Active fibroblasts can be recognized by their abundant rough endoplasmic reticulum. Inactive fibroblasts, which are also called **fibrocytes**, are smaller and spindle shaped. They have a reduced rough endoplasmic reticulum. Although disjointed and scattered when they have to cover a large space, fibroblasts when crowded often locally align in parallel clusters.

Fibroblasts make collagens, glycosaminoglycans, reticular and elastic fibers, glycoproteins found in the extracellular matrix and cytokine TSLP. Growing individuals' fibroblasts are dividing and synthesizing ground substance. Tissue damage stimulates fibrocytes and induces the mitosis of fibroblasts.

Unlike the epithelial cells lining the body structures, fibroblasts do not form flat monolayers and are not restricted by a polarizing attachment to a basal lamina on one side, although they may contribute to basal lamina components in some situations (e.g. subepithelial myofibroblasts in intestine may secrete the α-2 chain carrying component of the laminin which is absent only in regions of follicle associated epithelia which lack the myofibroblast lining). Fibroblasts can also migrate slowly over substratum as individual cells, again in contrast to epithelial cells. While epithelial cells form the lining of body structures, it is fibroblasts and related connective tissues which sculpt the "bulk" of an organism.

The life span of a fibroblast, as measured in chick embryos, is 57 ± 3 days.

Secondary actions

Mouse embryonic fibroblasts (MEFs) are often used as "feeder cells" in human embryonic stem cell research. However, many researchers are gradually phasing out MEFs in favor of culture media with precisely defined ingredients of exclusively human derivation. Further, the difficulty of exclusively using human derivation for media supplements is most often solved by the use of "defined media" where the supplements are synthetic and achieve the primary goal of eliminating the chance of contamination from derivative sources.

Chapter 9

Artificial Organ

An **artificial organ** is a man-made device that is implanted or integrated into a human to replace a natural organ, for the purpose of restoring a specific function or a group of related functions so the patient may return to as normal a life as possible. The replaced function doesn't necessarily have to be related to life support, but often is.

Implied by this definition is the fact that the device must not be continuously tethered to a stationary power supply, or other stationary resources, such as filters or chemical processing units. (Periodic rapid recharging of batteries, refilling of chemicals, and/or cleaning/replacing of filters, would exclude a device from being called an artificial organ.) Thus a dialysis machine, while a very successful and critically important life support device that completely replaces the duties of a kidney, is not an artificial organ. At this time an efficient, self-contained artificial kidney has not become available.

Reasons

Reasons to construct and install an artificial organ, an extremely expensive process initially, which may entail many years of ongoing maintenance services not needed by a natural organ, might include:

- Life support to prevent imminent death while awaiting a transplant (e.g. artificial heart)
- Dramatic improvement of the patient's ability for self care (e.g. artificial limb)
- Improvement of the patient's ability to interact socially (e.g. cochlear implant)
- Cosmetic restoration after cancer surgery or accident

The use of any artificial organ by humans is almost always preceded by extensive experiments with animals. Initial testing in humans is frequently limited to those either already facing death, or who have exhausted every other treatment possibility. (Rarely

testing may be done on healthy volunteers who are scheduled for execution pertaining to violent crimes.)

Although not typically thought of as organs, one might also consider replacement bone, and joints thereof, such as hip replacements, in this context.

Types

There are now many artificial organs that have been implanted in humans, with varying degrees of success.

Brain

Brain pacemakers, including deep brain stimulators, send electrical impulses to the brain in order to relieve depression, epilepsy, tremors of Parkinson's disease, and other conditions such as increased bladder secretions. Rather than replacing existing neural networks to restore function, these devices often serve by disrupting the output of existing malfunctioning nerve centers to eliminate symptoms.

Cardia and pylorus valves

Artificial cardia and pylorus can be used to fight esophageal cancer, achalasia and gastroesophageal reflux disease. This pertains to gastric repairs, specifically of the valves at either end of the stomach.

Corpora cavernosa

To treat erectile disfunction, both corpora cavernosa can be irreversibly surgically replaced with manually inflatable penile implants. This is a drastic therapeutic surgery meant only for men suffering from complete impotence that has resisted all other treatment approaches.

An implanted pump in the (groin) or (scrotum) can be manipulated by hand to fill these artificial cylinders, normally sized to be direct replacements for the natural corpus cavernosa, from an implanted reservoir in order to achieve an erection.

Ear

While natural hearing, to the level of musical quality, is not typically achieved, most recipients are pleased, with some finding it useful enough to return to their surgeon with a request to do the other ear.

Eye

The most successful function-replacing artificial eye so far is actually an external miniature digital camera with a remote unidirectional electronic interface implanted on the retina, optic nerve, or other related locations inside the brain. The present state of the art yields only very partial functionality, such as recognizing levels of brightness,

swatches of color, and/or basic geometric shapes, proving the concept's potential. While the living eye is indeed a camera, it is also much more than that.

Various researchers have demonstrated that the retina performs strategic image preprocessing for the brain. The problem of creating a 100% functional artificial electronic eye is even more complex than what is already obvious. Steadily increasing complexity of the artificial connection to the retina, optic nerve or related brain areas advances, combined with ongoing advances in computer science, is expected to dramatically improve the performance of this technology.

For the person whose damaged or diseased living eye retains some function, other options superior to the electronic eye may be available.

Heart

While considered a success, the use of artificial hearts is limited to patients awaiting transplants whose death is imminent. The current state of the art devices are unable to *reliably* sustain life beyond about 18 months.

Artificial pacemakers are electronic devices which can either intermittently augment (defibrillator mode), continuously augment, or completely bypass the natural living cardiac pacemaker as needed, are so successful that they have become commonplace.

