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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – I – Artificial Organs and Tissue Engineering-SBMA3003

UNIT I – INTRODUCTION

An **artificial organ** is a man-made device that is implanted or integrated into a human — interfacing with living tissue — to replace a natural organ, for the purpose of duplicating or augmenting a specific function or a group of related functions so the patient may return to a normal life as soon as possible. For example, replacement bones and joints, such as those found in hip replacements, could also be considered artificial organs.

PURPOSE

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

providing life support to prevent imminent death while awaiting a transplant (e.g.artificial heart);

- dramatically improving the patient's ability for self care (e.g. artificial limb);
- improving the patient's ability to interact socially (e.g. cochlear implant); or
- Improving a patient's quality of life through cosmetic restoration after cancer surgery or an 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.

EXAMPLES

Artificial limbs - Artificial arms and legs, or prosthetics, are intended to restore a degree of normal function to amputees. Mechanical devices that allow amputees to walk again or continue to use two hands have probably been in use since ancient times, the most notable one being the simple peg leg. Since then, the development of artificial limbs has progressed rapidly. New plastics and other materials, such as carbon fiber have allowed artificial limbs to become stronger and lighter, limiting the amount of extra energy necessary to operate the limb. Additional materials have allowed artificial limbs to look much more realistic. Prostheses can roughly be categorized as upper- and lower-extremity and can take many shapes and sizes.

New advances in artificial limbs include additional levels of integration with the human body. Electrodes can be placed into nervous tissue, and the body can be trained to control the prosthesis. This technology has been used in both animals and humans.

BLADDER - The two main methods for replacing bladder function involve either redirecting urine flow or replacing the bladder *in situ*. Standard methods for replacing the bladder involve fashioning a bladder-like pouch from intestinal tissue. An alternative emerging method involves growing a bladder from cells taken from the patient and allowed to grow on a bladder-shaped scaffold.

BRAIN - Neural prostheses are a series of devices that can substitute a motor, sensory or cognitive modality that might have been damaged as a result of an injury or a disease.

Neurostimulators, including deep brain stimulators, send electrical impulses to the brain in order to treat neurological and movement disorders, including Parkinson's disease, epilepsy, treatment resistant depression, and other conditions such as urinary incontinence. 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

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 theretina, optic nerve, or other related locations inside the brain. The present state of the art yields only partial functionality, such as recognizing levels of brightness, swatches of color, and/orbasic geometric shapes, proving the concept's potential

HEART - The artificial heart is typically used to bridge the time to heart transplantation, or to permanently replace the heart in case heart transplantation is impossible. Artificial pacemakers represent another cardiovascular device which can be implanted to either intermittently augment (defibrillator mode), continuously augment, or completely bypass the natural living cardiac pacemaker as needed. Ventricular assist devices are another alternative, acting as mechanical circulatory devices that partially or completely replace the function of a failing heart, without the removal of the heart itself.

PANCREAS - An artificial pancreas is used to substitute endocrine functionality of a healthy pancreas for diabetic and other patients who require it. It can be used to improve insulin replacement therapy until glycemic control is practically normal as evident by the avoidance of the complications of hyperglycemia,and it can also ease the burden of therapy for the insulin-dependent. Approaches include using an insulin pump under closed loop control, developing a bio-artificial pancreas consisting of a biocompatible sheetof encapsulated beta cells, or using gene therapy

ENHANCEMENT

Research is proceeding in areas of vision, memory, and information processing. Some current research focuses on restoring short-term memory in accident victims and long-term memory in dementia patients.

One area of success was achieved when Kevin Warwick carried out a series of experiments extending his nervous system over the internet to control a robotic hand and the first direct electronic communication between the nervous systems of two humans.

This might also include the existing practice of implanting subcutaneous chips for identification and location purposes (ex. RFID tags).

EVOLUTION OF ORGAN REPLACEMENT TECHNOLOGY

Artificial organs have different limitations. Seen on the scale of human evolution, they are still primitive devices, tested for 40 years at most. Yet they have transformed the prognosis of many heretofore fatal diseases, which are now allowed to evolve past what used to be their natural termination point. In order to design artificial organs, inventive engineers, physiologists, and surgeons think in terms of functional results, not anatomical structures. As a result, artificial organs have but a distant similarity to natural ones. They are mostly made of synthetic materials (often called **biomaterials**) which do not exist in nature. They use different mechanical, electrical, or chemical processes to achieve the same functional objectives as natural organs. They adapt but imperfectly to the changing demands of human activity. They cannot easily accommodate body growth and therefore are more beneficial to adults than to children. Most critically, artificial organs, as is the case for all machines, have a limited service expectancy because of friction, wear, or decay of construction materials in the warm, humid, and corrosive environment of the human body. Such considerations limit their use to patients whose life expectancy matches the expected service life of the replacement part or to clinical situations where repeated implantations are technically feasible. In spite of these obstacles, the astonishing reality is that millions of people are currently alive thanks to cardiac pacemakers, cardiac valves, artificial kidneys, or hydrocephalus drainage systems, all of which address life-threatening conditions. An even larger number of people enjoy the benefits of hip and knee prostheses, vascular grafts, intraocular lenses, and dental implants, which correct dysfunction, pain, inconvenience, or merely appearance. In short, the clinical demonstration of the central dogma of substitutive medicine over the span of two generations can be viewed demographically as the first step in a evolutionary jump which humans cannot yet fully appreciate.

Hybrid artificial organs, or bioartificial organs, are more recent systems which include living elements (organelles, cells, or tissues) as part of a device made of synthetic materials. They integrate the technology of natural organ transplantation and the refinements which living structures have gained through millions of years of evolution with the purposeful design approach of engineering science and the promises of newly developed synthetic materials. Table provides a current snapshot in the continuing evolution of substitutive medicine.

Depending upon medical needs and anticipated duration of use, artificial organs can be located outside of the body yet attached to it (paracorporeal prostheses or assist devices) or implanted inside the body in a appropriate location (internal artificial organs or implants). The application of artificial organs may be temporary, that is, a bridge procedure to sustain life or a specific biologic activity while waiting for either recovery of natural function (e.g., the heart-lung machine), or permanent organ replacement (e.g., left ventricular assist devices). It can be intermittent and repeated at intervals over extended periods of time when there is no biologic necessity for continuous replacement of the missing body functions (e.g., artificial kidney). It can pretend to be permanent, at least within the limits of a finite life span.

Up to 1950, organ replacement technology was relatively crude and unimaginative. Wooden legs, corrective glasses, and dental prostheses formed the bulk of artificial organs. Blood transfusion was the only accepted form of transplantation of living tissue. Suddenly, within a decade, the artificial kidney, the heart–lung machine, the cardiac pacemaker, the arterial graft, the prosthetic cardiac valve, and the artificial hip joint provided the first sophisticated examples of engineering in medicine. More recently, the membrane lung, the implantable lens, finger and tendon prostheses, total knee replacements, and soft-tissue implants for maxillo-facial, ear, or mammary reconstruction have reached the stage of broad clinical application. Ventricular assist devices and the total artificial heart have been extensively tested in animals and validated for clinical evaluation. Artificial skin is increasingly used in the treatment of ulcers and burns. Soft- and hard-tissue substitutes function effectively for several years. Sexual and sensory prostheses offer promises for the replacement of complex human functions. Interfacing of devices with the peripheral and central nervous systems appears as promising today as cardiovascular devices were 30 years ago. Perhaps the brightest future belongs to "information prostheses" which bring to the human body, signals which the organism can no longer generate by itself (e.g., pacemaker functions), signals which need to be modulated differently to correct a disease state (e.g., electronic blood pressure regulators) or signals which cannot be perceived by the nervous system through its usual channels of information gathering (e.g., artificial eye or artificial ear).

Biomaterials

The materials of the first generation of artificial organs — those which are widely available at the moment — are for the most part standard commodity plastics and metals developed for industrial purposes. Engineers have long recognized the limitations of construction materials in the design and performance of machines. However, a new awareness arose when they started interacting with surgeons and biologic scientists in the emerging field of medical devices. In many cases the intrinsic and well established physical properties of synthetic materials such as mechanical strength, hardness, flexibility, or permeability to fluids and gases were not as immediately limiting as the detrimental effects deriving from the material's contact with living tissues. As a result, fewer than 20 chemical compounds among the 1.5 million candidates have been successfully incorporated into clinical devices. Yet some functional implants require material properties which exceed the limits of current polymer, ceramic, or metal alloy technology. This is an indirect tribute to the power of evolution, as well as a challenge to scientists to emulate natural materials with synthetic compounds, blends, or composites.

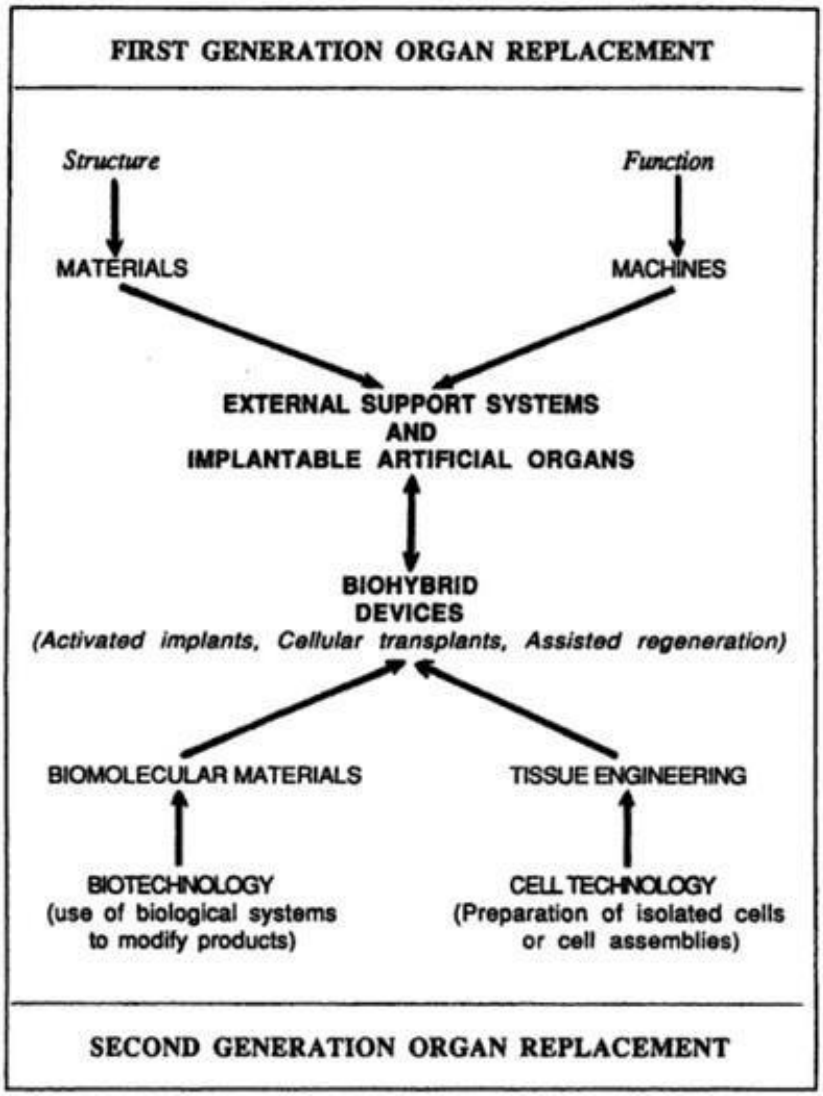

Fig. Outlook of organ replacement

TABLE VI.1 Evolution of Organ Replacement Technology: A 1995 Perspective

Current status	Artificial organs	Transplantation
Broadly accepted clinically	Heart-lung machine	Blood transfusion
	Large-joint prostheses	Corneal transplants
	Bone fixation systems	Banked bone
	Cardiac pacemakers	Bone marrow
	Implantable defibrillators	Kidney — living related donor
	Large vascular grafts	Kidney — cadaveric donor
	Prosthetic cardiac valves	Heart
	Intra-aortic balloon pump	Liver
	Intraocular lenses	
	Middle ear ossicle chain	
	Hydrocephalus shunts	
	Dental implants	
	Skin and tissue expanders	
	Maintenance hemodialysis	
	Chronic ambulatory peritoneal dialysis	
Accepted with reservations	Breast implants	Whole pancreas
	Sexual prostheses	Single and double lung
	Small joint prostheses	Combined heart-lung
	ECMO in children	
Limited clinical application	ECMO in adults	Cardiomyoplasty
	Ventricular assist devices	Pancreatic islets
	Cochlear prostheses	Liver lobe or segment
	Artificial tendons	Small Intestine
	Artificial skin	
	Artificial limbs	
Experimental stage	Artificial pancreas	Bioartificial pancreas
	Artificial blood	Bioartificial liver
	Intravenous oxygenation	CNS implants of secreting tissue
	Artificial esophagus	Gene therapy products
	Total artificial heart	
	Nerve guidance channels	
Conceptual stage	Artificial eye	Striated muscle implants
	Neurostimulator	Smooth muscle implants
	Blood pressure regulator	Cardiac muscle implants
	Implantable lung	Functional brain implants
	Artificial trachea	Bioartificial kidney
	Artificial gut	
	Artificial fallopian tube	

Design considerations and evaluation process:

Artificial organs can only replace those bodily functions which have been incorporated into their design. Therefore, in the design of an artificial organ, the first task is to establish the specification for the device i.e. the function or functions which must be fulfilled by a human- made construct and the physical constraints that apply because the device must interface with the human body.

Defining specifications and constraints is the first step in the conceptualization of an artificialorgan. Only when this is done can one think realistically about design alternatives, the limitations of available materials, and the clinical constraints which will apply, of which the key ones are connections to the body and duration of expected service.

Once all these considerations have been integrated, the next step is typically the construction of a prototype. Ideally the device should achieve everything it was expected to do, but usuallyit exhibits some level of performance and durability which falls short of design specifications,either because of some misjudgement in terms of required function or because of some unanticipated problem arising at the interface between the device and the body.

The following step of development may be called optimization. At this point, new experiments are needed to establish the reliability and effectiveness of the device in animal models. This is the stage of validation of the device, which is first conducted in acute experiments and must later be extended to periods of observation approximating the duration of intended use in humans.

The final stage of design, for many artificial organs, is individualization, that is, the ability to fit the needs of diverse individuals. Human come in a wide range of body sizes. In some cases, the prostheses must fit very strict dimensional criteria, which imply that they must be fabricated over an extended range of sizes.

Evaluation process:

The evaluation process of an artificial organ typically is done in six phase:

1. In vitro bench testing
2. Ex vivo appraisal
3. In vivo studies with health experimental animals
4. In vivo studies with animal models of disease
5. General clinical use.

EVALUATION PROCESS

In vivo bench testing:

In vivo bench testing of a completed prototype has three major purposes:

1. To observe the mode of operation of the device and assess its performance under tightly controlled circumstances
2. To define performance in quantitative terms over a wide range of environmental orinput conditions
3. To assess the device's reliability and durability in a manner which can be extrapolatedto the intended clinical use

For all its value, there are limitations to the in vitro testing of device. Devices are made to work while in contact with body fluids or body tissues. This complex environment modifies materials in ways which are not always predictable. To duplicate this effect as closely as possible a laboratory bench system can be made to match the body's environment in terms of temperature and humidity. Operating pressures and external forces can also be imitated but not perfectly reproduced (eg. complex pulsatile mature of cardiovascular events.). Other fluid dynamic conditions such as viscosity, wall shear stress and compliances of device surrounding structures call for sophisticated laboratory system and can only be approximated.The chemical environment is the most difficult to reproduce in view of the complexity of body fluids and tissue structures. Some in vitro testing systems make use of body fluids such as plasma or blood. This in turn brings in additional intricacies because these fluids are not stable outside of the body without preservatives and must be kept sterile if the experiment isto last more than a few hours.

Accelerated testing is a standard component in the evaluation of machine. It is critical for permanent implants with moving parts which are subject to the repeated action of external forces. Fatigue testing provides important information on progressive wear or catastrophic failure of device components. For examples, the human heart beats about 40 million time per year. Manufacturers and regulatory agencies conduct testing of prosthetic cardiac valve over

at least 400 million cycles. With a testing apparatus functioning at 1200 cycles per minute, this evaluation can be compressed by a factor of about 15, that is to about a year.

Ex vivo appraisal:

Because of the difficulty of keeping blood in its physiologic state in a container, the evaluation of some blood processing or blood contacting devices in performed by connecting them through the skin to an artery or vein or both if the blood must be returned to the cardiovascular system to avoid excessive haemorrhage. Such experiments retain the advantage of keeping the device under direct observation while allowing longer experiments than are feasible in vitro, particularly if the animal does not require general anaesthesia. It is also possible in some cases to evaluate several devices in parallel or sequentially under quite realistic conditions and therefore to conduct comparative experimental animals prevents studies for periods of service as long as can be expected with permanent implants in man.

In vivo evaluation with health experimental animals:

There comes a stage in the development of most devices where they must be assessed to their target location in a living body. The matching of device size and shape with available experimental sites in the location in a living body. The matching of device size and shapewith available experimental sites in the appropriate animal species is a necessary condition. Such experiments typically last weeks, months, or years and provide information about body-device and tissue-material interactions either through non-invasive measurement techniquesor through device retrieval at the end of the observation period. Rodents, felines, and dogs raised for research purposes are usually too small for the evaluation of human sized devices. Farm animals such as sheep, goats, pigs and claves are commonly used. Here again the limited life expectancy of experimental animals prevents studies for periods of service aslong as can be expected with permanent implants in man.

In vivo evaluation with animal models of disease:

A first approximation of the effectiveness of a device in replacing a physiologic function can be

obtained after removing the target organ in a normal animal. However, when the organ failure is only the cardinal sign of a complex systemic disease, the interactions between device and the persisting manifestations of the disease occur spontaneously in some species and in other cases can be obtained by chemical, physical or surgical intervention, where such models of disease exist n animals which can be fitted with a device, useful information is obtained which helps to refine the final prototype.

Controlling clinical trials:

Although some devices can be evaluated with little risk in normal volunteers who derive no health benefit from the experiments, our culture frowns on this approach and legal considerations discourage it. Once reliability and effectiveness have been established throughanimal experiments and the device appears to meet a recognized clinical need, a study protocol is typically submitted to an appropriate ethics committee or institutional review board and, upon their approval, a series of clinical trials is undertaken. The first step often concentrates on the demonstration of safety of the device with a careful watch for side effectsor complications. If the device passes this first hurdle, a controlled clinical trial will be carried out with patients to evaluate effectiveness as well as safety on a scale which allows statistical comparison with a control form of treatment. This protocol may extend from a few months to several years depending upon the expected benefits of the device and the natural history of the disease.

General clinical use:

Once a device is deemed successful by a panel of experts, it may be approved by regulatory agencies for commercial distribution. Increasingly a third stage of clinical evaluation appears necessary, namely post market surveillance, that is a system of clinical outcomes analysis under conditions of general availability of the device to a wide range of doctors and patients.

Post market surveillance is a new concept which is not yet uniformly codified. It may take theform of a data collection and analysis network, a patient registry to allow continuing follow up a statistical of a data analysis, a device-tracking system aimed at early identification of unforeseen types of failure, or ancillary controls such as inspection of facilities and review of patient histories in institutions where devices are used. Protocols of surveillance on al large scale are difficult and costly to implement and their cost-effectiveness is therefore open to question. They are also impaired by the shortage of broadly available and minimally invasive diagnostic methods for assessing the integrity or function of a device prior to catastrophic failure. Worthwhile post market surveillance requires a constructive collaboration between patients, doctors, device manufacturers, government regulatory agencies, and study groups assessing health care policy in the public and private sectors.

TYPES OF TISSUE GRAFTS

Transplantation involves the removal of cells, tissues or organs from one individual and then placing them into another individual. If the graft is returned to the same patient it is termed an **autograft**, while if it is placed in another individual of the same species it is termed an **allograft**. Tissue transferred to another species is termed a **xenograft**. If it is placed in the same anatomic location from which it was derived the transplantation procedure is termed **orthotopic**, while if the location to which it is moved is different from the original anatomic site, it is termed **heterotopic**. If tissue is transplanted from one individual to another unrelated individual there is high probability that the vascular supply to the graft will be destroyed and that it will be **rejected**.

First set rejection occurs seven to ten days after a graft is transferred between unrelated individuals. A subsequent skin graft transplanted from the same

Both **acute and chronic rejection** are processes that can occur simultaneously and are characterized by the cell types present. **Hyperacute rejection** is characterized by occlusion of vascular channels, by deposition of platelets and fibrin networks and begins within minutes of surgical completion of the suturing of donor and host vessels (Table 1.21). **Blood clotting** and **platelet aggregation** (thrombosis) occurs prior to the development of inflammation and is mediated by pre-existing antibodies that attach to endothelial cells, which subsequently activate complement. Endothelial cells secrete a form of von Willebrand factor which mediates platelet adhesion and aggregation and activates blood clotting. In early experimental transplantation procedures, hyperacute rejection occurred as a result of mismatching of blood types.

Table 1.21 Differences between hyperacute, acute and chronic rejection†

Type of rejection	Characterization
Hyperacute	Occlusion of vascular channels blood clotting and platelet aggregation mediated by circulating antibodies that activate complement
Acute humoral	Mediated by IgG antibodies to endothelial cell antigens and involves complement
Acute cellular	Necrosis of parenchymal cells in presence of lymphocytes and macrophages
Chronic	Deposition of collagen and loss of normal tissue

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UNIT – I

PART – A

S.No	Questions
1	Define artificial organ. Give example.
2	List out the phases in design consideration
3	List out the phases in evaluation process.
4	State organ replacement technology.
5	What are the factors affecting blood compatibility?
6	Define artificial organ with example
7	State the limitations of organ replacement technology.
8	What are the advantages of organ replacement technology?
9	What is tissue product matching?
10	Draw the flow chart of organ replacement.
11	On what organ replacement is designed anatomically and functionally
12	What events take place for evaluation of organ replacement?

PART B

S.No	Questions
1	Highlight the various concepts considered in the design of a fully functional artificial organ
2	Explain in detail the advantages and limitations of organ replacement technology.
3	Explain in detail about Evaluation process.
4	Give a detailed account on Biology of transplantation of tissue products matching with neat illustration.
5	Explain in detail about graft rejection and its types with a neat labeled diagram.
6	Narrate factors to be considered for the evolution of organ replacement technology.
7	Demonstrate the design considerations in organ replacement? What are the problems associated with different types of grafts.
8	Analyze how the evaluation process takes place during organ replacement.

UNIT – II – Artificial Organs and Tissue Engineering-SBMA3003

Unit-2
ARTIFICIAL HEART AND LUNG ASSIST DEVICES

A ventricular assist device (VAD) is a mechanical pump that's used to support heart function and blood flow in people who have weakened hearts. The device takes blood from a lower chamber of the heart and helps pump it to the body and vital organs, just as a healthy heart would.

Ventricles are the lower chambers of your heart.A VAD can help support your heart:

- During or after surgery, until your heart recovers.
- While you're waiting for a heart transplant.
- If you're not eligible for a heart transplant.
    - A VAD has several basic parts. A small tube carries blood out of your heart into a pump. Anothertube carries blood from the pump to your blood vessels, which deliver the blood to your body.
    - A VAD also has a power source that connects to a control unit. This unit monitors the VAD's functions. It gives warnings, or alarms, if the power is low or the device isn't working well.
    - Some VADs pump blood like the heart does, with a pumping action. Other VADs keep up a continuous flow of blood. With a continuous flow VAD, you might not have a normal pulse, but your body is getting the blood it needs.
    - Research has shown that, compared with other VADs, continuous flow VADs may decrease hospital stays and complications and improve survival. However, more research is needed.

Types of Ventricular Assist Devices

- The two basic types of VADs are a left ventricular assist device (LVAD) and a right ventricular assist device (RVAD). If both types are used at the same time, they're called a biventricular assist device (BIVAD).
- The LVAD is the most common type of VAD. It helps the left ventricle pump blood to the aorta. The aorta is the main artery that carries oxygen-rich blood from your heart to your body.

- RVADs usually are used only for short-term support of the right ventricle after LVAD surgery or other heart surgery. An RVAD helps the right ventricle pump blood to the pulmonary artery. Thisis the artery that carries blood from the heart to the lungs to pick up oxygen.
- A BIVAD might be used if both ventricles don't work well enough to meet the body's needs. Another treatment option for this condition is a total artificial heart (TAH). A TAH is a devicethat replaces the ventricles.
- VADs have two basic designs. A transcutaneous VAD has its pump

An implantable VAD has its pump located inside of the body and its power source located outside of the body. A cable connects the pump to the power source through a small hole in the abdomen. Implantable VADs are used mainly for people who are waiting for heart transplants or as a long-term solution for people who can't have heart transplants. Until recently, VADs were too big to fit in many people's chests, especially women and children. Only people who had large chests could get them. However, recent advances have resulted in smaller, more reliable devices. This now makes treatment with VADs an optionfor more people. Researchers also have made advances in how well VADs work and how much they improve people's quality of life. In the past, VADs mostly were used for people who had end-stage heart failure. Now VADs also can help people who have earlier stages of heart failure. Children who have heartfailure also can be treated with VADs. VADs approved for use in adults sometimes are used in children if the children are large enough for the device. Also, the Food and Drug Administration recently approved a VAD designed for smaller children.

LUNG ASSISTING DEVICES

The Novalung Interventional Lung Assist device is a membrane ventilator that allows for oxygen and carbon dioxide gas exchange to occur by simple diffusion. It has been used in patients with severe acute lung failure due to ARDS, inhalation injury, severe pneumonia, chest injury, foreign body aspiration, and after thoracic surgical interventions. The concept of “protective ventilation” was described decades ago, but with the introduction of extracorporeal ventilation devices such as the Novalung it may reach new dimensions. It potentially helps to avoid or reduce ventilator associated lung injury and remote secondary organ failure, which is related to injurious mechanical ventilation.

Technical aspects of the equipment

The iLA consists of a plastic gas exchange module with diffusion membranes made from polymethylpentene (PMP). These PMP fibers are woven into a complex configuration of hollow fibers. The PMP material is woven to bundles in a low resistance configuration mat arranged in well defined stacks, which provides maximum blood/gas mixing. Gas transfer takes place without the direct contact with blood. In addition, the PMP membrane surface in contact with blood is treated with a heparin coatingto provide a biocompatible and non-thrombogenic surface. Blood flows over the exterior surface of the device's fibers; the ventilating gas (commonly O_2) flows inside these fibers. In this way the NovalungiLA mimics the native lung. This allows for the blood exiting the device to have the normal amount of oxygen and carbon dioxide that exits the normal lung. In the arterio-venous portion of this pumpless shuntcarbon dioxide exchange is the primary function due to arterial inflow blood, while a veno-venous attachment, which requires the support of a mechanical pump, additionally allows full oxygenation support.

Clinical use and results

The Novalung has been used in over 1200 patients in Europe to enable advanced protective ventilation. We have recently reported on the successful use of the Novalung iLA as a bridge to lung transplantation in patients with severe ventilation-refractory respiratory acidosis and hypercapnea. The use of the device allows for a safer form of ventilation ('protective ventilation'), because the patients' carbon dioxide levelsand pH can be adjusted to normal levels with the device. Extracorporeal life support with the Novalung iLA has been applied up to 32 days at the Hannover Thoracic Transplant and Cardiac Assist Program.

The driving force for this mode is the left ventricular output. In other situations, which include low cardiac output or hypoxic lung failure, a blood pump is required to divert a relatively larger amount of blood from the venous system through the Novalung, which can be returned into the systemic arterial circulation (veno-arterial mode) or the central veins (veno-venous mode), respectively. The optimal extracorporeal circuit design and configuration for circulatory support is determined by the underlying disease state and the treating physician's choice.

ARTIFICIAL HEART VALVE

An **artificial heart valve** is a device implanted in the heart of a patient with valvular heart disease. When one of the four heart valves malfunctions, the medical choice may be to replace the natural valve with an artificial valve. This requires open-heart surgery.

There are three main types of artificial heart valves: the mechanical, the biological, and the tissueengineered valves.

- Mechanical heart valve
    - Percutaneous implantation
        - Stent framed
        - Not framed
    - Sternotomy/Thoracotomy implantation
        - Ball and cage
        - Tilting disk
        - Bi-leaflet
        - Tri-leaflet
- Tissue (biological) heart valves
    - Allograft/isograft
    - Xenograft
- Tissue-Engineered heart valves

MECHANICAL VALVES

Mechanical heart valves (MHV) are prosthetics designed to replicate the function of the natural valvesof the human heart. The human heart contains four valves: tricuspid valve, pulmonary valve, mitral valve and aortic valve. Their main purpose is to maintain unimpeded forward flow through the heart and from the heart into the major blood vessels connected to the heart, the pulmonary artery and the aorta. As a result of a number of disease processes, both acquired and congenital, any one of the four heart valvesmay malfunction and result in either stenosis (impeded forward flow) and/or backward flow (regurgitation). Either process burdens the heart and may lead to serious problems including heart failure. A mechanical heart valve is intended to replace a diseased heart valve with its prosthetic equivalent.

There are two basic types of valves that can be used for valve replacement, mechanical and tissue

valves. Modern mechanical valves can last indefinitely (the equivalent of over 50,000 years in an accelerated valve wear tester). However, current mechanical heart valves all require lifelong treatment with anticoagulants (blood thinners), e.g. warfarin, which requires monthly blood tests to monitor. This process of thinning the blood is called anticoagulation. Tissue heart valves, in contrast, do not require the use of anticoagulant drugs due to the improved blood flow dynamics resulting in less red cell damage and hence less clot formation. Their main weakness however, is their limited lifespan. Traditional tissue valves, made of pig heart valves, will last on average 15 years before they require replacement (but typically less in younger patients).

There are three major types of mechanical valves – caged-ball, tilting-disk and bileaflet valve – with many modifications on these designs.

Caged ball valve

The first artificial heart valve was the caged-ball, which utilizes a metal cage to house a silicone elastomerball. When blood pressure in the chamber of the heart exceeds that of the pressure on the outside of the chamber the ball is pushed against the cage and allows blood to flow. At the completion of the heart's contraction, the pressure inside the chamber drops and is lower than beyond the valve, so the ball moves back against the base of the valve forming a seal.

Tilting-disc valve - Tilting disk valves have a single circular occluder controlled by a metal strut. They are made of a metal ring covered by an ePTFE fabric, into which the suture threads are stitched in order tohold the valve in place. The metal ring holds, by means of two metal supports, a disc which opens and closes as the heart pumps blood through the valve. The disc is usually made of an extremely hard carbon material (pyrolytic carbon), in order to allow the valve to function for years without wearing out. The Medtronic-Hall model is the most common tilting-disc design in the US. In some models of mechanical valves, the disc is divided into two parts, which open and close as a door.

Bileaflet valve - Bileaflet heart valves consist of two semicircular leaflets that rotate about struts attached to the valve housing. This design was introduced in 1979and while they take care of some of the issues that were seen in the other models, bileaflets are vulnerable to backflow and so they cannot be consideredas ideal. Bileaflet valves do, however, provide much more natural blood flow than caged-ball or tilting- disc implants. One of the main advantages of these valves is that they are well tolerated by the body. Onlya small amount of blood thinner is needed to be taken by the patient

each day in order to prevent clotting of the blood when flowing through the valve. These *bileaflet* valves have the advantage that they have a greater effective opening area (2.4–3.2 square cm c.f. 1.5–2.1 for the single-leaflet valves). Also, they are the least thrombogenic of the artificial valves. Mechanical heart valves are today very reliable and allow the patient to live a normal life. Most mechanical valves last for at least 20 to 30 years

Durability

Mechanical heart valves have been traditionally considered to be more durable in comparison to their bioprosthetic counterparts. The struts and occluders are made out of either pyrolytic carbon or titanium coated with pyrolytic carbon, and the sewing ring cuff is Teflon (PTFE), polyester or Dacron. The major load arises from transvalvular pressure generated at and after valve closure, and in cases where structural failure does happen, it is usually as a result of occluder impact on the components. Impact wear and friction wear dictate the loss of material in MHV. Impact wear usually occurs in the hinge regions of bileaflets, between the occluder and ring in tilting-discs, and between the ball and cage in caged-ball valves. Friction wear occurs between the occluder and strut in tilting-discs, and between the leaflet pivots and hinge cavities in bileaflets. MHV, made out of metal are also susceptible to fatigue failure owing to the polycrystalline characteristic of metals, but this is not an issue with pyrolytic carbon MHV because this material is not crystalline in nature

CARDIAC PACEMAKER

A pacemaker or **artificial pacemaker** is a medical device which uses electrical impulses, delivered by electrodes contracting the heart muscles, to regulate the beating of the heart.

The primary purpose of a pacemaker is to maintain an adequate heart rate, either because the heart's natural pacemaker is not fast enough, or because there is a block in the heart's electrical conduction system. Modern pacemakers are externally programmable and allow a cardiologist to select the optimum pacing modes for individual patients. Some combine a pacemaker and defibrillator in a single implantable device. Others have multiple electrodes stimulating differing positions within the heart to improvesynchronisation of the lower chambers (ventricles) of the heart

A pacemaker is a small, battery-operated device that senses when your heart is beating irregularly or tooslowly. It sends a signal to your heart that makes your heart beat at the correct pace.

Most pacemakers have 2 parts:

- The generator contains the battery and the information to control the heartbeat.

- The leads are wires that connect the heart to the generator and carry the electrical messages to theheart.

A pacemaker must be implanted under the skin. This procedure takes about 1 hour in most cases. You will be given a sedative to help you relax. You will be awake during the procedure.

A small incision (cut) is made, most often on the left side of the chest below your collarbone. The pacemaker generator is then placed under the skin at this location. The generator may also be placed inthe abdomen, but this is less common.

Using live x-rays to see the area, the doctor puts the leads through the cut, into a vein, and then into the heart. The leads are connected to the generator. The skin is closed with stitches. Most people go home within 1 day of the procedure.

Two kinds of pacemakers -- transcutaneous and transvenous pacemakers -- are used only in medical emergencies. They are not permanent pacemakers

Pacemakers may be used for people who have heart problems that cause their heart to beat too slowly. A slow heartbeat is called bradycardia. Two common problems that cause a slow heartbeat are sinus node disease and heart block.

When your heart beats too slowly, your body and brain may not get enough oxygen. Symptoms may be light-headedness, tiredness, fainting spells, and shortness of breath.

Some pacemakers can be used to stop a heart rate that is too fast (tachycardia) or that is irregular.

Other types of pacemakers can be used in severe heart failure. These are called biventricular pacemakers. They help coordinate the beating of the heart chambers.

Most biventricular pacemakers implanted today can also work as implantable cardioverter defibrillators (ICD), which restore a normal heartbeat.

How does a pacemaker work?

A pacemaker consists of a battery, a computerized generator, and wires with sensors at their tips. (The sensors are called electrodes.) The battery powers the generator, and both are surrounded by a thin metalbox. The wires connect the generator to the heart.

A pacemaker helps monitor and control your heartbeat. The electrodes detect your heart's electrical activity and send data through the wires to the computer in the generator.

If your heart rhythm is abnormal, the computer will direct the generator to send electrical pulses to yourheart. The pulses travel through the wires to reach your heart.

Newer pacemakers can monitor your blood temperature, breathing, and other factors. They also can adjustyour heart rate to changes in your activity.

The pacemaker's computer also records your heart's electrical activity and heart rhythm. Your doctor willuse these recordings to adjust your pacemaker so it works better for you.

Your doctor can program the pacemaker's computer with an external device. He or she doesn't have to useneedles or have direct contact with the pacemaker.

Pacemakers have one to three wires that are each placed in different chambers of the heart.

- The wires in a single-chamber pacemaker usually carry pulses from the generator to the rightventricle (the lower right chamber of your heart).
- The wires in a dual-chamber pacemaker carry pulses from the generator to the right atrium (the upper right chamber of your heart) and the right ventricle. The pulses help coordinate the timingof these two chambers' contractions.
- The wires in a biventricular pacemaker carry pulses from the generator to an atrium and both ventricles. The pulses help coordinate electrical signaling between the two ventricles. This type ofpacemaker also is called a cardiac resynchronization therapy (CRT) device.

The image shows a cross-section of a chest with a pacemaker. Figure A shows the location and general size of a double-lead, or dual-chamber, pacemaker in the upper chest. The wires with electrodes are inserted into the heart's right atrium and ventricle through a vein in the upper chest. Figure B shows an electrode electrically stimulating the heart muscle. Figure C shows the location and general size of a single-lead, or single-chamber, pacemaker in the upper chest.

PACEMAKER IMPLANTATION

A pacemaker is implanted to treat bradycardia (an abnormally slow heart rate). Pacemakers can also adjust the heart rate to meet the body's needs, whether during exercise or rest. Implantation of a pacemaker involves positioning leads (thin, insulated wires) in the heart and placing the device in a pocket of skin, usually in the shoulder area. Typically the implant procedure involves only local anesthetics and a sedative, rather than general anesthesia. Most people have a fairly quick recovery after apacemaker implant.

What Is a Pacemaker?

A pacemaker is a small implantable device that treats abnormal heart rhythms called arrhythmias.

Specifically, a pacemaker treats slow arrhythmias called bradycardia. Arrhythmias result from a problem in the heart's electrical system. Electrical signals follow a certain pathway through the heart. It is the movement of these signals that causes your heart to contract.

A pacemaker system has two parts, and each plays a role in treatment. The pacemaker leads are thin, insulated wires that carry electrical signals back and forth between the device and the heart. The leads cansense when the heart is beating too slowly and needs treatment. The pacemaker device, or pulse generator, is quite small, easily fitting in the palm of your hand. It contains computerized parts that run ona battery. The device treats your heart by sending very small amounts of electrical energy to the heart through the leads. Patients usually can't feel the treatment. The pacing system delivers treatment based on what it senses in your heart, even if you don't feel any symptoms. The pacemaker can be implanted belowthe collarbone on either the right or left side of the body. In some cases the device is implanted in the abdomen. Before confirming where to place the device, you and your doctor will talk about:

- Your age and overall health
- Whether you have had chest surgery
- Your activities and lifestyle

Pacemaker Implantation Procedure

Implanting the Leads: You lie on an exam table and an intravenous (IV) line is put into your arm. The IV delivers fluids and medications during the procedure. The medication makes you relaxed and groggy, but not unconscious. (General anesthesia is usually not needed.) During the procedure, you will be attached toseveral monitors. Your doctor numbs a small area of skin and inserts the leads through a small incision, usually near the collarbone. The doctor gently steers the leads through the blood vessels and into the heart. The doctor can see where the leads are going by watching a video screen with real-time, moving x- rays (fluoroscopy). Depending on the treatment your heart needs, either one or two leads are implanted in your heart. A pacemaker that uses one lead is called a single-chamber pacemaker. A pacemaker that uses two leads is called a dual-chamber pacemaker. With a dual-chamber pacing system, one lead goes in your top right chamber (the atrium) and the other lead goes in your bottom right chamber (the ventricle).

Testing the Leads and Device: Your doctor connects the implanted leads to the device and tests the system. In this way the doctor makes sure that both parts of the pacemaker system—the leads and the device—work properly. During the testing you may feel your heart beating faster. Implanting

the Device: Your doctor places the device just under the skin—usually near your collarbone—and then stitches the incision closed.

What Happens After the Procedure?

The pacemaker implant experience can vary from one person to another. Some people stay in the hospital overnight, while others go home the same day as the procedure. There is usually tenderness at the incisionsite, just as there is any time you have stitches. However, most people have a fairly quick recovery.

1. any Haemoglobin-based blood substitutes may increase the odds of deaths and heart attacks. According to studies of outcomes of transfusions given to trauma patients in 2008, blood substitutes yielded a 30% increase in the risk of death and about a threefold increase in the chance of having a heart attack for the recipients. More than 3,711 patients were tested in sixteen studies using five types of artificial blood. Public Citizen sued the U.S. Food and Drug Administration (FDA) to attain information on the duration of these studies which were found to have been conducted from 1998 until 2007. The FDA permits artificial blood transfusions in the US without informed consent under a special exemption from requirements of informed consent during traumatic care.

ARTIFICIAL SKIN

Artificial skin refers to a collagen scaffold that induces regeneration of skin in mammals. The term was used in the late 1970s and early 1980s to describe a new treatment for massive burns. It was later discovered that treatment of deep skin wounds in adult animals and humans with this scaffold induces regeneration of the dermis. It has been developed commercially under the name IntegraTM and is used in massively burned patients, during plastic surgery of the skin, and in treatment of chronic skin wounds.

The term “artificial skin” sometimes is used to refer to skin-like tissue grown in a laboratory, although this technology is still quite a way away from being viable for use in the medical field. 'Artificial skin' canalso refer to flexible semiconductor materials that can sense touch for those with prosthetic limbs,

The skin is the largest organ in the human body. Skin is made up of three layers, the epidermis,

dermis and the fat layer, also called the hypodermis. The epidermis is the outer layer of skin that keeps vitalfluids in and harmful bacteria out of the body. The dermis is the inner layer of skin that contains blood vessels, nerves, hair follicles, oil, and sweat glands. Severe damage to large areas of skin exposes the human organism to dehydration and infections that can result in death.

ARTIFICIAL PANCREAS

The **artificial pancreas** is a technology in development to help people with diabetes automatically controltheir 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, butit is the lack of insulin production which is the motivation to develop a substitute. While the current stateof insulin replacement therapy is appreciated for its life-saving capability, the task of manually managingthe blood sugar level with insulin alone is arduous and inadequate.

The goal of the artificial pancreas is two-fold:

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

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 willbehave as the endocrine pancreas and will be viable for years.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-producingcells

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 protectivecoating has been developed to block the immune response to transplanted cells, which relieves the burdenof 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 meshfibers;
- a semi-permeable protective layer around the sheet, to allow the diffusion of nutrients andsecreted hormones;
- a protective coating, to prevent a foreign body response resulting in a fibrotic reaction whichwalls 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 humanclinical trials within a few years.

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

- insulin, which lowers blood glucose by converting glucose into glycogen;
- amylin, which slows digestion and slows the rate of glucose entering the bloodstream, andtemporarily suppresses release of glucagon;
- and glucagon, which raises blood glucose by converting glycogen into 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 co-secreted 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 glucoseto be removed from the bloodstream and stored in the liver and muscle cells. 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 isto spread out the blood glucose peak after eating, reducing the quantity of insulin needed. As the bloodsugar level comes back toward normal, the beta cells will stop spurting insulin and amylin. As the glucoselevel 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. 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, thegoal 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-peptidewas 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.

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 ofcarbohydrate 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 ofinsulin, an 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 carbohydrateis difficult) whereas pancreatic insulin is released in proportional response to actual blood glucoselevels;
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 bloodsugar 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.

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UNIT II

PART A

S.No	Questions
1	What are the different types of grafts?
2	What is transplantation?
3	Highlight few basic engineering concerns related to design heart valve.
4	What is cardiac pacemaker?
5	Mention the function of SA node.
6	What is artificial pancreas?
7	What is artificial skin?
8	List the composition of Dialysate.
9	Write the importance of cardiac pacemaker.
10	Brief the implantation procedure of pacemaker.
11	Schematically represent the surgical removal of kidney from a living donor.
12	Define dialysis.
13	Mention the principle of Dialysis.
14	What is artificial kidney?
15	Explain the working mechanism of artificial kidney membrane dialysis.
16	How dialyzer cartridge can be reused?
17	Define Nephron and mass transfer.
18	Briefly explain the dialysis procedure.

PART B

S.No	Questions
1	Compare the salient features and drawbacks of mechanical versus tissue heart valves with examples.
2	What are the characteristics an ideal heart valve should possess?
3	Narrate the design requirements of prosthetic cardiac valves. What are the problems associated with different types of valves?
4	What is artificial heart? Explain the procedures involved in cardiac pacemakers
5	Write in detail about skin and pancreatic transplantation.
6	Explain in detail about the kidney dialysis procedure and add a note on reuse of cartridge.
7	Explain the principle and working of artificial kidney membrane dialysis.
8	Review the fundamental aspects of dialysis procedure with specific reference.
9	Describe how the dialysis procedure takes place during dialysis
10	The surface of the kidney dialysis membrane is coated with poly HEMA. Discuss the advantages and disadvantages
11	Justify Nephron Mass transfer play a vital role of renal function of kidney.