Ventricular assist devices are mechanical circulatory devices that partially or completely replace the function of a failing heart, without the removal of the heart itself.

Limbs

Artificial arms with semi-functional hands, some even fitted with working opposable "thumbs" plus 2 "fingers", and legs with shock absorbing feet capable of allowing a trained patient to even run, have become available. While the meaning of "full mobility" is debated, steady progress is made.

Liver

HepaLife is developing a bioartificial liver device intended for the treatment of liver failure using stem cells. The artificial liver, currently under development, is designed to serve as a supportive device, either allowing the liver to regenerate upon acute liver failure, or to bridge the patient's liver functions until a transplant is available. It is only made possible by the fact that it uses real liver cells (hepatocytes), and even then, it is not a permanent substitute for a liver.

On the other hand, Researchers Dr. Colin McGucklin, Professor of Regenerative Medicine at Newcastle University, and Dr. Nico Forraz, Senior Research Associate and Clinical Sciences Business Manager at Newcastle University, say that pieces of artificial liver could be used to repair livers injured in the next five years. These artificial livers could also be used outside the body in a manner analogous to the dialysis process used to keep alive patients whose kidneys have failed.

Lungs

With some almost fully functional, artificial lungs promise to be a great success in near future. An Ann Arbor company MC3 is currently working on this type of medical device.

Pancreas

For the treatment of diabetes, numerous promising techniques are currently being developed, including some that incorporate donated living tissue housed in special materials to prevent the patient's immune system from killing the foreign live components.

Bladder

Artificial bladders represent a unique success in that these are autologous laboratory-grown living replacements, as opposed to most other artificial organs which depend upon electro-mechanical contrivances, and may or may not incorporate any living tissue.

Ovaries

Reproductive age patients who develop cancer often receive chemotherapy or radiation therapy which damages oocytes and leads to early menopause. An artificial human ovary has been developed at Brown University with self-assembled microtissues created using novel 3-D petri dish technology. The artificial ovary will be used for the purpose of in vitro maturation of immature oocytes and the development of a system to study the effect of environmental toxins on folliculogenesis.

Beyond restoration

It is also possible to construct and install an artificial organ to give its possessor abilities which are not naturally occurring. Research is proceeding, particularly in areas of vision, memory, and information processing, however this idea is still in its infancy.

Some current research focuses on restoring inoperative short-term memory in accident victims and lost access to long-term memory in dementia patients. Success here would lead to widespread interest in applications for persons whose memory is considered healthy to dramatically enhance their memory of far beyond what can be achieved with mnemonic techniques. Given that our understanding of how living memory actually works is incomplete, it is unlikely this scenario will become reality in the near future.

One area of success was achieved in 2002 when a British Scientist, Kevin Warwick, had an array of 100 electrodes fired into his nervous system in order to link his nervous system into the internet. With this in place he carried out a series of experiments including extending his nervous system over the internet to control a robotic hand, a form of extended sensory input and the first direct electronic communication between the nervous systems of two humans.

Another idea with significant consequences is that of implanting a Language Translator for diplomatic and military applications. While machine translation does exist, it is presently neither good nor small enough to fulfill its promise.

This might also include the existing (and controversial when applied to humans) practice of implanting subcutaneous "chips" (integrated circuits) for identification and location purposes. An example of this is the RFID tags made by VeriChip Corporation.

Chapter 10

Prosthesis

A man with two prosthetic arms playing table football.

In medicine, a **prosthesis**, **prosthetic**, or **prosthetic limb** (Greek: *πρόσθεσις* "addition") is an artificial device extension that replaces a missing body part. It is part of the field of biomechatronics, the science of using mechanical devices with human muscle, skeleton, and nervous systems to assist or enhance motor control lost by trauma, disease, or defect.

Prostheses are typically used to replace parts lost by injury (traumatic) or missing from birth (congenital) or to supplement defective body parts. Inside the body, artificial heart valves are in common use with artificial hearts and lungs seeing less common use but under active technology development. Other medical devices and aids that can be considered prosthetics include hearing aids, artificial eyes, palatal obturator, gastric bands, and dentures.

Prosthetics are specifically *not* orthotics, although given certain circumstances a prosthetic might end up performing some or all of the same functionary benefits as an orthotic. Prostheses (or "A" prosthesis) are technically the complete finished item. For instance, a C-Leg knee alone is *not* a prosthesis, but only a prosthetic *part*. The complete prosthesis would consist of the stump attachment system - usually a "socket", and all the attachment hardware parts all the way down to and including the foot. Keep this in mind as often nomenclature is interchanged.

History

Prosthetic toe from ancient Egypt

Prosthetics have been mentioned throughout history. Egyptians were the early pioneers of the idea. Roman bronze crowns have also been found, but their use could have been more aesthetic than medical. The first recorded mention of a prosthetic was done by the historian Herodotus, who tells the story of a Hegistratus, a Persian soldier, who cut off his own foot to escape his captors and replaced it with a wooden one. Pliny the Elder also recorded that a Roman general who had his arm cut off had an iron one made to hold his shield up when he returned to battle. A famous and quite refined historical prosthetic arm was that of Götz von Berlichingen, made in the beginning of the 16th century. Around

the same time, François de la Noue is also reported to have had an iron hand, as is, in the 17th century, René-Robert Cavalier de la Salle. During the Dark Ages, prosthetics remained quite basic in form. Debilitated knights would be fitted with prosthetics so they could be fitted with a shield. Only the wealthy were able to afford anything that would assist in daily function. During the Renaissance, prosthetics also underwent a rebirth. Prosthetics development using iron, steel, copper, and wood started. Functional prosthetics began to make an appearance in the 1500s. Gotz von Berlichingen, a German mercenary, developed a pair of iron hands that could be moved by relaxing a series of releases and springs. Record written by an Italian surgeon also notes the existence of amputee who had an arm that allowed him to remove his hat, open his purse, and sign his name. Improvement in amputation surgery and prosthetic design came at the hands of Ambroise Paré. Among his inventions was an above-knee device that was a kneeling peg leg and foot prosthesis that had a fixed position, adjustable harness, and knee lock control. The functionality of his advancements showed what future prosthetics would function.