UNIT – III – Artificial Organs and Tissue Engineering-SBMA3003

Transplantation types

1.1Autograft

Autografts are the transplant of tissue to the same person. Sometimes this is done with surplus tissue, tissue that can regenerate, or tissues more desperately needed elsewhere (examples include skin grafts, vein extraction for CABG, etc.). Sometimes an autograft is done to remove the tissue and then treat it or the person before returning it (examples include stem cell autograft and storing blood in advance of surgery).

1.2 Allograft and allotransplantation

An allograft is a transplant of an organ or tissue between two genetically non-identical members of the same species. Most human tissue and organ transplants are allografts. Due to the genetic difference between the organ and the recipient, the recipient's immune system will identify the organ as foreign and attempt to destroy it, causing transplant rejection.

1.3 Isograft

A subset of allografts in which organs or tissues are transplanted from a donor to a genetically identical recipient (such as an identical twin). Isografts are differentiated from other types of transplants because while they are anatomically identical to allografts, they do not trigger an immune response.

1.4 Xenograft and xenotransplantation

A transplant of organs or tissue from one species to another. An example is porcine heart valve transplant, which is quite common and successful. Another example is attempted piscine-primate(fish to non-human primate) transplant of islet (i.e. pancreatic or insular tissue) tissue. The latter research study was intended to pave the way for potential human use if successful. However, xenotransplantion is often an extremely dangerous type of transplant because of the increased risk of non-compatibility, rejection, and disease carried in the tissue.

Success of transplantation between identical twins proposes that the success rate depends on theamount of sharing of histo compatibility genes.

Histocompatibility genes are responsible for the production of antigens on cell surface. With reference to the surface antigens, the grafts or transplants are differentiated in to four types.

They are as follows:

1. Auto graft or Autogenic graft

2. Isograft or Syngraft or Syngenetic graft,

3. allografts or Homografts,

4. Xenograft.

1. Auto graft or Autogenic graft:

When tissue is transplanted from one site to another in the same individual, the transplant is referred as "auto graft" or "autogenic graft" (From Greek Auto=Self).

Immune system of recipient accepts the auto graft very easily, because antigens of recipient cells and the transplanted tissue are alike.

2. Isograft or Syngraft or Syngentic graft:

The graft taken from a genetically identical person is known as Isograft or Syngraft or Syngentic graft. This kind of transplantation is possible between two genetically identical twins.

Since development of identical twins takes place from a single zygote, identical twins share samegenes that are responsible for the production of antigens.

3. Allograft or Homograft:

If the transplantation is carried between genetically different members of the same species then graft is called as "allograft". The allograft is formally named as "Homograft".

The histo compartbility antigens of allograft are dissimilar with the host histo compartbility antigens. Hence immune system of recipient/ host identifies the graft as foreign and induces an immune response against to it, resulting rejection of graft.

4. Xenograft:

If the transplantation between individuals of two different species is carried, for eg. Transplanting monkey liver to human, the graft is referred as "Xenograft".

Since the histocompatibility genes are quite different, hosts body rejects the graft more vigorously.

3. Blood transfusion

Blood transfusion is generally the process of receiving blood or blood products into one's circulation intravenously. Transfusions are used for various medical conditions to replace lost components of the blood. Early transfusions used whole blood, but modern medical practice commonly uses only components of the blood, such as red blood cells, white blood cells, plasma, clotting factors, and platelets.

Red blood cell transfusion was considered when the hemoglobin level fell below 10 g/dL or hematocrit falls below 30% (the "10/30 rule"). Because each unit of blood given carries risks, a trigger level lower than that at 7–8 g/dL is now usually used as it has been shown to have better patient outcomes. The administration of a single unit of blood is the standard for hospitalized people who are not bleeding, with this treatment then followed with re-assessment and consideration of symptoms and hemoglobin concentration. Patients with poor oxygen saturation may need more blood. The advisory caution to use blood transfusion only with more severe anemia is in part due to evidence that outcomes are worsened if larger amounts are given. One may consider transfusion for people with symptoms of cardiovascular disease such as chest pain or shortness of breath. In cases where patients have low levels of hemoglobin but are cardiovascularly stable, parenteral iron is a preferred option based on both efficacy and safety

Blood transfusions typically use sources of blood: one's own (autologous transfusion), or someone else's (allogeneic or homologous transfusion). The latter is much more common thanthe former. Using another's blood must first start with donation of blood. Blood is most commonly donated as whole blood intravenously and collecting it with an anticoagulant. In developed countries, donations are usually anonymous to the recipient, but products in a blood bank are always individually traceable through the whole cycle of donation, testing, separation into components, storage, and administration to the recipient. This enables management and investigation of any suspected transfusion related disease transmission or transfusion reaction.

Blood transfusion is generally the process of receiving blood products into one's circulation intravenously. Transfusions are used for various medical conditions to replace lost components of the blood. Early transfusions used whole blood, but modern medical practice commonly uses only components of the blood, such as red blood cells, white blood cells, plasma, clotting factors, and platelets.

Procedure

Before a blood transfusion is given, there are many steps taken to ensure quality of the blood products, compatibility, and safety to the recipient. In 2012, a national blood policy was in place in 70% of countries and 62% of countries had specific legislation that covers the safety and quality of blood transfusion.

Blood donation

Blood transfusions typically use sources of blood: one's own (autologous transfusion), or someone else's (allogeneic or homologous transfusion).

The latter is much more common than the former.

Using another's blood must first start with donation of blood. Blood is most commonly donated as whole blood intravenously and collecting it with an anticoagulant.

In developed countries, donations are usually anonymous to the recipient, but products in a blood bank are always individually traceable through the whole cycle of donation, testing, separation into components, storage, and administration to the recipient. This enables management and investigation of any suspected transfusion related disease transmission or transfusion reaction.

In developing countries the donor is sometimes specifically recruited by or for the recipient, typically a family member, and the donation occurs immediately before the transfusion.

Processing and Testing

Donated blood is usually subjected to processing after it is collected, to make it suitable for use in specific patient populations. Collected blood is then separated into blood components by centrifugation: red blood cells, plasma, platelets, albumin protein, clotting factor concentrates, cryoprecipitate, fibrinogen concentrate, and immunoglobulins (antibodies). Red cells, plasma and platelets can also be donated individually via a more complex process called apheresis.

- All donated blood is tested for infections. The current protocol tests donated blood for HIV-1, HIV-2, HTLV-1, HTLV-2, Hepatitis B, Hepatitis C, Syphilis (*Treponema pallidum*),. In addition, platelet products are also tested for bacterial infections due to its higher inclination for contamination due to storage at room temperature..
- All donated blood is also tested for ABO and Rh groups, along with the presence of any red blood cell antibodies.
- Pathogen Reduction treatment that involves, for example, the addition of riboflavin with subsequent exposure to UV light has been shown to be effective in inactivating pathogens (viruses, bacteria, parasites and white blood cells) in blood products. By inactivating white blood cells in donated blood products, riboflavin and UV light treatment can also replace gamma-irradiation as a method to prevent graft-versus-host disease (TA-GvHD).

Compatibility testing

Before a recipient receives a transfusion, compatibility testing between donor and recipient bloodmust be done. The first step before a transfusion is given is to Type and Screen the recipient's blood. Typing of recipient's blood determines the ABO and Rh status. The sample is then Screened for any alloantibodies that may react with donor blood. It takes about 45 minutes to complete (depending on the method used). The blood bank scientist also checks for special requirements of the patient (e.g. need for washed, irradiated or CMV negative blood) and the history of the patient to see if they have a previously identified antibodies and any other serological anomalies.

Adverse effects

In the same way that the safety of pharmaceutical products are overseen by pharmacovigalence, the safety of blood and blood products are overseen by Haemovigilance. This is defined by the World Health Organization (WHO) as a system "...to identify and prevent occurrence or recurrence of transfusion related unwanted events, to increase the safety, efficacy and efficiency of blood transfusion, covering all activities of the transfusion chain from donor to recipient."

Thesystem should include monitoring, identification, reporting, investigation and analysis of adverseevents near-misses and reactions related to transfusion and manufacturing.

Transfusions of blood products are associated with several complications, many of which can begrouped as immunological or infectious. There is also increasing focus (and controversy) on complications arising directly or indirectly from potential quality degradation during storage.

Immunogic reaction

- *Acute hemolytic reactions* occur with transfusion of red blood cells, and occurs in about 0.016 percent of transfusions, with about 0.003 percent being fatal. This is due to destruction of donor red blood cells by preformed recipient antibodies. Symptoms include fever, chills, chest pain, back pain, hemorrhage, increased heart rate, shortness of breath, and rapid drop in blood pressure. When suspected, transfusion should be stopped immediately, and blood sent for tests to evaluate for presence of hemolysis.
- *Delayed hemolytic reactions* occur more frequently (about 0.025 percent of transfusions) andare due to the same mechanism as in acute hemolytic reactions.

Most blood transfusions go very smoothly. Sometimes mild problems can occur. Very rarely, serious problems occur.
Allergic Reactions - Some people have allergic reactions to the blood given during transfusions. This can happen even when the donated blood is the correct blood type. Allergic reactions can bemild or severe. Symptoms may include: Anxiety Chest or back pain Trouble breathing Fever, A quick pulse or low blood pressure, Nausea (feeling sick to your stomach)

A nurse or doctor will stop the transfusion at the first signs of an allergic reaction. The health care team will figure out the severity of the reaction, what treatments are needed, and whether they can safely restart the transfusion.

4. ORGAN TRANSPLANTATION

Moving an organ from a donor's body to a patient's body, or to create organs from the patient's own stem cells (regenerative medicine as an emerging field) in order to replace the recipient's damaged or absent organ, that is what the term Organ Transplantation refers to, including the following organs.

- Thymus
- Intestine
- Lungs
- Pancreas
- Liver
- Kidneys
- Heart

It also involves to transplantation of tissues such as,

- Bones
- Muscoloskeletal grafts
- Cornea
- Skin
- Heart valves
- Nerves
- Veins

It is one of the most complex and challenging areas of medicine, because of the ever-present riskthat the recipient body rejects the transplant, making the removal necessary.

Benefits and Outcomes of Organ Transplant

Organ transplant is the last possibility to address a state of organ failure. Kidney for instance, is the most frequently carried out organ transplant worldwide, and it is considered the best treatment for its cost effectiveness and life quality prospects it restores.

Organ transplantation requires long term health evaluation of the patient. Only academic communities and medical scientists have the right to monitor the outcomes of transplants and regulate donations.

Three Essential Processes

In modern times, doctors and patients face an enormous demand for transplants which has long surpassed the supply of organs. Patients must wait a long time, years in some cases, for a chance to get hold of a donated organ. That's why scientists are working along with politicians to solve this problem.

Organ distribution is therefore the first essential step, followed by the transplant surgery and the follow-up or post-surgery recovery.

Evaluation Process

The following are some components of the transplant evaluation process:

- Psychological evaluation – in which the medical team assesses significant psychological and social issues such as stress, financial situation and family support.
- Blood tests – essential in the selection process to joining the donor's list. They are performed to determine donor match, priority in the list and to improve chances against organ rejection.
- Diagnosis – to assess health status. Includes X-rays, ultrasound, biopsy, dental examinations, among other diagnostic tests depending on the transplant surgery required.

Organ Distribution

When a particular organ fails, transplant can be the only chance for the patient. For procedures

like kidney and liver transplant, a willing donor might be found among family members or friends. A very small number of transplants come from people donating as a result of a good, Samaritan gesture. Nevertheless, there is still the necessity of being appropriate for donor-recipient match, a process of selection achieved through serotyping. Then it is possible to proceed with the surgery state.

Patients must find a transplant team or a group of organ surgeons and health professionals, who decide if the patient is a good candidate, based on the attitude, psychological state, medical history and other factors, to be included in the national waiting list for transplantation.

When the organ becomes available, based on the criteria of all relevant information, a recipient, the best match for the organ is chosen. Then the hospital prepares for surgery.

Surgical Procedure

The fully anesthetized patient is injected with anticoagulant to keep the blood from clotting during the transplantation procedure. Doctors connect the hearth-lung machine, in the case of a heart transplant, or other life-support devices to enable the surgeon to remove the organ withoutdisrupting body functions.

Three types of rejection

Following a transplant surgery, the following three types of rejection might occur:

- Chronic rejection – might last months or years.
- Acute rejection – a few days after transplant and it is the immune response to foreign matter.
- Hyperacute rejection – as soon as the organ is connected to the new body.

Types of Transplant

- Autograft – transplant of tissue from one area of the body to another, using surplus tissue which is regenerative.
- Allograft – transplant of tissue or organ between non-identical members of a species. This transplant might cause rejection due to genetic difference.
- Xenograft or xenotransplant – a transplant from one species to another. Very risky due torejection.

Individual organs- kidney, liver, heart, lung, bone, skin, hair and pancreas

4.1Kidney transplant:

- Renal transplantation is the preferred treatment for patients with end-stage renal disease.It offers better quality of life and confers greater longevity than long-term dialysis.
- EMPs encounter transplant pts at 2 critical stages:
- Initial doctors to identify potential donors from a pool of critically ill patients who areadmitted to hospital.
- They care for pts once they have been transplanted and present with complicationsrelated to their immunosuppressive therapy, infections or ARF.
- Diabetic nephropathy accounts for 40% of the diseases resulting in renal transplantation.This subgroup of pts are also more prone to complications after renal transplantation.
- The spectrum of diseases in transplant pts is different from the general population.
- The classical presentation of common medical disorders may be modified byimmunosuppressive medication.

The Transplantation Process

- Transplant coordinators should be called early for any pt who may meet brain deathcriteria in the new future.
- Absolute C/Is for organ donation include HIV, sepsis, non-CNS malignancy and severeCVS disease.
- Age is also a relative C/I (i.e. organs not harvested from pts >75 years of age).
- The pretransplantation workup of a potential donor includes testing for CMV, HSV,EBV, HIV, Hep A, B, C, D + E and HTLV type 1.

- Following brain death, a number of physiological changes occur that need to be rectifiedif donor organ perfusion is to be preserved.
- Increased cerebral oedema after trauma or stroke results in catecholamine release andHT.
  With brainstem necrosis, catecholamine levels drop rapidly resulting in hypotension.This should be corrected with fluid and vasopressors
- About 75% of organ donors develop diabetes insipidus due to pituitary necrosis and thisleads to hypovolaemia.
- Systemic thermal control is often lost due to hypothalamic ischaemia which results incoagulopathy, hepatic dysfunction and cardiac dysfuction.
- Allograft : graft between genetically dissimilar individuals of the same species.
- Autograft : graft in which donor and recipient are the same individual.
- Xenograft : Donor and recipient belong to different species.

The Surgical Procedure

- Wet ischaemia time (time from cessation of circulation to removal of organ and itsplacement in cold storage) should not exceed 30 mins.
- Transplanted kidney is placed in the R or L lower quadrant of the abdomen in anextraperitoneal position. On examination, the transplant is easily palpable.
- The transplant renal a is anastomosed to the ipsilateral internal or external iliac a, therenal v to internal or external iliac v and the transplant ureter to the bladder.
- Generally a single kidney is transplanted.
- When small, paediatric or older cadaveric donor kidneys with age-related loss of renal fxn are transplanted, both kidneys from the donor might be placed in a single recipient to provide adequate fxnal renal mass.
- Living donor transplants fxn immediately after transplant, +/- 30% of cadaveric transplants have delayed graft fxn because of more prolonged ischaemic cold preservation. These pts need continued dialysis support until the kidney starts to function.

4.2 Liver transplant:

- 1960 :Initial Liver Transplant (LT) techniques done using dogs.
- 1963 : First human LT attempt by Starzl.
- 1967 : First successful LT by Starzl.
- Early 1980's: LT became clinical reality.
- 1983 : Definitive therapy for end-stage liver disease (ESLD).
- 1988: Development of the University of Wisconsin (UW) solution (graft preservation).
- 1992: First Liver xenotransplants x 2 (baboon) by AG Tzakis

Potential Indications for LT

- Viral hepatitis
- Malignant neoplasm of liver and intrahepatic bile ducts
- Benign neoplasm of liver and biliary passages
- Carcinoma of liver and biliary system
- Neoplasm of uncertain behavior in liver and biliary passages
- Neoplasm of unspecified nature in digestive system
- Glycogenesis
- Pure hypercholesterolemia
- Lipidoses
- Disorders of copper metabolism
- Cystic fibrosis, disorders of porphyrin metabolism, other disorders of purine

and pyrimidine metabolism, amyloidosis, disorders of bilirubin excretion, mucopolysaccharidosis, other deficiencies of circulating enzymes

- Congenital factor VIII disorder

- Congenital factor IX disorder
- Budd-Chiarri syndrome
- Acute and subacute necrosis of liver
- Alcoholic fatty liver
- Alcoholic cirrhosis of liver
- Chronic hepatitis
- Cirrhosis of the liver without mention of alcohol
- Biliary cirrhosis
- Other chronic nonalcoholic liver disease
- Unspecified liver disease without mention of alcohol
- Other sequelae of chronic liver disease
- Other specified disorders of gallbladder
- Biliary atresia, other anomalies of gallbladder, bile ducts, and liver
- Perinatal jaundice due to hepatocellular damage
- Other specified perinatal disorders of digestive system
- Injury to liver
- Encephalopathy, unspecified
- Portal vein thrombosis

Liver recipient procedure Orthotropic LT

- Total Hepatectomy (venovenous bypass)
- Caval anastomosis (conventional, Piggyback technique).
- Reperfusion
- Portal vein anastomosis
- Hepatic Artery anastomosis (end to end, infrarenal aortic jump graft)
- Biliary reconstruction (duct – to duct, Roux-en-Y hepatico-jejunostomy).

Living Donor/Split LT

- Living-donor LT: part of the liver from a living donor is resected and transplanted into arecipient.
- Split LT: a whole adult liver is transected into 2 pieces to provide grafts for 2 recipients

Postoperative Care

- Liver function tests monitoring
- Fibrinogen level is the most important indicator of graft function in first 24 hours (>100).
- Gradual normalization of ALT, AST, T. Bili, PT.
- Early elevation of liver enzymes (LEs) followed by quick normalization is reflective ofpreservation injury (cold preservation).
- Primary non functional liver: marked increase of (LEs) and T. Bili (Tx: re-transplant).
- Thrombocytopenia: Platelet count decrease in the first week after LT and increase duringthe second week. (Platelet sequestration in the liver and spleen, preservation injury).
- Doppler US Liver: intra-operatively, and daily until POD# 5. (Velocity and RI of HA,PV, HV flow).
- Daily monitoring of immunosuppressive drug levels.

Immunosuppressant regimens

- ✓ ISP drugs necessary to prevent rejection.
- ✓ The risk of rejection is highest (up to 40%) during the first 3-6 months aftertransplantation and decreases significantly thereafter.
- ✓ ISP induction: Prograf (FK) + Steroids (OR)
- ✓ Maintenance:

  Prograf + Steroids
  Prograf + Campath (steroid free protocol)Rapamycin + Steroids
  OKT-3 (severe rejection)

4.3 Bone Marrow Transplant

Located in the interior of our bones, bone marrow is one of the areas that we are never concerned with until we have some complaint. However, this flexible, spongy and well-protectedtissue is essential for our organism.

A vital component of the bone marrow are stem cells which are immature cells that are able to form a variety of different cells in our body (e.g.: neural cells). Stem cells are responsible for the production of the cellular elements of the blood: red blood cells (carry oxygen), platelets (ensure blood clotting) and lymphocytes (immune functions).

What Are The Most Common Diseases?

- Aplastic anemia (damaged bone marrow and dropped red blood cell production)
- Leukemia (abnormal white cell production)
- Bone marrow cancer

Moreover, cancer radiation and chemotherapy can also severely damage bone marrow. To avoid it, before radiation or chemotherapy treatment of cancer patients their stem cells are harvested from the bone marrow to protect them and after the treatment they are re-injected to restore immune functions.

Diagnosis

Examination of bone marrow tissue can happen by biopsy and bone marrow aspiration to gain information about the source of blood production. The procedure is rather unpleasant but unavoidable.

Bone Marrow Transplant

Bone marrow transplantation can be the only solution to treat some severe diseases, such as:

- bone marrow cancer
- leukemia
- multiple myeloma
- certain blood diseases
- autoimmune diseases

In the procedure stem cells are taken from a healthy donor and infused into the patient to help ideal blood cell production.

Bone Marrow Transplant Procedure

We can distinguish three kinds of bone marrow transplants:

- Autologous (the process of removing and reinjecting the patient's own bone marrow before cancer treatment)
- Umbilical cord blood transplant (stem cells are removed from the baby's umbilical cordfor later use)
- Allogenic bone marrow transplant (from donor to patient)

In allogenic procedures, first the matching donor is identified by blood tests (usually family members with similar genes).

Patients' own bone marrow is suppressed by radiation and chemotherapy. It is important in order to remove malfunctioning stem cells and to suppress the immune system that will resist the transplanted cells less.

Stem cells are taken from a donor, who receives general anesthesia while the bone marrow is surgically removed from hip bones.

The stem cells are infused into the bloodstream with a catheter, similarly to a blood transfusion.

The stem cells will find their way to the bone marrow. Bone marrow transplant has many risks and usually involves a lengthy post-treatment.

4.4. Pancreas Transplant

One of the most important functions of the pancreas is to produce insulin, which is a vital hormone that regulates the absorption of glucose (commonly known as blood sugar) into the cells. The main problem with type 1 diabetes is the lack of insulin production in the pancreas, resulting in the increase of blood sugar levels up to dangerous life-threatening conditions.

By far the principal cause for pancreas transplantation is type 1 diabetes. Pancreas transplant defines the surgical procedure in which a healthy donor pancreas is transplanted into a patient whose pancreas has failed or no longer function properly. Pancreas transplant may have a particularly significant number of side effects and complications and that is why the procedure isonly reserved for patients with serious diabetes complications.

Kidney transplant is quite often done in conjunction with pancreas transplants.

Pancreas Transplant – Causes and Risks

Pancreas transplantation is not a standard treatment, because anti-rejection medications, which are usually required for organ donations, in this case can trigger extremely serious complications.

Doctors should make and attempt with all treatments available for pancreatic diseases before recommending pancreas transplantation.

The most common causes for pancreas transplant are:

- Type I diabetes
- Poor blood sugar control
- Insulin reactions
- Severe kidney damage

Pancreas transplant is not a treatment option for Type II diabetes because the problem is not related to insulin production in the pancreas, but in the inability to use insulin properly.

When kidney damage is due to type 1 diabetes, pancreas transplant can be combined with kidney transplantation. These procedures aim to prevent further diabetes-related damage in the future.

Among the risks for pancreas transplant there are some that are commonly related to any type of surgery:

- Infection
- Bleeding
- Blood clots

Severe complications involved in pancreas transplant:

- Hyperglycemia (excess sugar in the blood)
- Urinary complications
- Failure of the donated pancreas
- Rejection of the donated pancreas

Side effects due to anti-rejection medication are frequents such as:

- High cholesterol
- Bone thinning
- High blood pressure
- Skin sensitivity
- Puffiness
- Weight gain
- Acne
- Swollen gums
- Excessive hair growth

Pancreas Transplantation Procedure

The first thing to do is to choose a transplant center, which should be selected from your insurance company's list or from your own selection.

A few things are important to consider:

- Learn about pancreas transplant history of the clinic
- Ask about recipient survival rates
- Compare statistics with the Scientific Registry of Transplant Recipients
- Consider post-op services like support groups, local housing, travel arrangements andreferrals

After this, the transplant team will perform an assessment of the patient's eligibility for pancreas transplant. Among the items to consider we find and overall health (can the patient tolerate life-long post-transplant medication?) and life-style habits. Before the procedure, patients have to prepare for numerous lab tests.

When the patient has been accepted, the candidate will be placed on the national waiting list. From this point until the actual pancreas transplantation, waiting time depends on when a suitable donor is available.

During the pancreas transplant, an inpatient surgical procedure done under general anesthesia, anincision is made in the center of the abdomen and the donor pancreas is placed into the lower abdomen. The next step requires the attachments of a piece of donor intestine and of the blood vessels.

The procedure usually lasts three hours or a bit more. After the pancreas transplant, patients stay in the clinic for a few days, till their condition stabilizes and medication routine is established.

4.5 Heart Transplant Surgery

Heart transplant surgery is a major procedure to replace a malfunctioning heart with a healthy donor heart. About 50% of heart transplant patients live 10 years or longer with the new heart, people who otherwise would have little chance of survival on medication or with minor heart surgeries. All potential complications considered, the practice of cardiac transplant is remarkablysuccessful.

Who Is Eligible For Heart Transplant Surgery?

Patients who have tried all other medical and surgical options and are determined to take the necessary lifestyle changes. Eligible patients are usually younger than 65 and have no other life threatening medical problem. The following conditions may call for heart transplantation -

- Inherited and congenital heart defects
- Coronary artery disease
- Cardiomyopathy (weakening heart muscles)
- Diseases of the heart valves

Heart Transplant Procedure

When all other medical means have failed to improve, the physican refers the patient to a heart transplant cencter to evaluate the case and the subject'general health status.

Then the patient is added to the heart transplant waiting list. As there is a global shortage of donor hearts, waiting lists are usually long.

When there is a recently deceased donor, doctors must consider the following aspects before appointing a patient for heart transplant surgery –

- Severity and urgency of the heart failure
- Size of the donor heart
- Blood type

The donor heart must be transplanted within 4 hours of removal so doctors and patients usually do not have much time for contemplation, a decision must be made immediately.

The heart transplant surgery itself is not very long; it takes about 4-5 hours. During the procedure the patient is connected to a heart-lung machine to maintain circulation while the diseased heartis changed to the donor heart. The newly implanted heart receives an electric shock to

initiate its beating, but sometimes it starts automatically once the blood flows again in the veins. For a few days after the operation patients experience heavy breathing, pain and chest pressure, but these side-effects cease after about a week or two. After patients are discharged from hospital, constant check-up is necessary for another three months.

During this period patients will be administered medication to repress your immune system, to reduce the risk that the immune system attacks the foreign tissues. Weakened immune responses should be compensated with antibacterial and antiviral medication.

In the recovery period patients should get used to new lifestyle habits, healthy, regular eating andphysical activity. Most cardiac transplant patients can resume their normal activities within 3-6 months but they are instructed to avoid stress and strenuous workout.

The Risks of Heart Transplant Surgery

- Immune rejection of the new heart may occur in the first year post-surgery. In order to monitor it, regular biopsy is taken from the heart. The signs of rejection are very similar to that of the flu: headache, fever, weakness, dizziness and vomiting.
- Artificial weakening of the immune system can be a double-edged sword, which can result in viral and bacterial infections.

In spite of the efficiency of the procedure and the relatively few cases of complication, heart transplant surgery has many downsides that need to be addressed in the future –

- Costs are extremely high, usually several hundreds of dollars
- Insurers' reluctance to cover the costs
- Limited eligibility of patients
- Scarce donor hearts
- Slow channels that do not reach the patient in time.

4.6 Skin Graft

Skin grafting is a type of graft surgery involving the transplantation of **skin**. The transplanted tissue is called a **skin** graft. **Skin grafting** is often used to treat: Extensive wounding or trauma. Burns.

Skin grafting is a surgical procedure that involves removing the skin from one area of the body and moving it, or transplanting it, to a different area of the body. This surgery may be done if a part of your body has lost its protective covering of skin due to burns, injury, or illness.

A skin graft is placed over an area of the body where skin has been lost. Common reasons for a skin graft include:

- skin infections
- deep burns
- large, open wounds

- bed sores or other ulcers on the skin that haven't healed well

There are two basic types of skin grafts: split-level thickness and full-thickness grafts.

Split-Level Thickness Grafts

- A split-level thickness graft involves the removal of the top two layers of skin, the epidermis and the dermis. These layers are taken from the donor site, which is the area where the healthy skin is located.
- Split-level thickness grafts are used to cover large areas. These grafts tend to be fragile and typically have a shiny or smooth appearance. They may also appear paler than the adjoining skin. Split-level grafts don't grow with the rest of the skin, so children who get them may need additional grafts as they grow older.

Full-Thickness Grafts

- A full-thickness graft involves the removal of the muscles and blood vessels in addition to the top two layers of skin from the donor site.
- Full-thickness grafts are generally used for small wounds on highly visible parts of the body, such as the face. Unlike split-level thickness grafts, full-thickness grafts blend in well with the skin around them and usually grow with the person.

Risks for the skin graft surgery are:

- Bleeding
- Infection
- Loss of grafted skin
- Nerve damage
- Graft-versus-host disease

Rejection may occur in xenografts. To prevent this, the patient usually must be treated with long-term immunosuppressant drugs.

4.7 Hair Transplantation

Hair transplantation is a surgical technique that moves hair follicles from a part of the body called the 'donor site' to a bald or balding part of the body known as the 'recipient site'. It is primarily used to treat male pattern baldness. In this minimally invasive procedure, grafts containing hair follicles that are genetically resistant to balding, (like the back of the head) are transplanted to the bald scalp. Hair transplantation can also be used to restore eyelashes, eyebrows, beard hair, chest hair and to fill in scars caused by accidents or surgery such as face-lifts and previous hair transplants. Hair transplantation differs from skin grafting in that grafts contain almost all of the epidermis and dermis surrounding the hair follicle, and many tiny grafts are transplanted rather than a single strip of skin.

Since hair naturally grows in groupings of 1 to 4 hairs, current techniques harvest and transplant hair "follicular units" in their natural groupings. Thus modern hair transplantation can achieve a natural appearance by mimicking original hair orientation. This hair transplant procedure is called follicular unit transplantation (FUT). Donor hair can be harvested in two different ways: strip harvesting, and follicular unit extraction (FUE).

4.7.1 Pre-operative assessment and planning

At an initial consultation, the surgeon analyzes the patient's scalp, discusses their preferences andexpectations, and advises them on the best approach (e.g. single vs. multiple sessions) and what results might reasonably be expected. Pre-operative folliscopy will help to know the actual existing density of hair, so that postoperative results of newly transplanted hair grafts can be accurately assessed.

4.7.2Harvesting methods

Transplant operations are performed on an outpatient basis, with mild sedation (optional) and injected local anesthesia. The scalp is shampooed and then treated with an antibacterial agent prior to the donor scalp being harvested.

There are several different techniques for harvesting hair follicles, each with their own advantages and disadvantages. Regardless of the harvesting technique, proper extraction of the hair follicle is paramount to ensure the viability of the transplanted hair and avoid transection, the cutting of the hair shaft from the hair follicle. Hair follicles grow at a slight angle to the skin'ssurface, so transplanted tissue must be removed at a corresponding angle.

There are two main ways in which donor grafts are extracted today: strip excision harvesting, and follicular unit extraction.

4.7.2.1 Strip harvesting

Strip harvesting is the most common technique for removing hair and follicles from a donor site. The surgeon harvests a strip of skin from the posterior scalp, in an area of good hair growth. A single-, double-, or triple-bladed scalpel is used to remove strips of hair-bearing tissue from the donor site. Each incision is planned so that intact hair follicles are removed. The excised strip is about 1–1.5 x 15–30 cm in size. While closing the resulting wound, assistants begin to dissect individual follicular unit grafts, which are small, naturally formed groupings of hair follicles, from the strip. Working with binocular Stereo-microscopes, they carefully remove excess fibrousand fatty tissue while trying to avoid damage to the follicular cells that will be used for grafting. The surgeon then uses very small micro blades or fine needles to puncture the sites for receiving the grafts, placing them in a predetermined density and pattern, and angling the wounds in a consistent fashion to promote a realistic hair pattern. The technicians generally do the final partof the procedure, inserting the individual grafts in place.

Strip harvesting will leave a thin linear scar in the donor area, which is typically covered by a

patient's hair even at relatively short lengths. The recovery period is around 2 weeks and will require the stitches/staples to be removed by medical personnel or sub cuticular suturing can be done.

4.7.2.2 Follicular unit extraction (FUE)

With Follicular Unit Extraction or FUE harvesting, individual follicular units containing 1 to 4 hairs are removed under local anesthesia; this micro removal typically uses tiny punches of between 0.6mm and 1.0mm in diameter. The surgeon then uses very small micro blades or fine needles to puncture the sites for receiving the grafts, placing them in a predetermined density andpattern, and angling the wounds in a consistent fashion to promote a realistic hair pattern. The technicians generally do the final part of the procedure, inserting the individual grafts in place.

FUE takes place in a single long session or multiple small sessions. The FUE procedure is more time consuming than strip surgery. An FUE surgery time varies according to the surgeons experience, speed in harvesting and patient characteristics. The procedure can take anywhere from a couple hours to extract 200 grafts for a scar correction to a surgery over two consecutive days for a megasession of 2,500 to 3,000 grafts. With the FUE Hair Transplant procedure there are restrictions on patient candidacy. Clients are selected for FUE based on a fox test, though there is some debate about the usefulness of this in screening clients for FUE.

FUE can give very natural results. The advantage over strip harvesting is that FUE harvesting negates the need for large areas of scalp tissue to be harvested, so there is no linear incision on the back of the head and it doesn't leave a linear scar. Because individual follicles are removed, only small, punctuate scars remain which are virtually not visible and any post-surgical pain and discomfort is minimized. As no suture removal is required, recovery from Micro Grafting FUE isless than 7 days.

Disadvantages include increased surgical times and higher cost to the patient. It is challenging for new surgeons because the procedure is physically demanding and the learning curve to acquire the skills necessary is lengthy and tough. Some surgeons note that FUE can lead to a lower ratio of successfully transplanted follicles as compared to strip harvesting.

Follicular unit transplant

Follicular unit transplant (FUT) is the traditional hair transplant method which involves extracting a linear strip of hair bearing skin from the back or the side of the scalp. The strip is then dissected to separate individual grafts.

Robotic hair restoration

Robotic hair restoration devices utilize cameras and robotic arms to assist the surgeon with theFUE procedure.

5. Regeneration and humans

Regeneration in humans is the regrowth of lost tissues or organs in response to injury. This isin contrast to wound healing, which involves closing up the injury site with a scar. Some tissues such as skin and large organs including the liver regrow quite readily, while others have been thought to have little or no capacity for regeneration. However ongoing research, particularly in the heart and lungs, suggests that there is hope for a variety of tissues and organs to eventually become regeneration-capable.

Regeneration means the regrowth of a damaged or missing organ part from the remaining tissue. As adults, humans can regenerate some organs, such as the liver. If part of the liver is lost by disease or injury, the liver grows back to its original size, though not its original shape. And our skin is constantly being renewed and repaired. Unfortunately many other human tissues don't regenerate, and a goal in regenerative medicine is to find ways to kick-start tissue regeneration inthe body, or to engineer replacement tissues.

Understanding how regeneration works

Recent research in different regenerating animals has shown that there are various stem cell strategies for regenerating body parts built from multiple tissues, such as muscle, nerve and skin.

Naturally regenerating appendages and organsEndometrium

The endometrium after the process of breakdown via the menstruation cycle, re-epithelializes swiftly and regenerates. Though tissues with a non-interrupted morphology, like non-injured softtissue, completely regenerate consistently; the endometrium is the only human tissue that completely regenerates consistently after a disruption and interruption of the morphology.

Fingers

In May 1932, L.H. McKim published a report in *The Canadian Medical Association Journal*, that described the regeneration of an adult digit-tip following amputation. A house surgeon in theMontreal General Hospital underwent amputation of the distal phalanx to stop the spread of an infection. In less than one month following surgery, x-ray analysis showed the regrowth of bone while macroscopic observation showed the regrowth of nail and skin. This is one of the earliest recorded examples of adult human digit-tip regeneration.

Studies in the 1970s showed that children up to the age of 10 or so who lose fingertips in accidents can regrow the tip of the digit within a month provided their wounds are not sealed up with flaps of skin – the de facto treatment in such emergencies. They normally won't have a fingerprint, and if there is any piece of the finger nail left it will grow back as well, usually in a square shape rather than round.

In August 2005, Lee Spievack, then in his early sixties, accidentally sliced off the tip of his right middle finger just above the first phalanx. His brother, Dr. Alan Spievack, was researching

regeneration and provided him with powdered extracellular matrix, developed by Dr. Stephen Badylak of the McGowan Institute of Regenerative Medicine. Mr. Spievack covered the wound with the powder, and the tip of his finger re-grew in four weeks. The news was released in 2007. Ben Goldacre has described this as "the missing finger that never was", claiming that fingertips regrow and quoted Simon Kay, professor of hand surgery at the University of Leeds, who from the picture provided by Goldacre described the case as seemingly "an ordinary fingertip injury with quite unremarkable healing"

A similar story was reported by CNN. A woman named Deepa Kulkarni lost the tip of her little finger and was initially told by doctors that nothing could be done. Her personal research and consultation with several specialists including Badylak eventually resulted in her undergoing regenerative therapy and regaining her fingertip.

Kidney

Regenerative capacity of the kidney has been recently explored.

The basic functional and structural unit of the kidney is nephron, which is mainly composed of four components: the glomerulus, tubules, the collecting duct and peritubular capillaries. The regenerative capacity of the mammalian kidney is limited compared to that of lower vertebrates.

In the mammalian kidney, the regeneration of the tubular component following an acute injury iswell known. Recently regeneration of the glomerulus has also been documented. Following an acute injury, the proximal tubule is damaged more, and the injured epithelial cells slough off the basement membrane of the nephron. The surviving epithelial cells, however, undergo migration, dedifferentiation, proliferation, and redifferentiation to replenish the epithelial lining of the proximal tubule after injury. Recently, the presence and participation of kidney stem cells in the tubular regeneration has been shown. However, the concept of kidney stem cells is currently emerging. In addition to the surviving tubular epithelial cells and kidney stem cells, the bone marrow stem cells have also been shown to participate in regeneration of the proximal tubule, however, the mechanisms remain controversial. Recently, studies examining the capacity of bonemarrow stem cells to differentiate into renal cells are emerging.

Like other organs, the kidney is also known to regenerate completely in lower vertebrates such asfish. Some of the known fish that show remarkable capacity of kidney regeneration are goldfish, skates, rays, and sharks. In these fish, the entire nephron regenerates following injury or partial removal of the kidney.

Liver

The human liver is particularly known for its ability to regenerate, and is capable of doing so from only one quarter of its tissue, due chiefly to the unipotency of hepatocytes. Resection of liver can induce the proliferation of the remaining hepatocytes until the lost mass is restored, where the intensity of the liver’s response is directly proportional to the mass resected. Foralmost 80 years surgical resection of the liver in rodents has been a very useful model to the study of cell proliferation.

Future research and regenerative medicine

By defining the properties of stem cells that regenerate complex body parts, scientists are learning how injury causes these stem cells to regenerate the missing part instead of just forming scar tissue.

Ethical considerations of Tissue engineering

Different approaches address ethical considerations of tissue engineering: research ethics, socioeconomic issues and anthropological issues.

Research ethics

- When asking the consent of cell donors, it is important to inform them of the use of their tissue. But will researchers explain clearly what they will do with the cells and what kindof tests they will perform? Will the information provided be sufficient?
- Can the human body and its parts be subject to property rights?

Socioeconomic issues

- What will be the cost of tissue engineering products and treatments?
- Who will finance the research? The government or the private sector?
- Who will be given priority to receive these treatments? Young people with congenitaldiseases or the elderly who suffer from degenerative diseases?

Anthropological issues

- Is it ethically right to fight the negative effects of ageing? Is extending life always a goodthing?
- Have we thought about the consequences of having an ageing society?

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UNIT III

PART A

S.No	Questions
1	What are the donors?
2	Define transplantation.
3	What are the immunological considerations in transplantation?
4	Brief the ethical considerations in transplantation.
5	Define regeneration.
6	Define blood transfusion.
7	Mention the different types of transplants.
8	What are problems encountered during blood transfusion?
9	State the fundamentals of transplantation.
10	What are the immunological factors affecting organ transplants?

PART B

S.No	Questions
1	Explain in detail about the transplantation of kidney.
2	Explain in detail about the bone, hair and pancreatic transplantation.
3	How does the immunological factor affect the organ transplant process? Briefly explain blood transfusion.
4	Discuss the transplantation of liver and bone.
5	Define transplantation. Brief about procedure of renal transplantation.
6	Brief about the ethical considerations of organ transplantation.
7	Discuss briefly about regeneration and factors involved for the same.
8	Discuss the transplantation of kidney and include the need, technical feasibility, economic and social concepts in your discussion
9	Discuss about biological factors involved in blood transfusion.
10	Discuss transplantation of heart, lung and skin. Include question of need, technical, ethical, economic and social aspects in your discussion.

UNIT – IV – Artificial Organs and Tissue Engineering-SBMA3003

TISSUE ENGINEERING

The cellular approach principle is to use donor cells processed before implantation and either seeded into the scaffold (cell-seeded scaffold approach) to encourage the growth or regeneration of functional tissue, or used alone – the stem cell

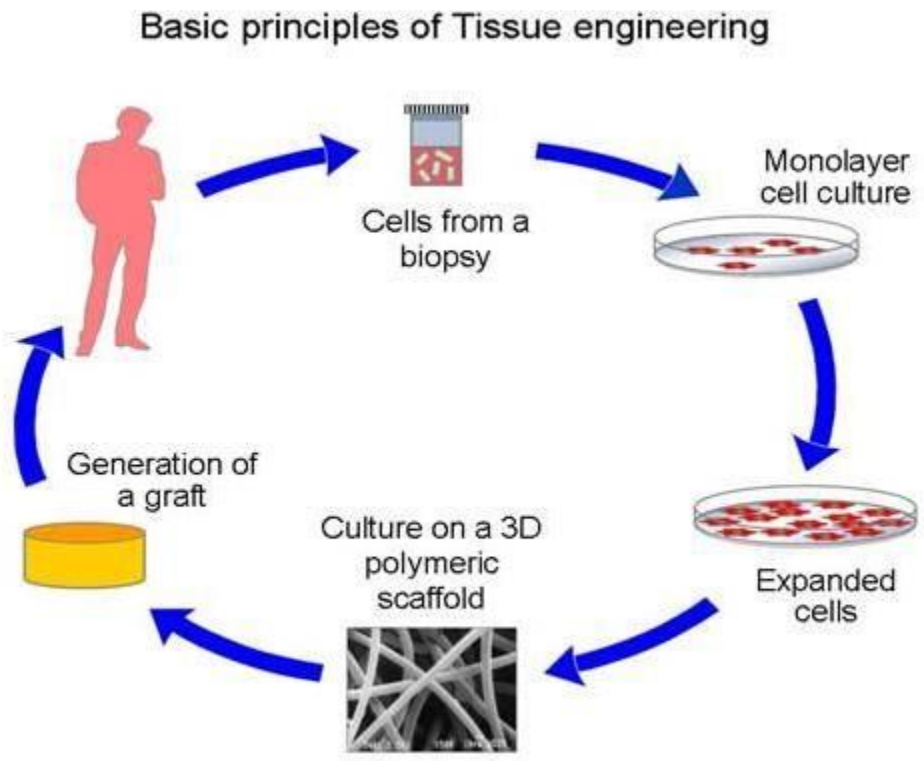

Fig.4.1 Cells and tissue types in tissue engineering – Stem cells

Various types of cells are used in tissue engineering that can be categorized in several ways. In broad terms, technologies in tissue engineering make use of both fully differentiated as well as progenitor cells at various stages of differentiation generally known as stem cells.

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 two main groups: “adult” and “embryonic” stem cells.