Other major improvements before the modern era:

- Pieter Verduyn - First nonlocking below-knee (BK) prosthesis.
- James Potts - Prosthesis made of a wooden shank and socket, a steel knee joint and an articulated foot that was controlled by catgut tendons from the knee to the ankle. Came to be known as “Anglesey Leg” or “Selpho Leg.”
- Sir James Syme - A new method of ankle amputation that did not involve amputating at the thigh.
- Benjamin Palmer - Improved upon the Selpho leg. Added an anterior spring and concealed tendons to simulate natural-looking movement.
- Dubois Parmlee – Created prosthetic with a suction socket, polycentric knee, and multi-articulated foot.
- Marcel Desoutter & Charles Desoutter – First aluminum prosthesis

At the end of World War II, the NAS (National Academy of Sciences) began to advocate better research and development of prosthetics. Through government funding, a research and development program was developed within the Army, Navy, Air Force, and the Veterans Administration.

The following organizations have been created to help and inform the general publics about prosthetics:

- American Orthotics and Prosthetic Association, American Board for Certification in Prosthetics and Orthotics, American Academy of Orthotics and Prosthetics – These three groups work together to take responsibility for the academic side of orthotics and prosthetics and provide certification of individuals and facilities working with orthotics and prosthetics.
- The International Society for Prosthetics and Orthotics – Founded in 1970 and headquartered in Copenhagen, this association helps with the progression in research and clinical practice worldwide. They hold an international conference every three years and publish their own technical journal.

- Association of Children's Orthotic-Prosthetic Clinics – The organization was started in 1950s to advocate research and development of children's prosthetics. They meet annually and have their own publication.
- Amputee Coalition of America – The organization was created in 1990 to improve the lives of amputees. Advocate the improvement of amputee lifestyle through education and also have their own publication, inMotion.

Lower extremity prosthetics

Lower extremity prosthetics describes artificially replaced limbs located at the hip level or lower. The two main subcategories of lower extremity prosthetic devices are 1.trans-tibial (any amputation transecting the tibia bone or a congenital anomaly resulting in a tibial deficiency) and 2.trans-femoral (any amputation transecting the femur bone or a congenital anomaly resulting in a femural deficiency). In the prosthetic industry a trans-tibial prosthetic leg is often referred to as a "BK" or below the knee prosthesis while the trans-femoral prosthetic leg is often referred to as an "AK" or above the knee prosthesis.

Other, less prevalent lower extremity cases include the following:

1. Hip disarticulations - This usually refers to when an amputee or congenitally challenged patient has either an amputation or anomaly at or in close proximity to the hip joint.
2. Knee disarticulations - This usually refers to an amputation through the knee disarticulating the femur from the tibia.
3. Symes - This is an ankle disarticulation while preserving the heel pad.

Lower extremity modern history

Socket technology for lower extremity limbs saw a revolution of advancement during the 1980s when Sabolich Prosthetics, John Sabolich C.P.O., invented the Contoured Adducted Trochanteric-Controlled Alignment Method (CATCAM) socket, later to evolve into the Sabolich Socket. The advancement was due to the difference in the socket to patient contact model. Prior, sockets were made in the shape of a square bucket with no specialized containment for either the patient's bony prominences' or muscular tissue. Sabolich's design held the patient's limb like a glove, locking it into place and distributing the weight evenly over the existing limb as well as the bone structure of the patient. This was the first instance of ischial containment and led to an extreme advancement in patient accomplishment. Because of Sabolich's dedication to research and development in lower extremity prosthetics, Sabolich Prosthetics saw the first above the knee prosthetic patients walk and run step over step with both one leg and two legs missing, walking down stairs, suction sockets, modern plastic and bio elastic sockets, sense of feel technology, and numerous other inventions in the prosthetic field.

The first microprocessor-controlled prosthetic knees became available in the early 1990s. The Intelligent Prosthesis was first commercially available microprocessor controlled prosthetic knee. It was released by Chas. A. Blatchford & Sons, Ltd., of Great Britain, in 1993 and made walking with the prosthesis feel and look more natural. An improved version was released in 1995 by the name Intelligent Prosthesis Plus. Blatchford released another prosthesis, the Adaptive Prosthesis, in 1998. The Adaptive Prosthesis utilized

hydraulic controls, pneumatic controls, and a microprocessor to provide the amputee with a gait that was more responsive to changes in walking speed.

C-Leg knee prosthesis

Two different models of the C-Leg prosthesis

The Otto Bock Orthopedic Industry introduced the **C-Leg** during the World Congress on Orthopedics in Nuremberg in 1997. The company began marketing the C-Leg in the United States in 1999. Other microprocessor-controlled knee prostheses include Ossur's Rheo Knee, released in 2005, the Power Knee by Ossur, introduced in 2006, the Plié Knee from Freedom Innovations and DAW Industries' Self Learning Knee (SLK).

The idea was originally developed by Kelly James, a Canadian engineer, at the University of Alberta. The C-Leg uses hydraulic cylinders to control the flexing of the knee. Sensors send signals to the microprocessor that analyzes these signals, and communicates what resistance the hydraulic cylinders should supply. C-Leg is an abbreviation of 3C100, the model number of the original prosthesis, but has continued to be applied to all Otto Bock microprocessor-controlled knee prostheses. The C-Leg functions through various technological devices incorporated into the components of the prosthesis. The C-Leg uses a knee-angle sensor to measure the angular position and angular velocity of the flexing joint. Measurements are taken up to fifty times a second. The knee-angle sensor is located directly at the axis of rotation of the knee.