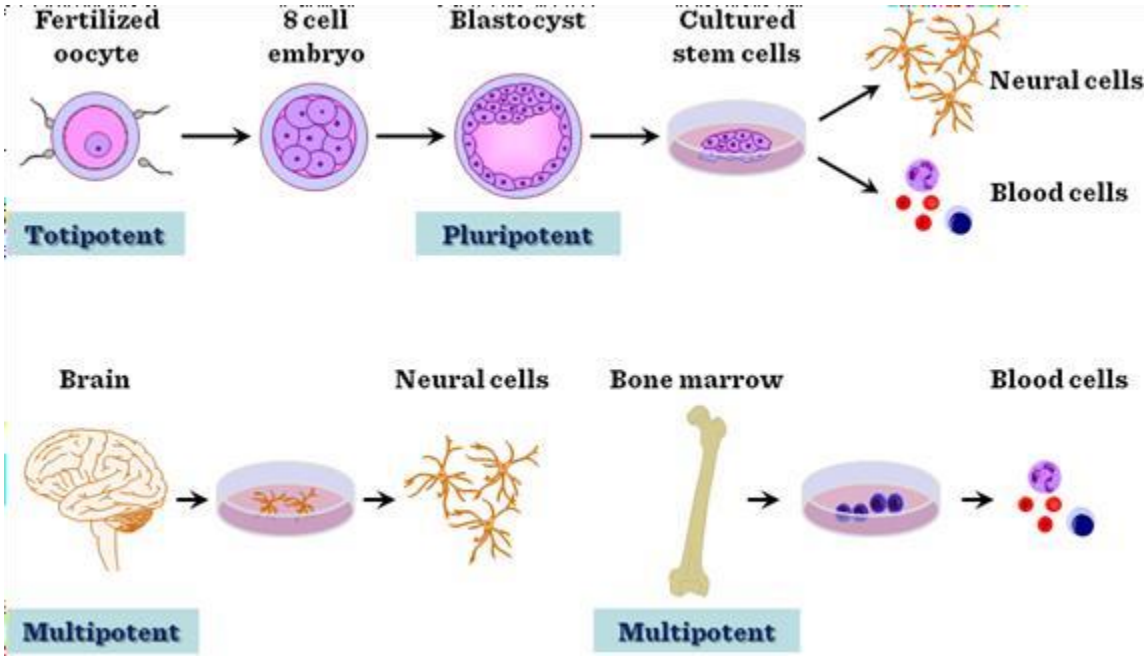

Fig. 4.2 Stem cell types

While adult stem cells are multipotent, embryonic stem cells are mostly pluripotent except of cells in the earliest stages of embryonic development (fertilized egg) that are totipotent. Stem cellniches where stem cells reside in an embryo or any given organ are defined as stem cell microenvironments. The fate of stem cells depends on the effect of the microenvironment. Stem cells – depending on the stimuli – have two main ways to replicate: either symmetricallyresulting in two daughter cells with stem cell characteristics or asymmetrically yielding a daughter cell with stem and another daughter cell with differentiating cell characteristics.

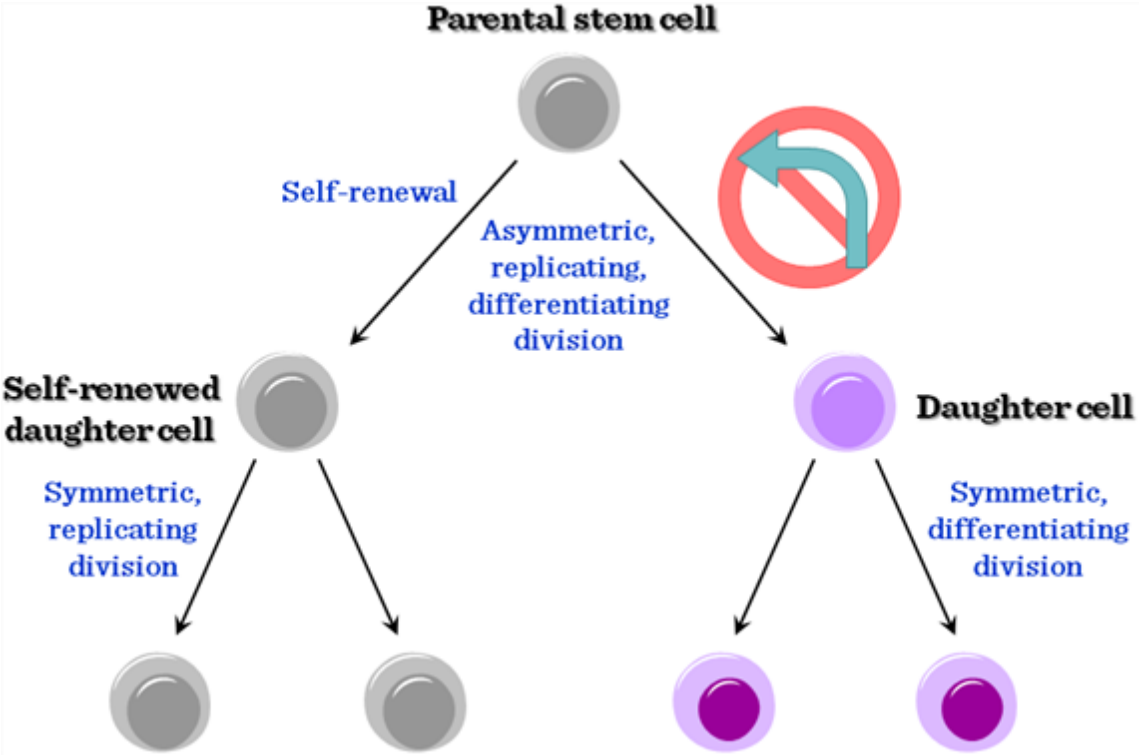

Fig. 4.3 Types of stem cell replications I

With the increasing number of divisions, the proliferation capacity of stem cells is decreasing favouring differentiated phenotypes.

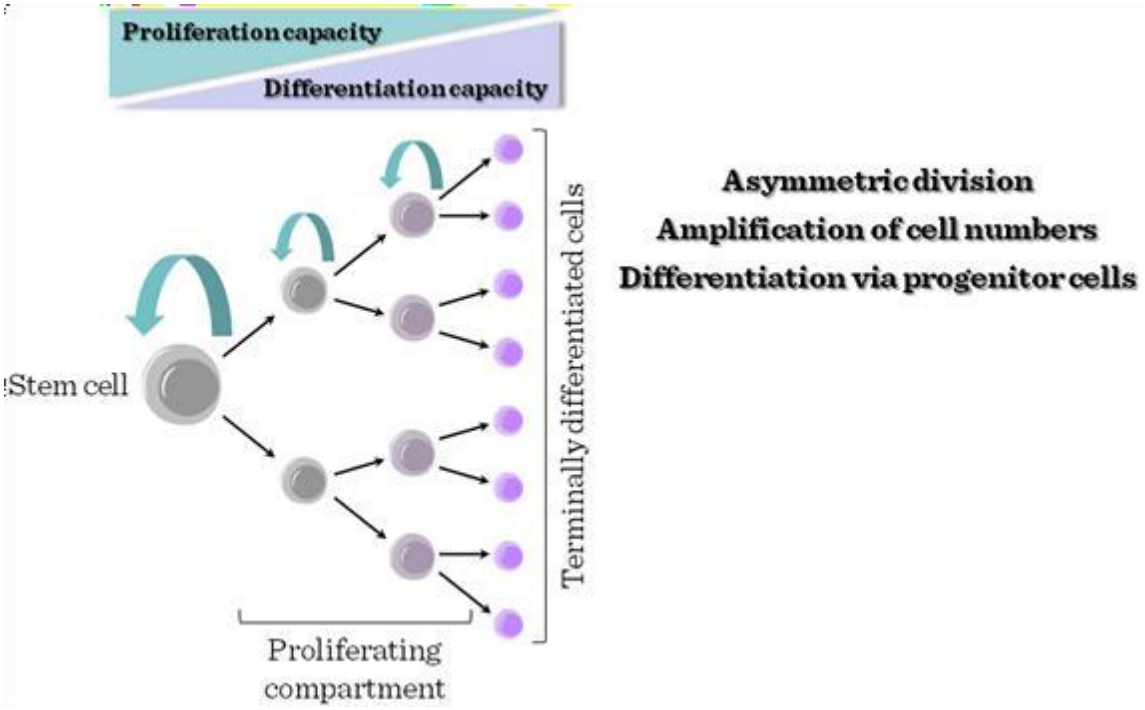

Fig. 4.4 Types of stem cell replications II

As a result, continuous proliferation signals can lead to depletion of stem cell sources.

Characteristic stem cell types

Embryonic stem cells (ES). Although ethical debate is ongoing about using embryonic stem cells, embryonic stem cells are still being used in research for the repair of diseased or damaged tissues, or to grow new organs for therapy or for drug testing. Especially, as rodent embryonic stem cells do not behave the same way as human embryonic stem cells and require different culture conditions to be maintained. Recently, it has been discovered, that if stem cells are taken at a later stage not at the early blastocyst stage (3–4 days after conception), when the developing embryo implants into the uterus, epiblast – the innermost cell layer – stem cells form (Figure II- 4), and these cells resemble human embryonic stem cells and had many of the same properties making it possible to use rodent rather than human cells in some research.

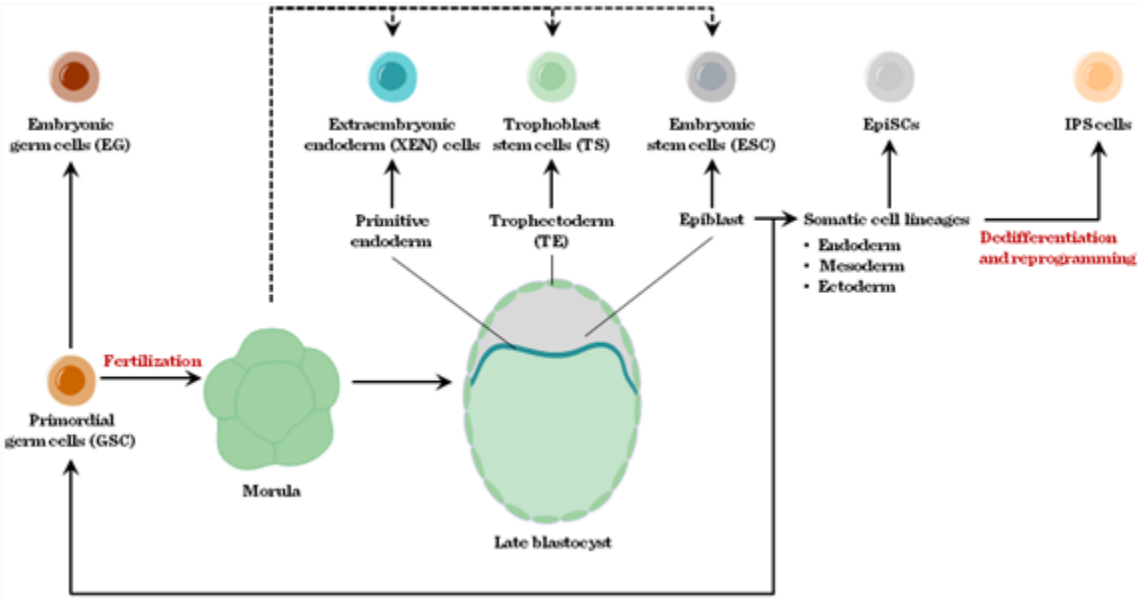

Fig.4.5 Epiblast stem cells

Adult stem cells. Adult or somatic stem cells (ASC) are present in every organ including the bone marrow, skin, intestine, skeletal muscle, brain, etc. where tissue specific microenvironments provide the necessary stem cell niches where stem cells reside and where the tissue gains its regenerative potential from.

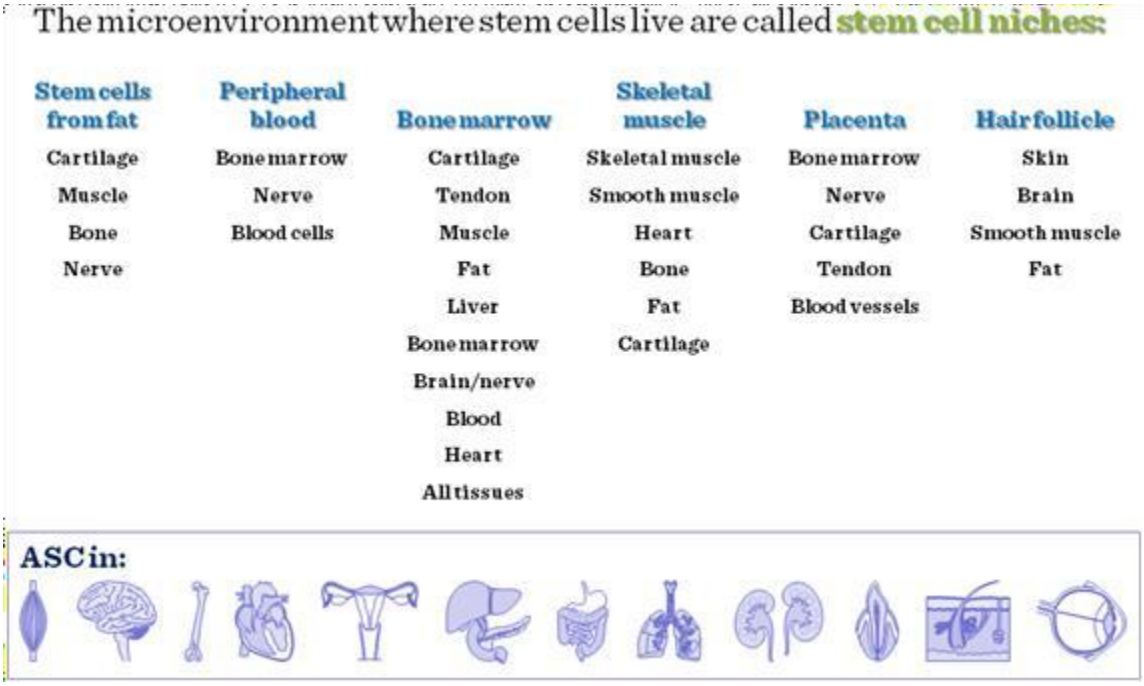

Fig. 4.6 Adult or somatic stem cells

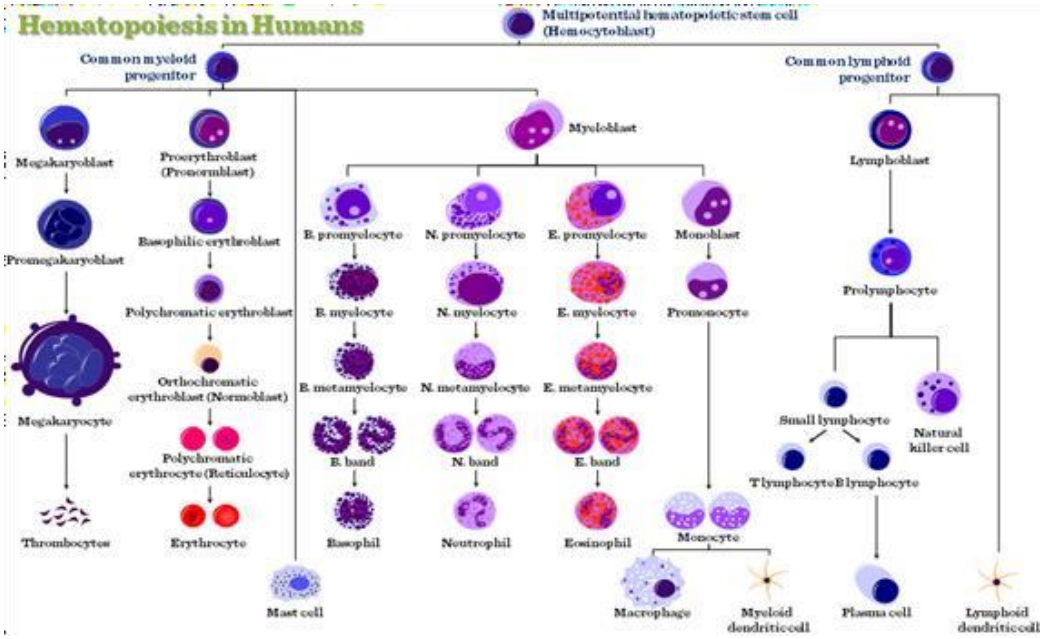

Fig. 4.7 Hematopoietic stem cells (HSCs)

Mature cells, when allowed to multiply in an incubator, ultimately lose their ability to function as differentiated cells. To proliferate, differentiate and function effectively in engineered tissues, cells must be easily procured and readily available; they must multiply well without losing their potential to generate new functional tissue; they should not be rejected by the recipient and not turn into cancer; and they must have the ability to survive in the low-oxygen environment normally associated with surgical implantation. Mature adult cells fail to meet many of these criteria. The oxygen demand of cells increases with their metabolic activity. After being expanded in the incubator for significant periods of time, they have a relatively high oxygen requirement and do not perform normally. A hepatocyte, for example, requires about 50 times more oxygen than a chondrocyte consequently much attention has turned to progenitor cells and stem cells. True stem cells can turn into any type of cell, while progenitor cells are more or less committed to becoming cell types of a particular tissue or organ. Somatic adult stem cells may actually represent progenitor cells in that they may turn into all the cells of a specific tissue. If somatic stem cells are intended to be used in regenerative therapy, healthy, autologous cells (one's own cells) would be the best source for individual treatment as tissues generated for organtransplant from autologous cells would not trigger adverse immune reactions that could result in tissue rejection. Also, organs engineered from autologous cells would not carry the risk of pathogen transmission. However, in genetic diseases suitable autologous cells are not available, while autologous cells from very ill (e.g.

suffering from severe burns and require skin grafts) or elderly people 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 while the result might remain unpredictable. Also, autologous cells for most procedures must be purified and cultured following sample taking to increase numbers before they can be used: this takes time, so autologous solutions might be too slow for effective therapy.

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.

Bone marrow stem cells (MSCs). Bone marrow stem cells can be subdivided into bone marrow stromal (endothelial, mesenchymal) and haematopoietic lineages. All the specific progenitor types can be purified from the bone marrow based on their respective cell surface markers. In thebone marrow microenvironment, these stem cells show a functional interdependency (Figure II- 7), secreting factors and providing the necessary cellular interactions to keep the stem cell niche suitable for all the progenitor cells.

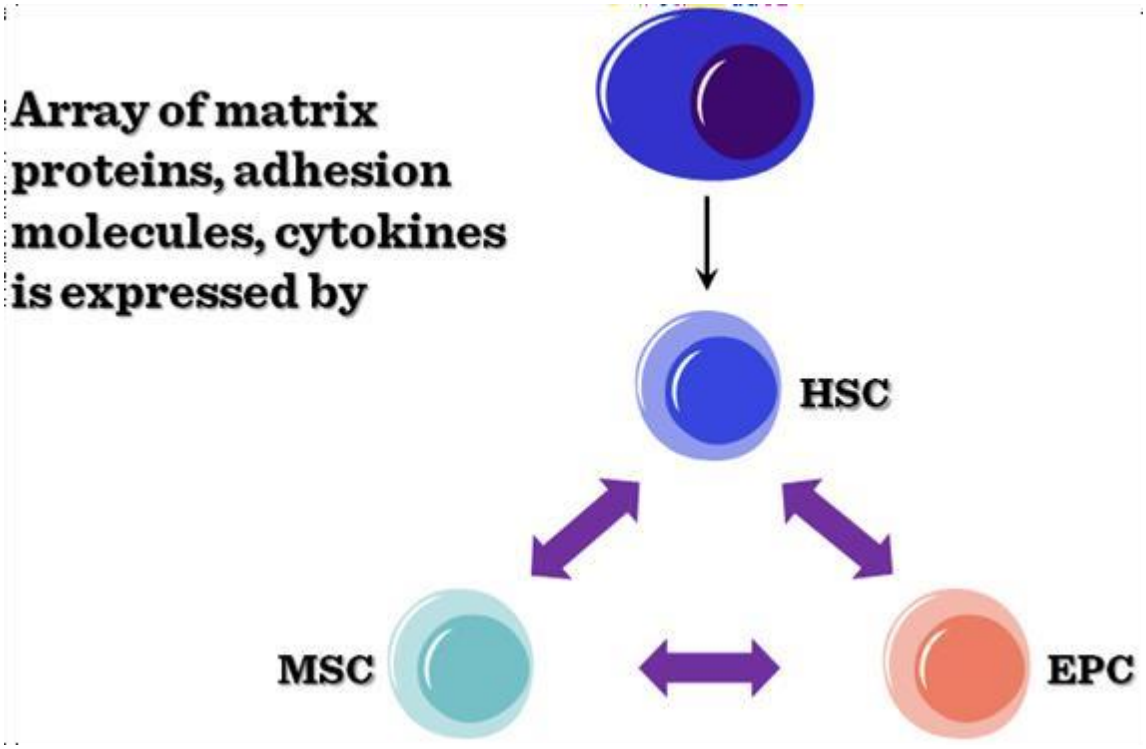

Fig. 4.8 Functional interdependency of bone marrow stem cellsThe ontogeny of tissue lineages in bone marrow is summarized in 8.

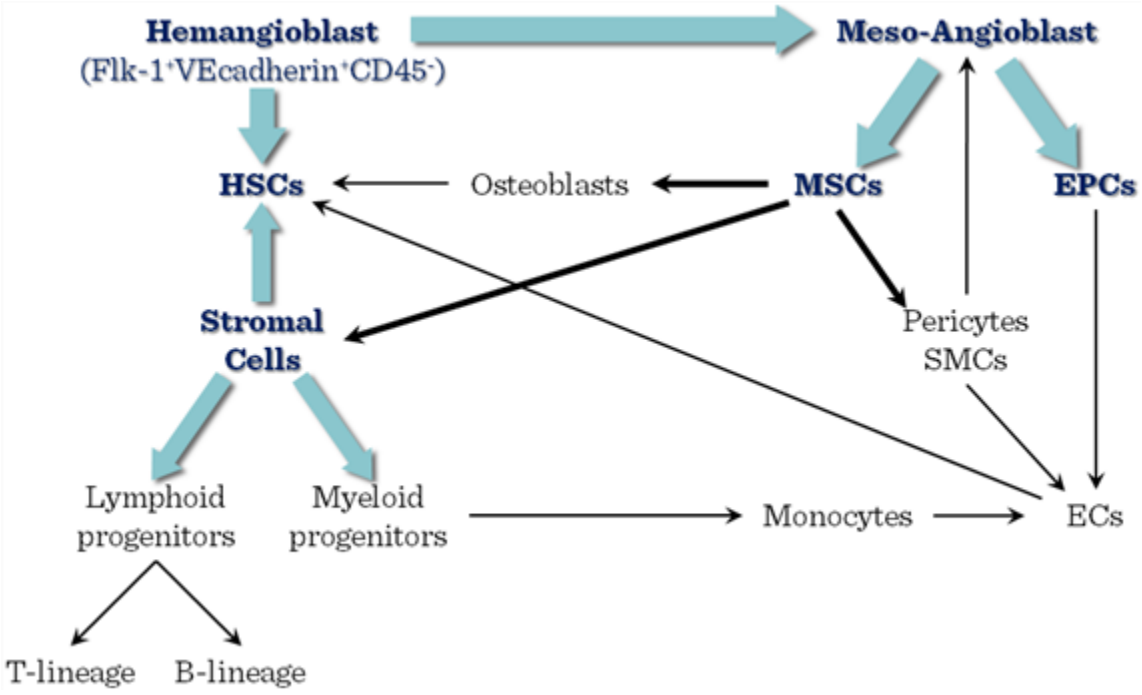

Fig. 4.9 Ontogeny of tissue lineages in bone marrow

Cord blood stem cells. The largest source of stem cells for regenerative medicine is the umbilical cord stem cell pool. Based on the number of babies born yearly (approximately 130 million), thisis not surprising. However, unless the cord blood is collected from the newborn to be used in potential diseases later on in his or her life –mainly childhood haematological disorders –, the application is not autologous, therefore cell types have to be matched just as in any other transplantation procedures. Cord blood is collected at birth and processed immediately (Figure II-9) to purify stem cells based on their cell surface markers or simply just to be frozen with added DMSO in liquid nitrogen.

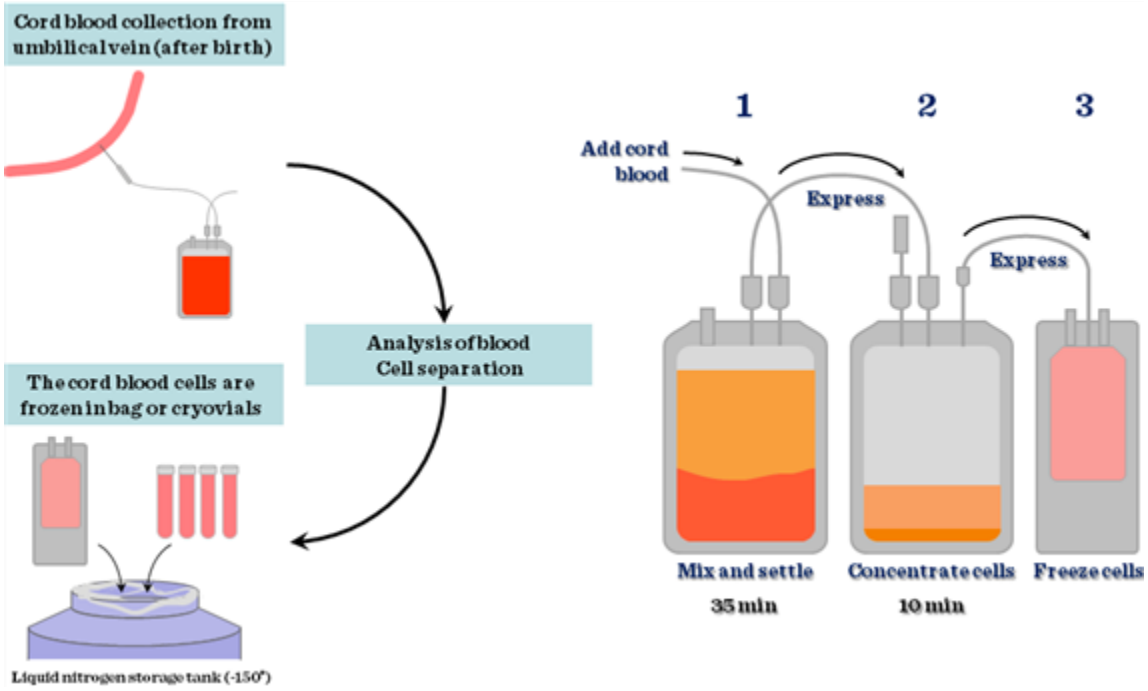

Fig. 4.10 Cord blood stem cells and foetal stem cells

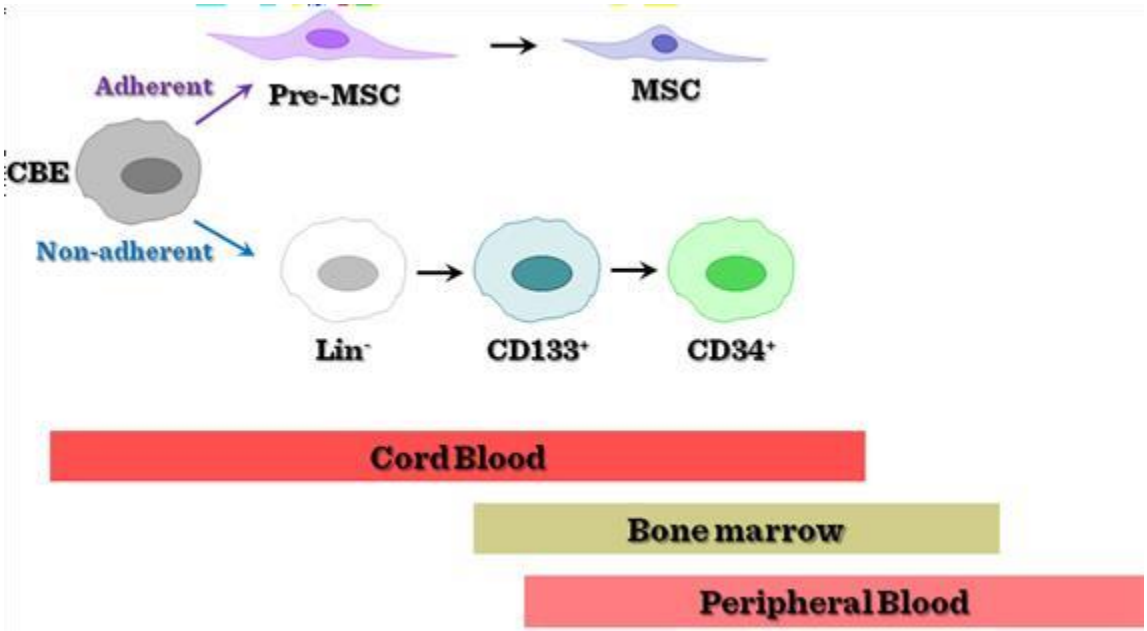

Fig. 4.11 Stem cell populations in cord blood

In the past 36 years 10,000 patients were treated for more than 80 different diseases using cord blood stem cells. Ideally, cord blood banks should be set up with active links to international data bases where all the stored cells would be characterized with HLA typing and made available when needed. Unfortunately, storage of stem cells is costly therefore this relatively simple sourcefor progenitor cells is not used to its full potential.

Adipose tissue derived stem cells. Recently there has been a trend to obtain mesenchymal stem cells from fat. Similarly to other differentiated tissue types, adipose tissues contain tissue specificstem cells. Adipose stem cells (ASC) can be easily isolated from adipose tissues (Figure II-11), and ASCs are multipotent, their immunophenotype is consistent (Figure II-12) and adipose stem cells are easily manipulated by genetic engineering.

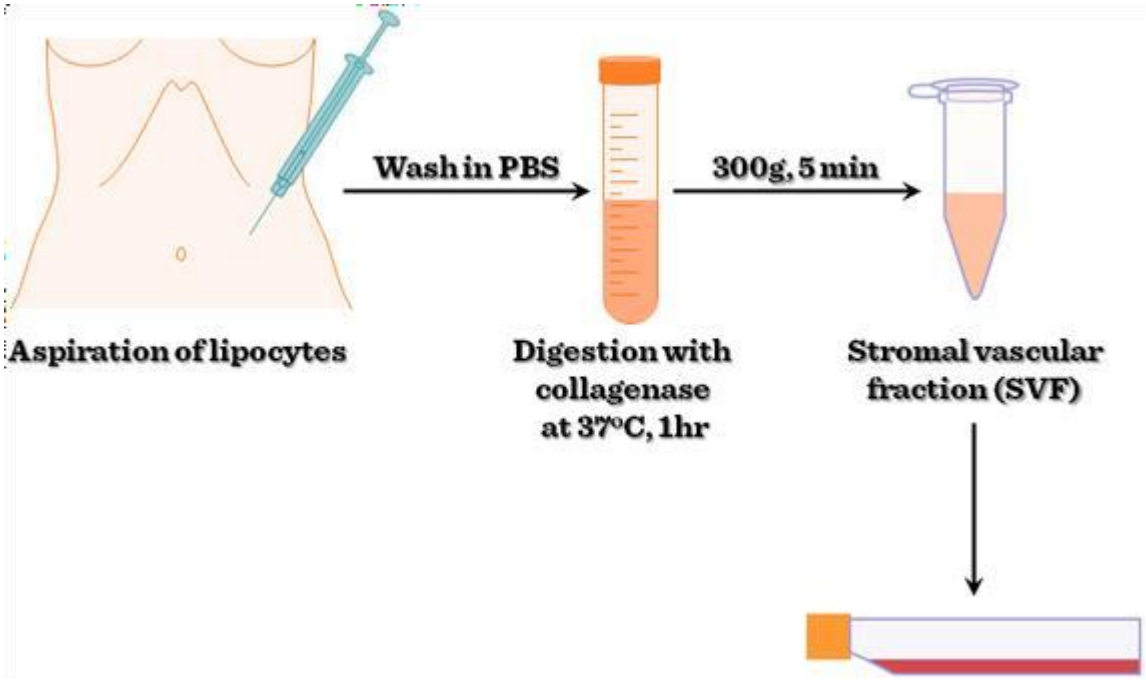

Fig. 4.12 Isolation procedures of ASCs

Figure II-12: Immunophenotype of ASCs (Positive markers)

The differentiation potential of ASCs is also wide. By using the right combination of extracts and factors, ASCs can differentiate into cardio-myocytes, skeletal myocytes, chondrocytes,osteoblasts, neuronal, endodermal and ectodermal lineages.

Application of stem cells (either embryonic or adult stem cells) (Figure II-13) is becoming broader since cellular manipulation can aid development of the required cell types.

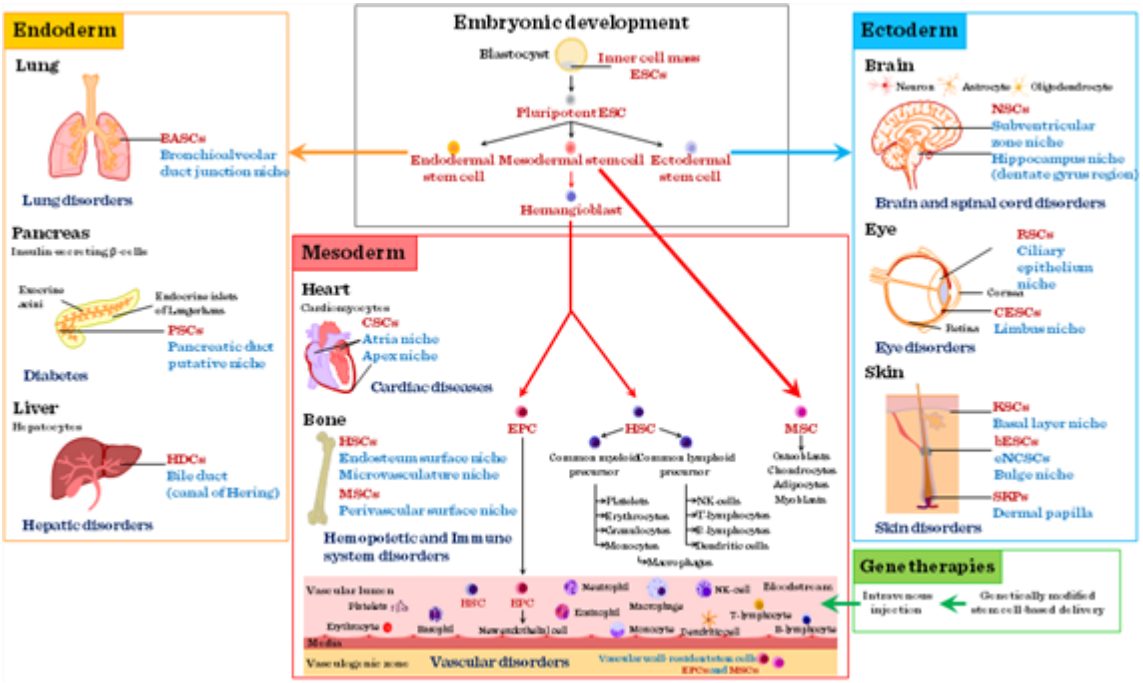

Figure 4.13: Application of ESCs and ASCs

Cells can be reprogrammed by modification of their original gene expression patterns or cellular differentiation can be manipulated by applying directed changes into the cellular growth environment in forms of growth factors, cytokines, cellular interactions (e.g. feeder layer). Naturally, the right factors need to be identified prior to any attempts to grow specific tissue types for commercial use. In fact tight regulatory issues surround tissue engineering aimed at clinical application of laboratory generated tissues in regenerative medicine. Apart from ethical issues of using embryonic stem cells, and working with human subjects to purify adult stem cells, the purification of stem cells requires GLP (good laboratory practice) and GMP (good manufacturing practice) conditions that make the production of such tissues very expensive.

Differentiated cells. Previously, it was believed that many differentiated cells of adult human tissues have only a limited capacity to divide. In contrast, some almost differentiated cells have widely been used in clinical tissue engineering. The “almost” status is frequently achieved by culturing purified differentiated cells in 2D culture conditions where they lose their final differentiation characteristics. Among differentiated cells used in tissue engineering are notably

fibroblasts, keratinocytes, osteoblasts, endothelial cells, chondrocytes, preadipocytes, and adipocytes. One of the most frequently used differentiated cell types are chondrocytes. In the following, chondrocytes shall serve as an example to describe the use of differentiated cells in tissue engineering applications.

Isolation of autologous chondrocytes for human use is invasive. It requires a biopsy from a non-weight-bearing surface of a joint or a painful rib biopsy. In addition, the ex vivo expansion of a clinically required number of chondrocytes from a small biopsy specimen, which may itself be diseased, is hindered by deleterious phenotypic changes in the chondrocyte. It is important that chondrocytes synthesize type II collagen, the primary component of the cross-banded collagen fibrils. The organization of these fibrils, into a tight meshwork that extends throughout the tissue, provides the tensile stiffness and strength of articular cartilage, and contributes to the cohesiveness of the tissue by mechanically entrapping the large proteoglycans. In growing individuals, the chondrocytes produce new tissue to expand and remodel the articular surface. With aging, the capacity of the cells to synthesize some types of proteoglycans and their response to stimuli, including growth factors, decrease. These age-related changes may limit the ability of the cells to maintain the tissue, and thereby contribute to the development of degeneration of the articular cartilage. For the organ repair or replacement process the in vitro amplified number of chondrocytes have to be grown in bioreactors to grow as weight bearing tissue. Although differentiated chondrocytes appear to be one of the best sources for engineered cartilage, to date, few tissue-engineered systems provide an autologous, minimally invasive, and easily customizable solution for the repair or augmentation of cartilage defects.

1. Bioreactors

One of the persistent problems of tissue engineering is the difficult production of considerable tissue mass. As engineered tissues normally lack blood vessels, it is difficult for cells in the inside of a larger tissue mass to obtain sufficient oxygen and nutrient supply to survive, and consequently to function properly.

Although self-assembly plays an important role in any tissue engineering methods, tissue growth and functional differentiation needs to be directed by the tissue engineeer. Creation of functional

tissues and biological structures in vitro needs to observe some basic requirements for cellular survival, growth and differentiation. In general, the basic requirements include continuous supply of oxygen, correct pH, humidity, temperature, nutrients and osmotic pressure. If the structure of the tissue is important from the point of view of tissue function, suitable scaffolds are often needed. Tissue engineered cultures present additional problems in maintaining culture conditions. In standard, simple maintenance cell cultures, molecular diffusion is often the sole means of nutrient and metabolite transport. However, as a culture becomes larger and more complex, for example in the case of engineered organs with larger tissue mass, other mechanisms must be employed to maintain the culture and to avoid cellular necrosis. It is important to create some sort of capillary networks within the tissue that allows relatively easy transport of nutrients and metabolites.

Similarly to directing growth and differentiation of stem cell cultures, complex tissue cultures also require added factors, hormones, metabolites or nutrients, chemical and physical stimuli to reach the desired functionality.

Just a couple of examples: chondrocytes for example respond to changes in oxygen tension as part of their normal development to adapt to hypoxia during skeletal development. Other cell types, such as endothelial cells, respond to shear stress from fluid flow, which endothelial cells encounter in blood vessels. Mechanical stimuli, such as pressure pulses seem to be beneficial to all kinds of cardiovascular tissue such as heart valves, blood vessels or pericardium. All the special requirements are intended to be resolved by the use of bioreactors.

A bioreactor in tissue engineering is a device that simulates a physiological environment in order to promote cell or tissue growth. 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 the required forces or stresses to the tissue two- or three-dimensional setups (e.g., flex and fluid shearing for heart valve growth). Several general-use and application- specific bioreactors are available commercially to aid research or commercial exploitation of engineered tissues. Bioreactors, however, require a large number of cells to start off with.

Cells to be used in bioreactors

m. In the dynamic environment of bioreactors, this parameter can be increased several fold. Static cell cultures are the most frequently applied cell culture methods. The maintenanceis easy, cheap and no specialized laboratory equipment is needed. Petri dishes or disposable plastic tissue culture flasks are used most frequently. Basically 2 types of conventional tissue culture exist: Adherent cells which grow in monolayer cultures and non-adherent cells that grow in suspension cultures. In both case, conventional cell cultures are capable to maintain cells at relatively low densities. The main problem rises concerning static cell cultures when large numbers of cells are needed and conventional cultures need to be scaled-up to be added to bioreactors. In the conventional cultures nutrient supply is maintained by frequent and periodic change of culture medium that is time consuming and involves a high risk of infection. The advantages of the dynamic cellular environment of bioreactors include the dynamic and continous supply of nutrients and oxygen. These features make the formation of larger 3D tissue structures possible. However, availability of nutrients and oxygen inside a 3D tissue construct is still problematic. While in 3D cell cultures direct cell-cell contacts enhance cellular communication, transport of oxygen, nutrients and metabolites is still a challenging issue. First, oxygen and nutrients should diffuse from the static medium to the surface cells. This concerns the oxygen content and the oxygen-carrying capacity of the medium. Diffusion from the surface cells to the deeper structures is also important. Critical parameters are the porosity of the culturedcell/tissue construct. Understandably, thickness of the tissue construct is a critical parameter. In static conditions tissue thickness should not exceed 100

Bioreactor design requirements

Although bioreactors cannot recreate the physiological environment, they need to reproduce as many parameters as possible. Bioreactors need to maintain desired nutrient and gas concentration in 3D constructs and to facilitate mass transport into and from 3D tissues. It is also important for bioreactors to improve even cellular distribution, which is also facilitated through the dynamic environment. Exposure of the construct to physical stimuli is also important during the engineering of load-bearing tissues, like cartilage, bone, tendon and muscle. Without mechanical stimuli these tissues cannot withstand physiological load and strain.

There is an additional critical parameter in dynamic tissue cultures: the shear force. Shear forces are particularly important, as cells are sensitive to shear stress, which may cause dedifferentiation, growth inhibition or apoptosis. Unfortunately, shear stress distribution is uneven in dynamic bioreactors. In dynamic bioreactors the highest stress is located around edges and sides of the moving vessel or around the moving edge of the stirrer and the edges of the scaffold which is static itself but the fluid is moving around it. (The measure unit of shear stressis the dyn/cm2. 1 dyn = 10 mN. The maximum shear stress for mammalian cells is 2.8 dyn/cm2, so in a well-designed bioreactor, shear stress values are well below this number) (Figure III-1).

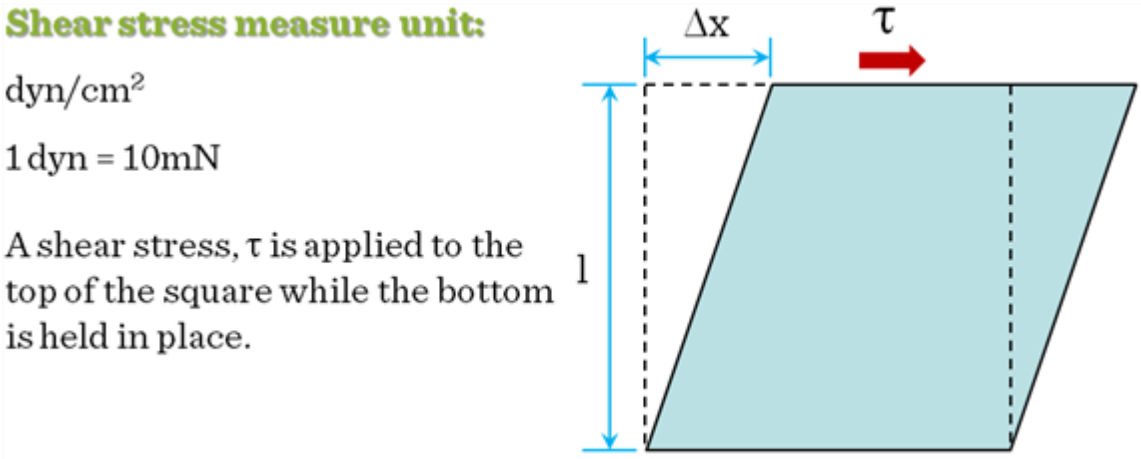

Fig. 4.14 Shear forces in dynamic fluids

Perhaps the most difficult task is to provide real-time information about the structure of the forming 3D tissue. Normally the histological, cellular structure of the 3D construct can only be judged after the culturing period is completed.