Moment sensors are located in the tube adapter at the base of the C-Leg. These moment sensors use multiple strain gauges to determine where the force is being applied to the knee, from the foot, and the magnitude of that force.

The C-Leg controls the resistance to rotation and extension of the knee using a hydraulic cylinder. Small valves control the amount of hydraulic fluid that can pass into and out of the cylinder, thus regulating the extension and compression of a piston connected to the upper section of the knee. The microprocessor receives signals from its sensors to determine the type of motion being employed by the amputee. The microprocessor then signals the hydraulic cylinder to act accordingly. The microprocessor also records information concerning the motion of the amputee that can be downloaded onto a

computer and analyzed. This information allows the user to make better use of the prosthetic.

The C-Leg is powered by a lithium-ion battery housed inside the prosthesis below the knee joint. (cell is actually located within the axis of the joint) On a full charge, the C-leg can operate for up to 45 hours, depending on the intensity of use. A charging port located on the front of the knee joint can be connected to a charging cable plugged directly into a standard outlet. A "pigtail" charging port adapter permits the relocation of the charging port to a location more accessible when the prosthesis has a cosmetic cover applied. The charger cord has lights that allow the user to observe the level of charge when connected to the knee. A 12 volt car charger adapter can also be purchased.

The C-Leg provides certain advantages over conventional mechanical knee prostheses. It provides an approximation to an amputee's natural gait. The C-Leg allows amputees to walk at near walking speed. Variations in speed are also possible and are taken into account by sensors and communicated to the microprocessor, which adjusts to these changes accordingly. It also enables the amputees to walk down stairs with a step-over-step approach, rather than the one step at a time approach used with mechanical knees. The C-Leg's ability to respond to sensor readings can help amputees recover from stumbles without the knee buckling. However, the C-Leg has some significant drawbacks that impair its use. The C-Leg is susceptible to water damage and thus great care must be taken to ensure that the prosthesis remains dry. Otto Bock recommends that each amputee use the C-Leg for up to two months before the system can fully become accustomed to the individual's unique gait. Becoming accustomed to the C-Leg is especially difficult when walking downhill, and amputees should seek help while becoming familiar with the system to avoid injury.

A wide range of amputees can make use of the C-Leg; however, some people are more suited to this prosthesis than others. The C-Leg is designed for use on people who have undergone transfemoral amputation, or amputation above the knee. The C-Leg can be used by amputees with either single or bilateral limb amputations. In the case of bilateral amputations, the application of C-Legs must be closely monitored. In some cases, those who have undergone hip disarticulation amputations can be candidates for a C-Leg. The prosthesis is recommended for amputees that vary their walking speeds and can reach over 3 miles per hour; however, it cannot be used for running. The C-Leg is practical for upwards of 3 miles daily, and can be used on uneven ground, slopes, or stairs. Active amputees, such as bikers and rollerbladers may find the C-Leg suited to their needs.

Certain physical requirements must be met for C-Leg use. The amputee must have satisfactory cardiovascular and pulmonary health. The balance and strength of the amputee must be sufficient to take strides while using prosthesis. The C-Leg is designed to support amputees weighing up to 275 pounds.

Robotic prostheses

In order for a robotic prosthetic limb to work, it must have several components to integrate it into the body's function: Biosensors detect signals from the user's nervous or muscular systems. It then relays this information to a controller located inside the device, and processes feedback from the limb and actuator (e.g., position, force) and sends it to

the controller. Examples include wires that detect electrical activity on the skin, needle electrodes implanted in muscle, or solid-state electrode arrays with nerves growing through them. One type of these biosensors are employed in myoelectric prosthesis.

Mechanical sensors process aspects affecting the device (e.g., limb position, applied force, load) and relay this information to the biosensor or controller. Examples include force meters and accelerometers.

The controller is connected to the user's nerve and muscular systems and the device itself. It sends intention commands from the user to the actuators of the device, and interprets feedback from the mechanical and biosensors to the user. The controller is also responsible for the monitoring and control of the movements of the device.

An actuator mimics the actions of a muscle in producing force and movement. Examples include a motor that aids or replaces original muscle tissue.

Cosmesis

Cosmetic prosthesis has long been used to disguise injuries and disfigurements. With advances in modern technology, cosmesis, the creation of lifelike limbs made from silicone or PVC has been made possible. Such prosthetics, such as artificial hands, can now be made to mimic the appearance of real hands, complete with freckles, veins, hair, fingerprints and even tattoos. Custom-made cosmeses are generally more expensive (costing thousands of US dollars, depending on the level of detail), while standard cosmeses come ready-made in various sizes, although they are often not as realistic as their custom-made counterparts. Another option is the custom-made silicone cover, which can be made to match a person's skin tone but not details such as freckles or wrinkles. Cosmeses are attached to the body in any number of ways, using an adhesive, suction, form-fitting, stretchable skin, or a skin sleeve.

Cognition

Unlike neuromotor prostheses, neurocognitive prostheses would sense or modulate neural function in order to physically reconstitute or augment cognitive processes such as executive function, attention, language, and memory. No neurocognitive prostheses are currently available but the development of implantable neurocognitive brain-computer interfaces has been proposed to help treat conditions such as stroke, traumatic brain injury, cerebral palsy, autism, and Alzheimer's disease. The recent field of Assistive Technology for Cognition concerns the development of technologies to augment human cognition. Scheduling devices such as Neuropage remind users with memory impairments when to perform certain activities, such as visiting the doctor. Micro-prompting devices such as PEAT, AbleLink and Guide have been used to aid users with memory and executive function problems perform activities of daily living.