In general, bioreactors should be as clear and simple as possible to make cleaning easy and to reduce the risk of infections. The assembly and disassembly of the device should be also simple and quick. It is extremely important that all parts of the bioreactor that comes into contact with the cell culture is made of biocompatible or bioinert materials. For example no chromium alloys or stainless steel should be used in biorecators. Also the material should withstand heat or alcohol sterilization and the presence of the continuously humid atmosphere. The design should also ensure the proper embedding of instruments like the thermometer, pH meter, the pump or rotator motor, etc.

Main types of bioreactors

Spinner flask bioreactors (Figure III-2) are maybe the simplest and the most frequently used bioreactor types.

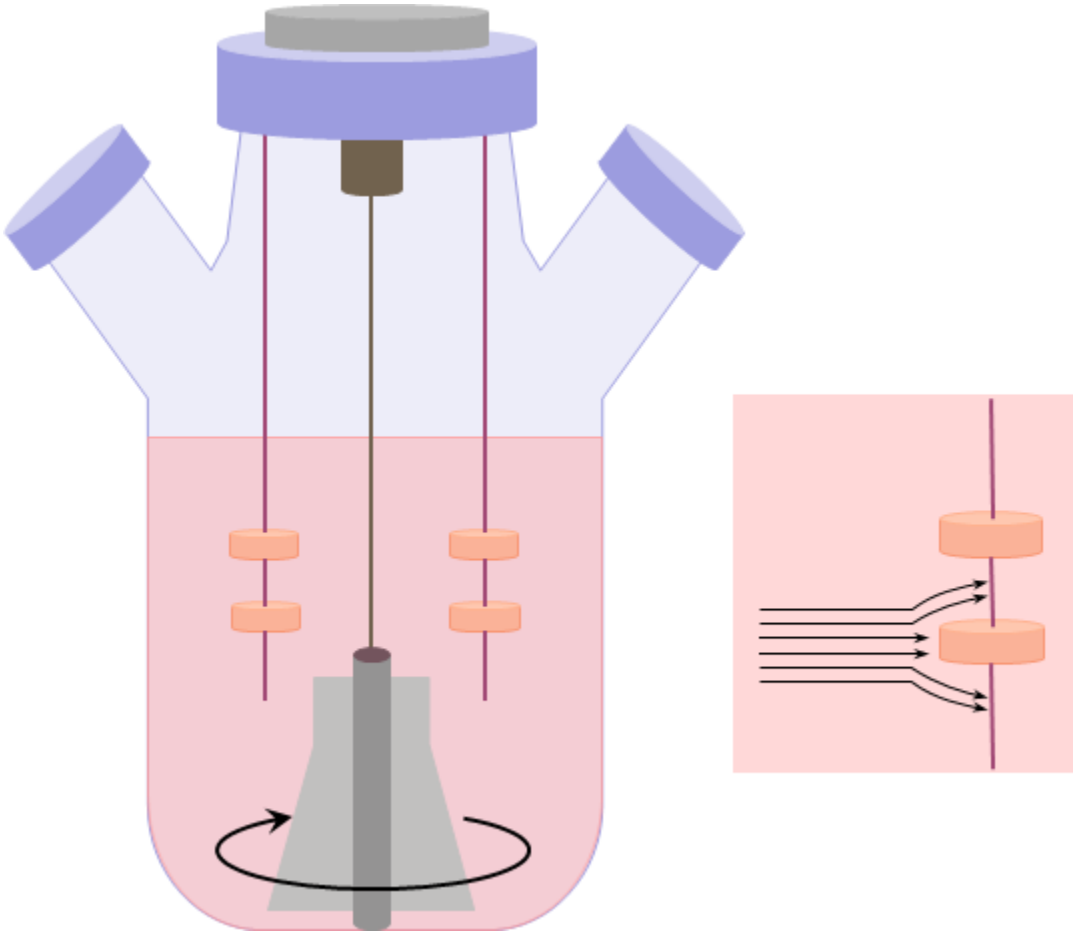

Fig. 4.15 Spinner flask bioreactors

m in static cultures. However, cell seeding efficiency is typically low in spinner flask bioreactors, this method usually fails to deliver homogeneous cell distribution throughout scaffolds and cells predominantly reside on the construct periphery.□Spinner bioreactor typesmix the oxygen and nutrients throughout the medium and reduce the concentration boundary layer at the construct surface. In a spinner flask, scaffolds are suspended at the end of needles ina flask of culture media. A magnetic stirrer mixes the media and the scaffolds are fixed in place with respect to the moveing fluid. Flow across the surface of the scaffolds results in turbulences and flow instabilities caused by clumps of fluid particles that have a rotational structure superimposed on the mean linear motion of the fluid particles. Via these added fluid motions

fluid transport to the centre of the scaffold is thought to be enhanced. Typically, spinner flasks are around 120 ml in volume (although much larger flasks of up to 8 liters have also been used). The most frequent stirring speed is 50–80 rpm and generally 50% of the total medium is changed every two days. The efficiency of the enhancement of mass transport is indicated that cartilage constructs have been grown in spinner flasks to thicknesses of 0.5 mm compared to that of 100 The rotating wall bioreactor (Figure III-3) was originally developed by NASA.

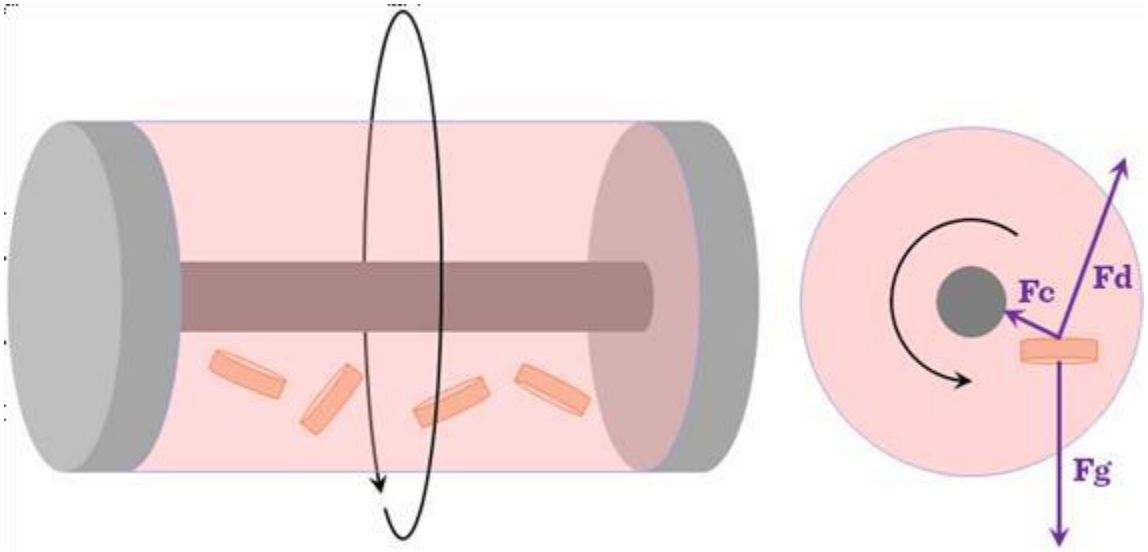

Fig. 4.16 Rotating wall bioreactors

It was designed with a view to protect cell culture experiments from high forces during space shuttle take off and landing. The device has proved useful in tissue engineering laboratories on Earth too. In a rotating wall bioreactor, scaffolds are free to move in media in a vessel. A rotatingwall vessel bioreactor consists of a cylindrical chamber in which the outer wall, inner wall, or both are capable of rotating at a constant angular speed. The vessel wall is then rotated at a speedsuch that a balance is reached between the downward gravitational force and the upward hydrodynamic drag force acting on each scaffold. The wall of the vessel rotates, providing an upward hydrodynamic drag force that balances with the downward gravitational force, resulting in the scaffold remaining suspended in the media. Dynamic laminar flow generated by a rotating fluid environment is an alternative and efficient way to reduce diffusional limitations of nutrients and wastes while producing low levels of shear compared to the stirring flask. Culture medium can be exchanged by stopping the rotation temporarily or by adding a fluid pump whereby media

is constantly pumped through the vessel. Fluid transport is enhanced in a similar fashion to the mechanism in spinner flasks and the rotational devices also provide a more homogeneous cell distribution compared to static or spinner bioreactor cultures. Gas exchange occurs through a gas exchange membrane. Typically, the bioreactor is rotated at speeds of 15–30 rpm. Cartilage tissueof 5 mm thickness has been grown in this type of bioreactor after seven months of culture. As tissue mass increases while cells grow in the bioreactor, the rotational speed must be increased inorder to balance the gravitational force and to ensure that the scaffold remains in suspension.

Compression bioreactors (Figure III-4) are another widely used type of bioreactors.

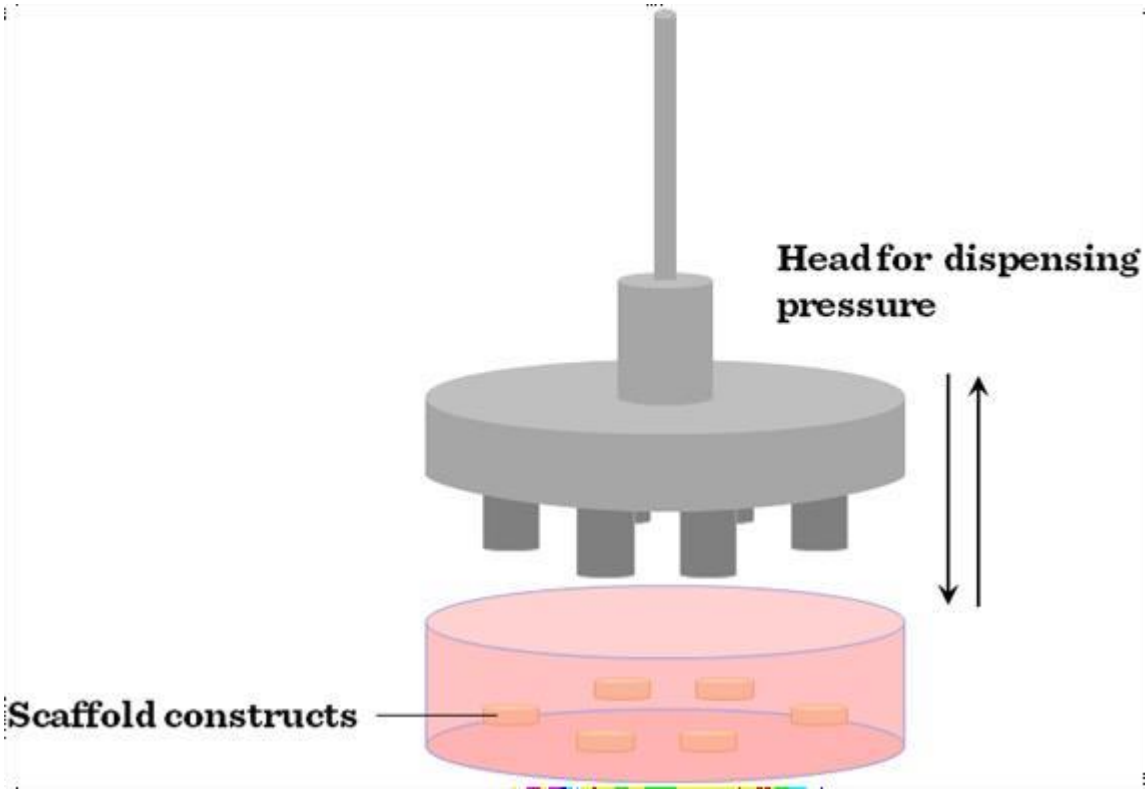

Fig. 4.17 Compression bioreactors

This class of bioreactor is generally used in cartilage engineering and can be designed so that both static and dynamic loading can be applied. In contrast to static loading that has a negative effect on cartilage formation dynamic loading is more beneficial and more representative of physiological tissue deposition. In general, compression bioreactors consist of a motor, a system providing linear motion and a controlling mechanism to provide different magnitudes and frequencies. A signal generator can be used to control the system including loading of cells while

transformers can be used to measure the load response and imposed displacement. The load can be transferred to the cell-seeded constructs via flat platens which distribute the load evenly. However, in a device for stimulating multiple scaffolds simultaneously, care must be taken that the constructs are of similar height or the compressive strain applied will vary as the scaffold height does. Mass transfer is improved in dynamic compression bioreactors over static culture (as compression causes fluid flow in the scaffold) which results in the improvement of the aggregate modulus of the resulting cartilage tissue to levels approaching those of native articular cartilage.

Tensile strain bioreactors have been used in an attempt to engineer a number of different types of tissues including tendon, ligament, bone, cartilage and cardiovascular tissue. Some designs are very similar to compression bioreactors, only differing in the way the force is transferred to the construct. Instead of flat platens as in a compression bioreactor, a way of clamping the scaffold into the device is needed so that a tensile force can be applied. Tensile strain has been used to differentiate mesenchymal stem cells along the chondrogenic lineage. A multistation bioreactor was used in which cell-seeded collagen-glycosaminoglycan scaffolds were clamped and loadedin uniaxial tension. Alternatively, tensile strain can also be applied to a construct by attaching theconstruct to anchors on a rubber membrane and then deforming the membrane. This system has been used in the culture of bioartificial tendons with a resulting increase in Young's modulus over non-loaded controls. (Young's modulus is a numerical constant that was named after an 18th-century English physician and physicist. The constant describes the elastic properties of a solid material undergoing tension or compression forces in one direction only).

Culture using flow perfusion bioreactors (Figure III-5) has been shown to provide more homogeneous cell distribution throughout scaffolds.

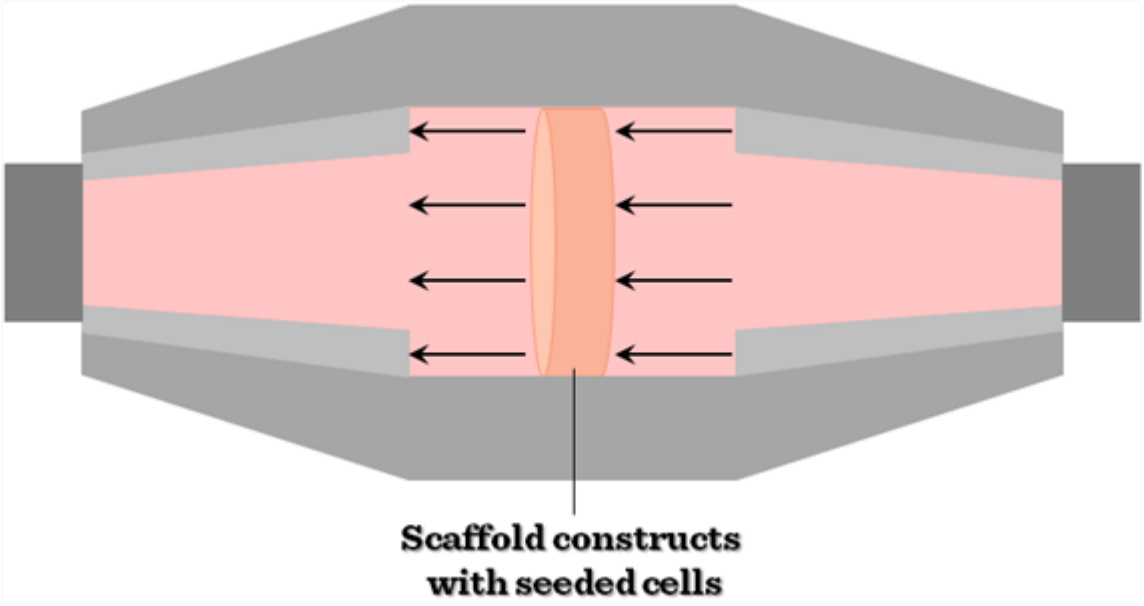

Fig. 4.18 Flow perfusion bioreactors

Collagen sponges have been seeded with bone marrow stromal cells and perfused with flow. This has resulted in greater cellularity throughout the scaffold in comparison to static controls, implying that better nutrient exchange occurs due to flow. Using a calcium phosphate scaffold, abundant extracellular matrix (ECM) with nodules of calcium phosphate was noted after 19 days in steady flow culture. In comparisons between flow perfusion, spinner flask and rotating wall bioreactors, flow perfusion bioreactors have proved to be the best for fluid transport. Using the same flow rate and the same scaffold type, while cell densities remained the same using all three bioreactors, the distribution of the cells changed dramatically depending on which bioreactor was used. Histological analysis showed that spinner flask and static culture resulted in the majority of viable cells being on the periphery of the scaffold. In contrast, the rotating wall vessel and flow perfusion bioreactor culture resulted in uniform cell distribution throughout the scaffolds. Flow perfusion bioreactors generally consist of a pump and a scaffold chamber joined together by tubing. A fluid pump is used to force media flow through the cell-seeded scaffold. The scaffoldis placed in a chamber that is designed to direct flow through the interior of the scaffold. The scaffold is kept in position across the flow path of the device and media is perfused through the scaffold, thus enhancing fluid transport. Media can easily be replaced in the media reservoir. However, the effects of direct perfusion can be highly dependent on the medium flow rate.

Therefore optimising a perfusion bioreactor for the engineering of a 3D tissue must address the careful balance between the mass transfer of nutrients and waste products to and from cells, the retention of newly synthesised ECM components within the construct and the fluid induced shear stresses within the scaffold pores.

Flow perfusion reactors in bone tissue engineering. Flow perfusion bioreactors have proved to be superior compared to rotating wall or spinner flask bioreactors to seed cells onto scaffolds. The expression levels of bone differentiation markers (namely Alkaline Phosphatase, Osteocalcin and the transcription factor Runx2) proved to be consistently higher in flow perfusion reactors than in any other type of bioreactors. Additionally, the mineralization of the scaffolds is also higher. To avoid the disadvantageous effects of high shear stress, the flow rate in the reactor needs to be set carefully. Experiments demonstrated that intermittent dynamic flow is more favourable than steady speed flow in bone tissue engineering.

Two chamber bioreactor – The most current and revolutionary achievement in tissue engineering was the implantation of a tissue engineered trachea which was developed in a special two- chamber bioreactor. The external surface of the de-cellularized donor trachea was seeded with autologous chondrocytes differentiated from hemapoetic SCs of the recipient and airway epithelium was seeded to the inner surface in a rotation wall-like bioreactor. The application of two separate “chambers” allowed simultaneous culture of different cell types. The construct was then used for the surgical replacement of the narrowed trachea in a tuberculosis patient.

Drawbacks to currently available bioreactors

Tissue engineering methodology is very labor intensive, specialized equipment and specially trained technicians are needed to perform the work. The current bioreactors are highly specialized devices which are difficult to assemble and disassemble. The cell output is low and the culturing times are long. Moreover, real-time monitoring of tissue structure and organization is not yet available. Problems with compression bioreactors involve mainly the mechanical parts,which are prone for leakage. Understandably, infection is a problem when such bioreactors are used. Added problem is the type of the scaffold to grow the cells on, as the applied scaffoldshave to withstand mechanical stimulation, so strong scaffolds are needed, which may have

longer degradation time once implanted. Naturally, this is not preferred. So a compromise has to be achieved between scaffold stiffness and resorption time.

2. Biomaterials

The requirements for biomaterials used in tissue engineering are quite strictly defined. Biocompatibility for example is high on the agenda, as scaffold and bioreactor materials have to be tissue friendly and not eliciting immunoresponse. Moreover, at best, the biomaterial should support cellular and tissue functions like adhesion, differentiation and proliferation via its specialsurface chemistry. Porosity is an important requirement concerning scaffolds. Generally the porosity should reach and even exceed 90% to allow even seeding of cells and to supportvascular in growth after implantation. Controlled biodegradation is also an important issue in some cases when the healthy tissue replaces the implanted biomaterial and the biomaterial gradually degrades in the body of the host. Biomaterials can be divided into natural and syntheticbiomaterials.

Natural biomaterials

The advantages of natural biomaterials (Figure IV-1) are that they mostly come from an in vivo source therefore large quantities are constantly available at a reasonable price.

Types of natural biomaterials

Further advantages of natural biomaterials are that they already have binding sites for cells and adhesion molecules so the biocompatibility is not a major issue. However, there are also some disadvantages. Due to natural variability in the in vivo source, the lot-to-lot variability is always

a concern. Additionally, potential impurities may also result in unwanted immune reactions, while their mechanical properties are also limited.

Collagen is the most frequently used and therefore the most studied biomaterial. There are rich in vivo sources, as all connective tissues of animals are rich in this ubiquitous protein. Collagen hasa fibrous structure, and its amino acid composition is unique. It provides binding sites for integrins called RGD sites (from the amino acid sequence arginine-glycine-aspartic acid). Collagen has a superior biocompatibility being a conserved protein. Additional benefit, that the immune system well tolerates collagen. It is capable of supporting a large spectrum cellular differentiation types, therefore collagen is well preferred as scaffold.

Another, easily accessible type of scaffold is fibrinogen. Fibrinogen is obtained from (human) plasma. Although in its uncleaved form is a soluble protein, upon cleavage with thrombin fibrinogen sets as a gel and forms a 3D meshwork which is 100% biocompatible and physiological in wound healing. It is often used as a biological glue when cells to be seeded onto scaffolds (e.g. non-woven mesh or fleece or other porous materials) are suspended first in a fibrinogen-containing solution. Then the solution is applied onto the scaffold and upon the addition of thrombin, a hydrogel is formed which enhances the cells' ability to attach to the 3D scaffold. Fibrin is also suitable for supporting ES cell differentiation as well as keeping differentiated cells in culture. Recent applications of fibrin include cardiovascular, cartilage,bone and neuronal tissue engineering.

Silk that is also used as scaffold material, is a protein produced within specialized glands of some arthropods. It has a special tertiary structure consisting of repeating amino acid motifs forming an overlapping beta-sheet structure giving the unique sturdiness for this protein. There have been many industrial attempts to mimic the features of silk. As a result the availability of recombinant analogues is increasing.

The silkworm's (Bombix mori) silk consists of two different protein components, namely Fibroin and Sericin. Sericin forms the outer layer on the fibroin core making it slippery and elastic. Fibroin that is biocompatible and possesses excellent mechanical properties is also used

as tissue engineering scaffold. Its use is widespread in bone, cartilage and ligament engineering. Additionally, silk fibroin can be modified chemically, e.g. the attachment of RGD groupsprovides binding sites for osteoblasts and enhances Ca++ deposition and bone cell differentiation. Moreover, silk promotes more intensive chondrogenesis than collagen used as a

scaffold material for cartilage engineering. The degradation rate of silk is very slow but finally bone tissue gradually replaces the silk scaffold.