Prosthetic enhancement

In addition to the standard artificial limb for everyday use, many amputees or congenital patients have special limbs and devices to aid in the participation of sports and recreational activities.

Within science fiction, and, more recently, within the scientific community, there has been consideration given to using advanced prostheses to replace healthy body parts with artificial mechanisms and systems to improve function. The morality and desirability of such technologies are being debated. Body parts such as legs, arms, hands, feet, and others can be replaced.

The first experiment with a healthy individual appears to have been that by the British scientist Kevin Warwick. In 2002, an implant was interfaced directly into Warwick's nervous system. The electrode array, which contained around a hundred electrodes, was placed in the median nerve. The signals produced were detailed enough that a robot arm was able to mimic the actions of Warwick's own arm and provide a form of touch feedback again via the implant.

In 2008, Oscar Pistorius was briefly ruled ineligible for the 2008 Summer Olympics due to an alleged mechanical advantage over runners who have ankles.

In early 2008, Oscar Pistorius, the "Blade Runner" of South Africa, was briefly ruled ineligible to compete in the 2008 Summer Olympics because his prosthetic limbs were said to give him an unfair advantage over runners who had ankles. One researcher found that his limbs used twenty-five percent less energy than those of an able-bodied runner moving at the same speed. This ruling was overturned on appeal, with the appellate court stating that the overall set of advantages and disadvantages of Pistorius' limbs had not been considered. Pistorius did not qualify for the South African team for the Olympics, but went on to sweep the 2008 Summer Paralympics, and has been ruled eligible to qualify for any future Olympics.

The "Luke arm" is an advanced prosthesis currently under trials as of 2008.

Types

A United States Marine with bilateral prosthetic legs leads a formation run.

There are four main types of artificial limbs. These include the transtibial, transfemoral, transradial, and transhumeral prostheses. The type of prosthesis depends on what part of the limb is missing.

Transtibial prosthesis

A transtibial prosthesis is an artificial limb that replaces a leg missing below the knee. Transtibial amputees are usually able to regain normal movement more readily than someone with a transfemoral amputation, due in large part to retaining the knee, which allows for easier movement. In the prosthetic industry a trans-tibial prosthetic leg is often referred to as a "BK" or below the knee prosthesis.

Transfemoral prosthesis

A transfemoral prosthesis is an artificial limb that replaces a leg missing above the knee. Transfemoral amputees can have a very difficult time regaining normal movement. In general, a transfemoral amputee must use approximately 80% more energy to walk than a person with two whole legs. This is due to the complexities in movement associated with the knee. In newer and more improved designs, after employing hydraulics, carbon fibre, mechanical linkages, motors, computer microprocessors, and innovative combinations of these technologies to give more control to the user. In the prosthetic industry a trans-femoral prosthetic leg is often referred to as an "AK" or above the knee prosthesis.

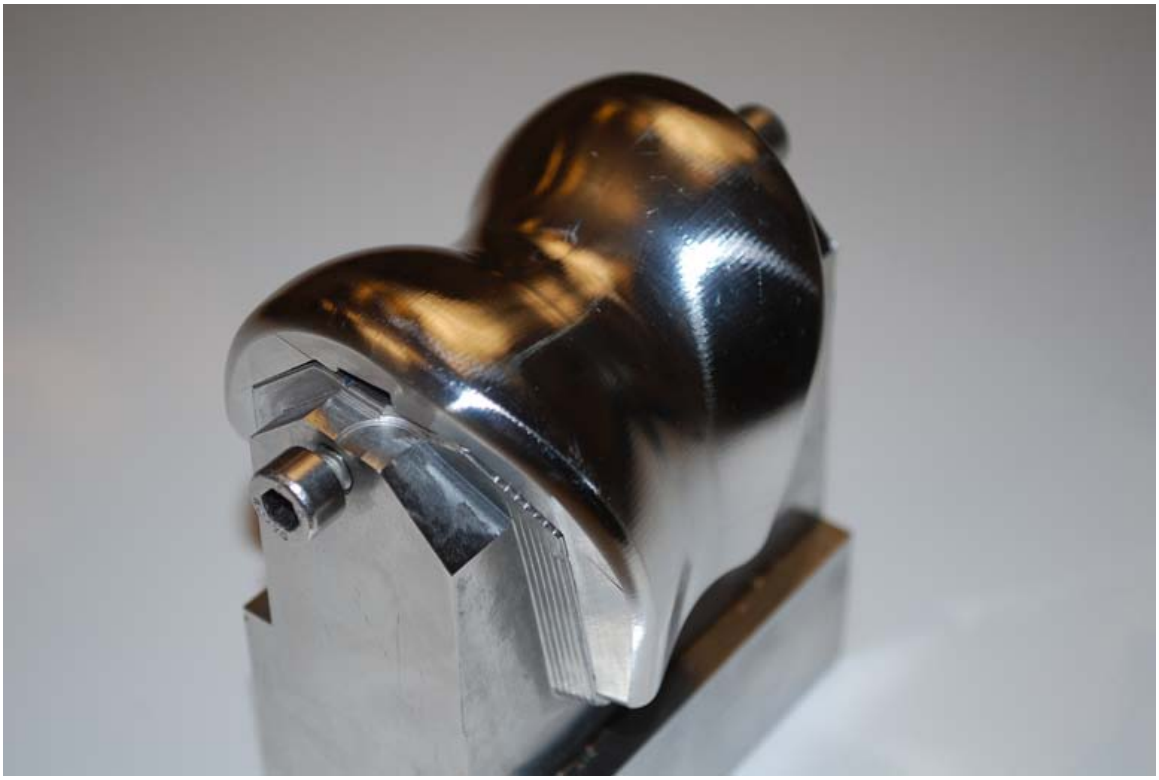
Transradial prosthesis

A transradial prosthesis is an artificial limb that replaces an arm missing below the elbow. Two main types of prosthetics are available. Cable operated limbs work by attaching a harness and cable around the opposite shoulder of the damaged arm. The other form of prosthetics available are myoelectric arms. These work by sensing, via electrodes, when the muscles in the upper arm moves, causing an artificial hand to open or close. In the prosthetic industry a trans-radial prosthetic arm is often referred to as a "BE" or below elbow prosthesis.

Transhumeral prosthesis

A transhumeral prosthesis is an artificial limb that replaces an arm missing above the elbow. Transhumeral amputees experience some of the same problems as transfemoral amputees, due to the similar complexities associated with the movement of the elbow. This makes mimicking the correct motion with an artificial limb very difficult. In the prosthetic industry a trans-humeral prosthesis is often referred to as a "AE" or above the elbow prothesis.

Current technology/manufacturing



Knee prosthesis manufactured using WorkNC Computer Aided Manufacturing software.

In recent years there have been significant advancements in artificial limbs. New plastics and other materials, such as carbon fiber, have allowed artificial limbs to be stronger and lighter, limiting the amount of extra energy necessary to operate the limb. This is especially important for transfemoral amputees. Additional materials have allowed artificial limbs to look much more realistic, which is important to transradial and transhumeral amputees because they are more likely to have the artificial limb exposed.

In addition to new materials, the use of electronics has become very common in artificial limbs. Myoelectric limbs, which control the limbs by converting muscle movements to electrical signals, have become much more common than cable operated limbs. Myoelectric signals are picked up by electrodes, the signal gets integrated and once it exceeds a certain threshold, the prosthetic limb control signal is triggered which is why inherently, all myoelectric controls lag. Conversely, cable control is immediate and physical, and through that offers a certain degree of direct force feedback that myoelectric control does not. Computers are also used extensively in the manufacturing of limbs. Computer Aided Design and Computer Aided Manufacturing are often used to assist in the design and manufacture of artificial limbs.

Most modern artificial limbs are attached to the stump of the amputee by belts and cuffs or by suction. The stump either directly fits into a socket on the prosthetic, or - more commonly today - a liner is used that then is fixed to the socket either by vacuum (suction sockets) or a pin lock. Liners are soft and by that, they can create a far better suction fit than hard sockets. Silicone liners can be obtained in standard sizes, mostly with a circular (round) cross section, but for any other stump shape, custom liners can be

made. The socket is custom made to fit the residual limb and to distribute the forces of the artificial limb across the area of the stump (rather than just one small spot), which helps reduce wear on the stump. The custom socket is created by taking a plaster cast of the stump or, more commonly today, of the liner worn over the stump, and then making a mold from the plaster cast. Newer methods include laser guided measuring which can be input directly to a computer allowing for a more sophisticated design.

One problems with the stump and socket attachment is that a bad fit will reduce the area of contact between the stump and socket or liner, and increase pockets between stump skin and socket or liner. Pressure then is higher, which can be painful. Air pockets can allow sweat to accumulate that can soften the skin. Ultimately, this is a frequent cause for itchy skin rashes. Further down the road, it can cause breakdown of the skin.

Artificial limbs are typically manufactured using the following steps:

1. Measurement of the stump
2. Measurement of the body to determine the size required for the artificial limb
3. Fitting of a silicone liner
4. Creation of a model of the liner worn over the stump
5. Formation of thermoplastic sheet around the model – This is then used to test the fit of the prosthetic
6. Formation of permanent socket
7. Formation of plastic parts of the artificial limb – Different methods are used, including vacuum forming and injection molding
8. Creation of metal parts of the artificial limb using die casting
9. Assembly of entire limb

Body-powered arms

Current body powered arms contain sockets that are built from hard epoxy or carbon fiber. Wrist units are either screw-on connectors featuring the UNF 1/2-20 thread (USA) or quick release connector, of which there are different models. Terminal devices contain a range of hooks, hands or other devices. Hosmer and Otto Bock are major commercial hook providers. Mechanical hands are sold by Hosmer and Otto Bock as well; the Becker Hand is still manufactured by the Becker family. Prosthetic hands may be fitted with standard stock or custom made cosmetic looking silicone gloves. But regular work gloves may be worn as well. Other terminal devices include the V2P Prehensor, a versatile robust gripper that allows customers to modify aspects of it, Texas Assist Devices (with a whole assortment of tools) and TRS that offers a range of terminal devices for sports. Cable harnesses can be built using aircraft steel cables, ball hinges and self lubricating cable sheaths. Current high tech allows body powered arms to weigh around half to only a third of the weight that a myoelectric arm has.

Actor Owen Wilson gripping the myoelectric prosthetic arm of a United States Marine

Myoelectric

A **myoelectric prosthesis** uses electromyography signals or potentials from voluntarily contracted muscles within a person's residual limb on the surface of the skin to control the movements of the prosthesis, such as elbow flexion/extension, wrist supination/pronation (rotation) or hand opening/closing of the fingers. A prosthesis of this type utilizes the residual neuro-muscular system of the human body to control the functions of an electric powered prosthetic hand, wrist or elbow. This is as opposed to an electric switch prosthesis, which requires straps and/or cables actuated by body movements to actuate or operate switches that control the movements of a prosthesis or one that is totally mechanical. It is not clear whether those few prostheses that provide feedback signals to those muscles are also myoelectric in nature. It has a self suspending socket with pick up electrodes placed over flexors and extensors for the movement of flexion and extension respectively.

The first commercial myoelectric arm was developed in 1964 by the Central Prosthetic Research Institute of the USSR, and distributed by the Hangar Limb Factory of the UK.

Robotic limbs

Advancements in the processors used in myoelectric arms has allowed for artificial limbs to make gains in fine tuned control of the prosthetic. The Boston Digital Arm is a recent artificial limb that has taken advantage of these more advanced processors. The arm allows movement in five axes and allows the arm to be programmed for a more customized feel. Raymond Edwards, Limbless Association Acting CEO, was the first amputee to be fitted with the i-LIMB by the National Health Service in the UK. The hand, manufactured by "Touch Bionics" of Scotland (a Livingston company), went on sale on 18 July 2007 in Britain. It was named alongside the Super Hadron Collider in Time magazine's top fifty innovations. Another robotic hand is the RSLSteeper bebionic

Another neural prosthetic is Johns Hopkins University Applied Physics Laboratory Proto 1. Besides the Proto 1, the university also finished the Proto 2 in 2010.

Robotic legs exist too: the Argo Medical Technologies ReWalk is an example or a recent robotic leg, targeted to replace the wheelchair. It is marketed as a "robotic pants".

Targeted muscle reinnervation (TMR) is a technique in which motor nerves which previously controlled muscles on an amputated limb are surgically rerouted such that they reinnervate a small region of a large, intact muscle, such as the pectoralis major. As a result, when a patient thinks about moving the thumb of his missing hand, a small area of muscle on his chest will contract instead. By placing sensors over the reinervated muscle, these contractions can be made to control movement of an appropriate part of the robotic prosthesis.

An emerging variant of this technique is called targeted sensory reinnervation (TSR). This procedure is similar to TMR, except that sensory nerves are surgically rerouted to skin on the chest, rather than motor nerves rerouted to muscle. The patient then feels any sensory stimulus on that area of the chest, such as pressure or temperature, as if it were occurring on the area of the amputated limb which the nerve originally innervated. In the future, artificial limbs could be built with sensors on fingertips or other important areas. When a stimulus, such as pressure or temperature, activated these sensors, an electrical signal would be sent to an actuator, which would produce a similar stimulus on the "rewired" area of chest skin. The user would then feel that stimulus as if it were occurring on an appropriate part of the artificial limb.

Recently, robotic limbs have improved in their ability to take signals from the human brain and translate those signals into motion in the artificial limb. DARPA, the Pentagon’s research division, is working to make even more advancements in this area. Their desire is to create an artificial limb that ties directly into the nervous system.

Direct bone attachment / osseointegration

Osseointegration is a new method of attaching the artificial limb to the body. This method is also sometimes referred to as exoprosthesis (attaching an artificial limb to the bone), or endo-exoprosthesis.

The stump and socket method can cause significant pain in the amputee, which is why the direct bone attachment has been explored extensively. The method works by inserting a titanium bolt into the bone at the end of the stump. After several months the bone attaches itself to the titanium bolt and an abutment is attached to the titanium bolt. The abutment extends out of the stump and the artificial limb is then attached to the abutment. Some of the benefits of this method include the following:

- Better muscle control of the prosthetic.
- The ability to wear the prosthetic for an extended period of time; with the stump and socket method this is not possible.
- The ability for transfemoral amputees to drive a car.

The main disadvantage of this method is that amputees with the direct bone attachment cannot have large impacts on the limb, such as those experienced during jogging, because of the potential for the bone to break.

Cost

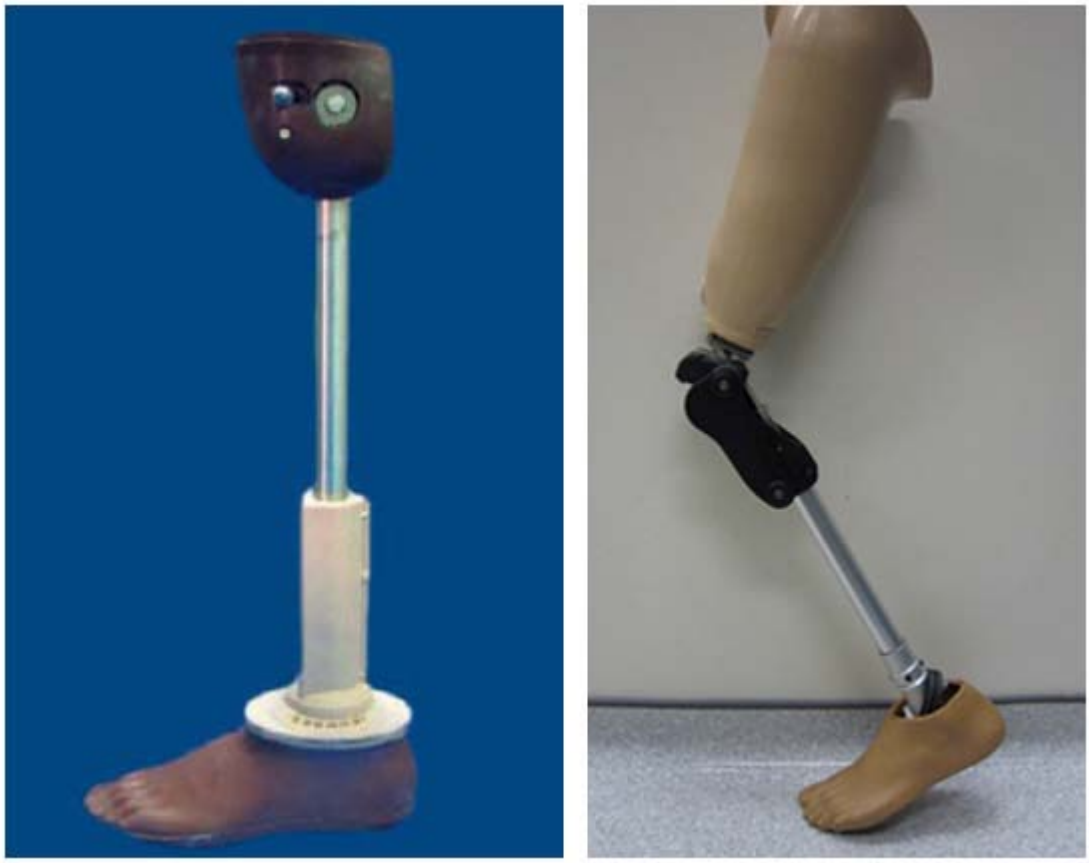
Transradial and transtibial prostheses typically cost between US $6,000 and $8,000. Transfemoral and transhumeral prosthetics cost approximately twice as much with a range of $10,000 to $15,000 and can sometimes reach costs of $35,000. The cost of an artificial limb does recur because artificial limbs are usually replaced every 3–4 years due to wear and tear. In addition, if the socket has fit issues, the socket must be replaced within several months. If height is an issue components can be changed, such as the pylons.

Low cost above knee prostheses often provide only basic structural support with limited function. This function is often achieved with crude, non-articulating, unstable, or manually locking knee joints. A limited number of organizations, such as the International Committee of the Red Cross (ICRC), create devices for developing countries. Their device which is manufactured by CR Equipments is a single-axis, manually operated locking polymer prosthetic knee joint.

Table. List of knee joint technologies based on the literature review.

Name of technology (country of origin)	Brief description	Highest level of evidence
ICRC knee (Switzerlan)	Single-axis with manual lock	Independent field
ATLAS knee (UK)	Weigh-activated friction	Independent field
POF/OTRC knee (US)	Single-axis with ext. assist	Field

DAV/Seattle knee (US)	Compliant polycentric	Field
LEGS M1 knee (US)	Four-bar	Field
JaipurKnee (US)	Four-bar	Field
LCKnee (Canada)	Single-axis with automatic lock	Field
None provided (Nepal)	Single-axis	Field
None provided (New Zealand)	Roto-molded single-axis	Field
None provided (India)	Six-bar with squatting	Technical development
Friction knee (US)	Weigh-activated friction	Technical development
Wedgelock knee (Australia)	Weigh-activated friction	Technical development
SATHI friction knee (India)	Weigh-activated friction	Limited data available



Low Cost Above Knee Prosthetic Limbs: ICRC Knee (left) and LC Knee (right)

There is currently an open Prosthetics design forum known as the "Open Prosthetics Project". The group employs collaborators and volunteers to advance Prosthetics technology while attempting to lower the costs of these necessary devices.

A plan for a low-cost artificial leg, designed by Sébastien Dubois, was featured at the 2007 International Design Exhibition and award show in Copenhagen, Denmark, where it won the Index: Award. It would be able to create an energy-return prosthetic leg for US $8.00, composed primarily of fiberglass.

Prior to the 1980s, foot prostheses merely restored basic walking capabilities. These early devices can be characterized by a simple artificial attachment connecting one's residual limb to the ground.

The introduction of the Seattle Foot (Seattle Limb Systems) in 1981 revolutionized the field, bringing the concept of an Energy Storing Prosthetic Foot (ESPF) to the fore. Other companies soon followed suit, and before long, there were multiple models of energy storing prostheses on the market. Each model utilized some variation of a compressible heel. The heel is compressed during initial ground contact, storing energy which is then returned during the latter phase of ground contact to help propel the body forward.

Since then, the foot prosthetics industry has been dominated by steady, small improvements in performance, comfort, and marketability. *Jaipur Foot*, an artificial limb from Jaipur, India, costs about US$ 40.

Design considerations

There are multiple factors to consider when designing a transtibial prosthesis. Manufacturers must make choices about their priorities regarding these factors.

Performance

Nonetheless, there are certain elements of foot mechanics that are invaluable for the athlete, and these are the focus of today's high-tech prosthetics companies:

- Energy storage and return – storage of energy acquired through ground contact and utilization of that stored energy for propulsion
- Energy absorption – minimizing the effect of high impact on the musculoskeletal system
- Ground compliance – stability independent of terrain type and angle
- Rotation – ease of changing direction
- Weight – maximizing comfort, balance and speed
- Suspension - how the socket will join and fit to the limb

Other

The buyer is also concerned with numerous other factors:

- Cosmetics
- Cost
- Ease of use
- Size availability

Emerging technology

Most companies choose to focus on two areas of performance: energy capabilities and ground compliance. Two particular models exemplify the innovation in these areas: the Elite foot (Endolite) and the Venture foot (College Park Industries).

The Elite foot relies on a polymeric material with a very specific set of elasticity and resistance requirements in order to optimize energy storage and return. It also uses an unprecedented three-pronged foot, which allegedly allows the foot to closely mold to the contours of any surface.

In contrast, the Venture foot retains the common one-point contact with the ground, but seeks to maximize performance (in both energy and compliance) with a complex metal heel component. This heel is equipped not just with a standard compressible foam piece, but also hinges which allow rotation on three different axes, allegedly yielding superior comfort (ground compliance) and a more precise mimicry of native foot biomechanics (energy capabilities).

Many other foot prostheses employ other useful innovative technology and designs. No one foot is perfect for all transtibial amputees. Hopefully, however, each amputee can find a foot that is best for his or her particular pattern of physical activity.