Polysaccharide-based biomaterials are polymers consisting of sugar monomers. Those used for tissue engineering purposes are of plant (seaweed) or animal origin. Some polysaccharides may trigger unwanted immune reactions so a careful selection is advised considering polysaccharide scaffold materials. Polysaccharides are most frequently used as hydrogels, which per se form a 3D meshwork so they can provide a scaffold for seeded cells. These hydrogels are frequently used as injectons: they can be dispensed directly to the site of injury so it supports wound healingand also cell growth and differentiation.

Main source of agarose, another scaffold material for tissue engineering, are red algae and seaweed. Agarose is the most frequently used polysaccharide scaffold consisting of a galactose- based backbone. It is immunologically inert, so no immune response is triggered. One of its great advantages lies in its versatility: the stiffness and mechanical parameters can be easily manipulated of agarose gels. It has been used for scaffolding cartilage, heart, nerve tissues and it also supports stem cell differentiation.

Alginate is the polysaccharide component of the cell walls of brown algae. It is an acidic compound, so in tissue engineering various cationic alginate salts are used. Sodium-alginate is a frequently used food additive (E-401) and its use is also widespread in gastronomy. Besides of gastronomic applications, sodium alginate is used in industry as a heavy metal-binding or fat- binding agent. The potassium salt is also used in the food industry as an emulsifier and stabilizer.In tissue engineering its calcium salt or Calcium-alginate has gained widespread application. Calcium alginate is a water-insoluble gel-like material, and it is generally used in the industry or laboratory for enzyme immobilization or encapsulation. In tissue engineering calcium-alginate proved to be useful for encapsulation of whole living cells, thus isolating them from the immune

system preventing rejection after transplantation. In a clinical trial, calcium-alginate was used for the encapsulation of pancreatic islet cells which preserved their ability to produce insulin according to the needs of the host as the encapsulation prevented the immune reaction against thegrafted cells.

Hyaluronan which is also termed as Hyaluronic acid is an animal-derived polysaccharide whichis extensively used as a scaffold material in tissue engineering. It is a non-sulfatated Glucose- Amino-Glycan (GAG) molecule and exists as a major component of the ECM in hyaliniccartilage and skin but it is present in other organs as well. As a natural ECM component, multiple cell surface receptor binding and cell adhesion sites available on the macromolecular complex. Hyaluronan has an important role in wound healing and tissue repair. Moreover, it supports embryonic stem cell differentiation, survival and proliferation. Like other polysaccharides, hyaluronan is used as a gel in nerve, cartilage and skin tissue engineering.

Chitosan is derived from the deacetylation of chitin which is a strongly cationic polysaccharide which is the main component of the arthropod exoskeleton. Chitosan is commercially derived from sea-dwelling crustaceans and it is widely used for bandages and wound dressing, utilizing its ability to enhance blood clotting. Chitosan scaffolds are mainly used in bone tissue engineering, as chitosan supports osteoblast differentiation. Moreover, chitosan-calcium- phosphate composite scaffolds form a pliable, injectable hydrogel at slightly acidic pH. Upon transition to physiological pH, it gels thus anchoring osteocytes to the scaffold. Native and collagen-modified chitosan can be used for tissue engineering, as both forms support progenitor differentiation into osteoblasts.

Synthetic biomaterials

In tissue engineering a large scale of synthetic biomaterials (Figure IV-2) are used besides that of natural origin.

Figure IV-2: Types of synthetic biomaterials

Their main advantages over natural biomaterials are the high reproducibility, availability on demand and constant quality supporting industrial-scale production. Moreover, by application of

slight changes of production, an easy control of mechanical properties, degradation rate, shape, composition, etc. can be adjusted to current needs. However, synthetic materials often lack sites for cell adhesion and the biocompatibility is frequently questionable. Biocompatibility andsupport of stem cell differentiation is not clear either, while immune reactions are also possible. Poly (lactic-co-glycolic acid) PLGA is an FDA approved scaffold material. PLGA is a mixedpolymer consisting of lactic and glycolic acids in various ratios. PLGA is a biodegradablematerial and its degradation rate can be modulated. It is one of the most frequently usedbiomaterials applied in neural tissue, bone and cartilage tissue engineering. PLGA isbiocompatible, triggers no immune reaction and supports embryonic stem cell differentiation,proliferation and survival. However one has to consider that the degradation products are acidic,therefore may alter cell metabolism.

Poly (ethylene glycol) PEG is a commonly used biocompatible polymer. This molecule has amphophilic properties having a hydrophilic head and a variably long hydrophobic tail. The PEGylation of proteins is a commonly used pharmaceutical technology to modulate the degradation and absorption of bioactive proteins like interferons. The chemical modification of PEG is also available e.g. heparin, peptides, or RGD motifs. PEG is also a frequently used scaffold material in bone, cartilage, nerve, liver and vascular tissue engineering. Application of PEG-formed hydrogels is very versatile. The chemical nature, the extent of crosslinking, and the potential application of modifications, like RGD or other amino-acid groups, makes PEG widely

used. PEG-based gels can be applied not only to entrap or anchor cells but also in storing and delivering bioactive molecules like BMP or TGFβ.

Peptide-based biomaterials consist of short amino acid sequences which usually are ampholitic thus making peptides capable of self-assembly. The application of these peptides allows the combination of the advantages of synthetic materials with that of natural scaffolds. Synthetic nature of the peptides eliminates batch-to-batch variability and consistent purity and quality can be achieved. Moreover, known binding sites for cell-surface adhesion molecules can be included in the sequence. For example, IKVAV amino acid sequence is from laminin and it facilitates neurite outgrowth. RGD sequence is a binding site for integins which promotes cellular adherence and migration.

Ceramic-based biomaterials are used in bone tissue engineering only. These scaffolds are totally or partly inorganic. Mostly they are shaped with heat forming porous, brittle materials. For example, bioactive glass is slowly biodegradable. With an ion-exchange mechanism it slowly turns to natural hydroxyapathite via surface degradation. So bioglass is biocompatible and usedas an implant material. Hydroxyapatite is the inorganic compound of bone. Sometimes it is used as a completely inorganic, porous scaffold, more often it is combined with other biocompatible, organic polymers, like PLGA, collagen, or chitosan. This combination also enhances the drug delivery capacity of these scaffolds to enhance bone formation.

Metals are used as implant materials. Mostly alumina and titanium alloys are used because of their biocompatibility. The high durability of metals is needed where implants are subjected to extreme mechanical load, like articular prostheses, dental implants or heart valves. These metals are bioinert materials, however, sometimes metallic implants may cause immunological reactionslike metal allergy in sensitive individuals. Also, as they are not biodegradable it is questionable whether metals can be listed amongst tissue engineering.

Engineering Biomaterials for Tissue Engineering - 10 to 100 micron size scale

The design and fabrication of biodegradable scaffolds are keystones to advancing the field of tissue engineering and organ regeneration. Similarly, the widespread application of microfabrication strategies has proven to be beneficial both in elucidating complex biological processes and improving cell function through a variety of avenues. Although a wide number of techniques and approaches have been developed to study the behavior of cells and their components *in vitro*, expanding micro-scale functionalities to tissue engineering scaffolds could

prove beneficial in controlling cell function *in vivo.* Incorporating micro-scale systems and strategies for tissue and organ regeneration into scaffolds requires the ability to develop advanced microfabrication techniques tailored specifically for biomaterials. This chapter is dedicated to describing current strategies for the microfabrication of biomaterials within the context of realizing an ultimate goal of fabricating biodegradable scaffolds with micron-and nanometer-scale features. In general, these processes and approaches implement modifications to traditional micro-scale fabrication techniques, such as replica molding, soft lithography, and electrospinning, thereby expanding processing capabilities to include either natural or synthetic biomaterials. More advanced techniques, such as solid free-form fabrication and the production of *in situ* cell-seeded scaffolds, are also reviewed. The next generation of scaffold fabrication will benefit from adapting nascent generalized materialsprocessing strategies to expand functionality and match corresponding advances in novel biomaterial development.

The design and engineering of suitable biodegradable scaffolds are central to the field of tissue engineering and organ regeneration. Traditional advancements in scaffold fabrication have focused on developing new types of biomaterial systems with more desirable characteristics, such as reduced toxicity or immune response, increased strength, and elastomeric properties. Parallel fabrication strategies have also been improved and developed to accommodate novel biopolymeric systems and to some extent have also been modified to improve and control a similarly defined parameter space, including, for example, biocompatibility, pore size, porosity, and pore connectivity. Integrating drug delivery techniques to administer appropriate growth factors or growth factor–encoding plasmids can lead to improved cell and tissue function and in some cases can promote improved tissue function and vascularization of the construct. This general approach has been shown to be useful in the application of designing systems to support the growth of small volumes of simple tissues of primarily one cell type, such as the epidermis, cartilage, and the bladder. Complex or highly vascularized organs and tissues such as the muscle, liver, and kidney require the integration of many cell phenotypes, where the functionality of the organ is highly dependent on spatially defined microenvironments and subsequent heterotypic cell–cell interactions. While there have been substantial advancements in producing vascularized scaffolds, the inability to produce large volumes of organs is still problematic. Controlling the mechanical, chemical, and spatial cellular microenvironments within a scaffold is essential to designing tissue engineering systems.

The integration of micro- and nanoscale technologies with biology and bioengineering has led to significant advancements in the field of tissue engineering. Probing cells and biological systems with tools that operate at the micron- and submicron-length scales have led to the elucidation of some of the fundamental parameters of the cellular microenvironment that influence cell processes and phenotype. Studying and controlling cell–matrix interactions is also of extreme importance. While the chemistry and biology of cell–matrix interactions have been studied extensively, the topography of this interface also plays an important role in regulating cell function. The extracellular matrix is known to contain nanometer-scale features, which provide cues that influence essential cell functions such as proliferation, migration, and spreading. Numerous synthetic systems with a variety of submicron-scale feature sizes and geometries have been used to study the behavior of cells in response to substrates rich in nanometer-scale topographical cues. Cells have also been known to respond to randomly oriented topography such as nano-scale roughness in addition to well-defined substrates with submicron-scale fabricated features. Topographic features on the order of 1 micron or smaller can influence a number of cell functions, including cell attachment, morphology, and directed migration, which are important cellular processes to control in fabricating cell–scaffold constructs. Cell alignment, for example, has been shown to play an important role in developing stronger tissues in the cases of smooth muscle cells, skeletal muscle, and fibroblasts. Topography has also been shown to influence the gene profile, including the up-regulation of fibronectin mRNA levels in fibroblasts. The generalized reaction of cell to topography has been extensively reviewed elsewhere.

An understanding of the interactions between cells and chemical, topographical, and spatial microenvironments is an important aspect for the rational design of tissue engineering systems. The corpus of work performed in the field of microfabrication for tissue engineering has focused primarily on studying cellular interactions in two dimensions. Translating the systems and techniques developed to control cell function in two dimensions must be expanded and applied to demonstrate similar control in three-dimensional scaffolds in order to utilize tissue engineering as a viable therapeutic option. The current paradigm of biomimicry in the fabrication of tissue engineering scaffolds requires the ability to control the cellular environment on a micron and submicron level. A wide range of top-down and bottom-up processes have been developed to meet the corresponding increase in demand of micro- and nanometer-scale precision in developing tissue and organ

regeneration systems. This chapter focuses on the design and fabrication of tissue-engineering scaffolds with micron- and submicron-scale features by surveying the current state of the art in a wide range of systems and approaches.

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UNIT IV

PART A

S.No	Questions
1	Define tissue engineering.
2	Define protein adsorption
3	Mention the ethical problem relating to Tissue engineering.
4	Mention the commercial problem relating to Tissue Engineering.
5	Do you think tissue engineering has future?
6	What is protein surface interaction?
7	What are the problems encountered with Bioreactor?
8	State Bioreactor.
9	What are the fundamentals and engineering principles of Bioreactor?
10	What are the causes for failure of Bioreactors?

PART B

S.No	Questions
1	Which stage of stem cell development is best suited for tissue engineering? Why?
2	Explain hormones and growth factor signaling in tissue engineering.
3	Explain bioreactor and their application in functional tissue engineering.
4	Discuss the recent developments in the use of tissue engineering in therapeutics.
5	What are scaffolds? Describe the methods which are used for the synthesis of scaffolds.
6	Using the bottom up approach and the fundamental principles of tissue engineering, How will you produce skin?
7	What is a Bioreactor? Why is cell seeding important in a Bioreactor? How do bioreactors function for engineering 3D-tissue construction?
8	What is tissue engineering? Mention the basic clinical goals and fundamental challenges of tissue engineering.
9	Describe how bioreactor can be used in Tissue engineering.
10	Mention the advantages and disadvantages of perfusion bioreactor or rotary bioreactor.

UNIT – V – Artificial Organs and Tissue Engineering-SBMA3003

STEM CELLS

Stem cells in tissue engineering. The concept of producing 'spare parts' of the body for replacement of damaged or lost organs lies at the core of the varied biotechnological practices referred to generally as tissue engineering.

Tissue engineering based on stem cells has gained interest recently as attempts are made to engineer scaffold environments mimicking the stem cell niche, which contains a reservoir of multipotent stem cells that can maintain normal tissue or restore unhealthy cell populations in response to mechanisms of quiescence, self-renewal, and differentiation of the stem cells. These cell behaviors are governed by soluble signals that are systemic or presented by local niche cells. In this review, current and emergent approaches based on stem cells in the field of tissue engineering are presented for specific applications of human tissues and organs. The combination of stem cells and tissue engineering opens new perspectives in tissue regeneration for stem cell therapy because of the potential to control stem cell behavior with the physical and chemical characteristics of the engineered scaffold environment.

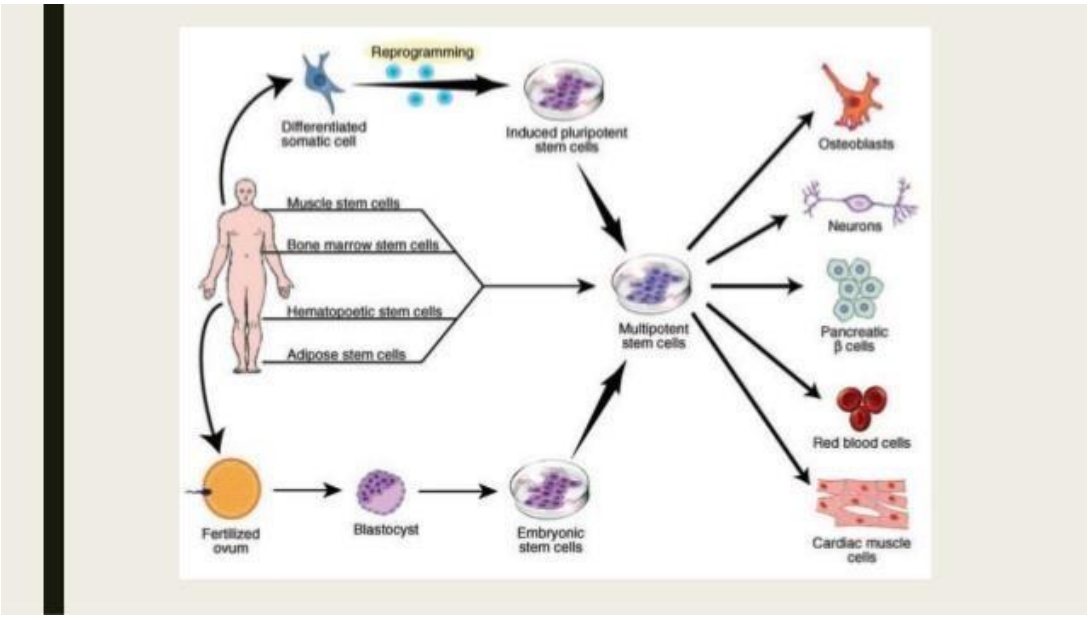

Fig. 5.1 **Stem cells** in tissue engineering

Chronic limitations of traditional transplantation surgeries still exist due to the lack of appropriate donor tissues, risk of disease transmission, and potential for immune rejection.Tissue engineering, the multidisciplinary application of biology, chemistry, physics, engineering,and medical science, offers an alternative method to overcome these issues [1,2]. For therapeutic application of tissue engineering, engineered tissue is grown either within a patient or outside the patient and subsequently transplanted into the patient. Figure 1 provides a schematic representation of the process of tissue regeneration in tissue engineering. Human cells areharvested from a patient and after in vitro cell culture, cells are seeded onto scaffolds with medium containing chemical stimuli, such as growth factors and differentiation-inducing factors.Scaffolds are three-dimensional (3D) matrices that support cellular growth processes, such ascell adhesion, migration, proliferation, and differentiation, by which cells are colonized onto the scaffold. The cell-colonized scaffold is then implanted into the patient, to regenerate biocompatible, immunocompatible, and biofunctional tissues or organs inside the patient body. Cells and scaffolds are essential to regenerate new tissues with tissue engineering. Cells become the primary component of the engineered tissue and the scaffold provides cells with an appropriate physical and chemical environment where they

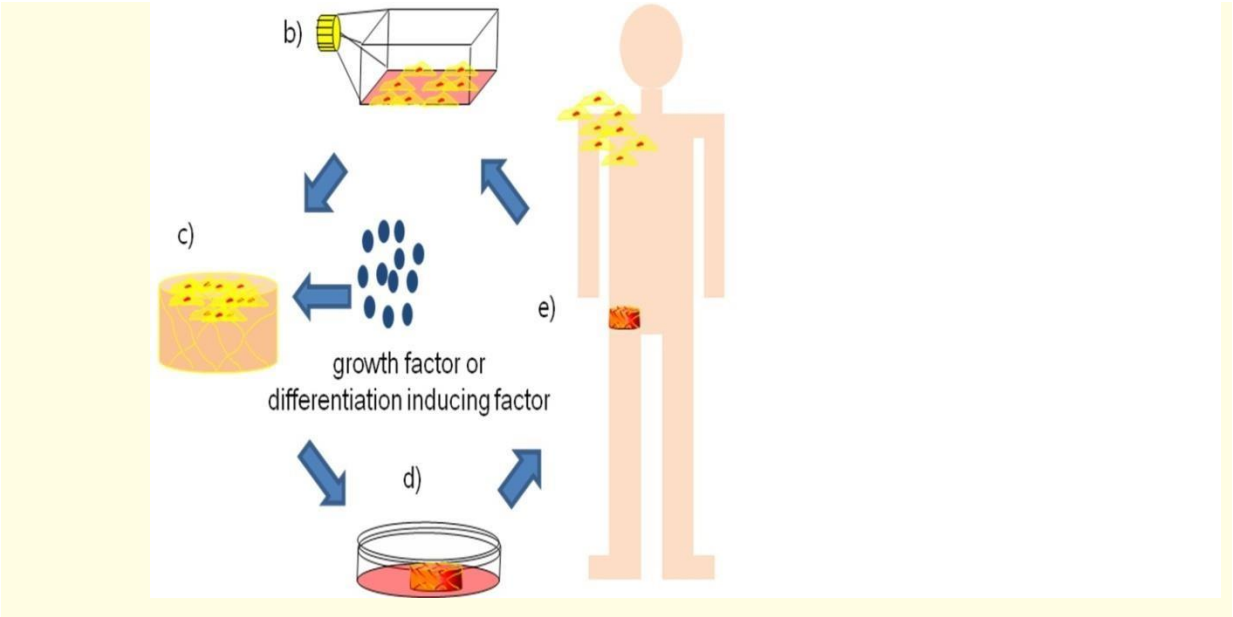

Fig. 5.2 Tissue harvested from a patient's body to obtain cells

can attach to the surface of the scaffold, migrate through the scaffolds' pores, and then proliferate. In some instances, such as stem cell therapy, collaboration of cells and scaffolds with differentiation-inducing factors is essential for stem cells to differentiate into engineered cell lineages and to develop new tissues.

A scaffold is a 3D matrix that provides the framework and initial structural support for cells to attach, proliferate, and differentiate, facilitating the formation of an extracellular matrix (ECM) Characteristics of an ideal scaffold include: 1) contains a network of interconnecting pores so that cells can attach, proliferate, and migrate throughout the entire scaffold; 2) has channels through which oxygen and nutrients are provided to cells and waste products are carried out; 3)is biocompatible with a high affinity for cells to attach and proliferate; and 4) has appropriate mechanical properties Various processing techniques have been used for fabricating scaffolds which have biocompatibility and appropriate surface properties to support cellular attachment, proliferation and differentiation. Examples of scaffold fabrication methods includeemulsion/freeze-drying, solvent casting/particular leaching, computer-aided design/computer-aided manufacturing, electrospinning, nanofiber self-assembly, and photolithography.

For cell based tissue engineering, cells are usually seeded onto scaffolds which are made of materials such as acellular tissue matrices, naturally derived materials (natural biomaterials), and synthetic polymers (synthetic biomaterials). Acellular tissue matrices may be animal or human- derived with all cells removed during manufacture and natural biomaterials extracted fromanimal sources, such as fibrin, collagen, gelatin, chitosan, alginate hyaluronic acid etc. Synthetic biomaterials fabricated from laboratories or factories, such as polycaprolactone (PCL) [40], polylactic acid (PLA) poly(glycolic acid) (PGA), poly(d,l-lactic-co-glycolic acid) (PLGA) polyvinyl alcohol (PVA) polyethyleneglycol (PEG) polyurethanes, carbon nanotubes (CNT), TiO_2 nanotubes, etc. are also widely used. Synthetic biomaterials have tunable mechanical properties, however, the biocompatibility of natural biomaterials is better than synthetic

materials, thus, hybrids of natural and synthetic materials are also used for scaffold fabrication. To support tissue regeneration for in vitro stem cell study, differentiation-inducing factors can beloaded into scaffolds to promote and to induce differentiation of stem cells, but these factors under specific circumstances remain indispensable. Achieving success in tissue engineering is attributed only to stem cells and scaffolds, suggesting that the effects of differentiation factors may be substituted with suitable scaffold structures.

2. STEM CELLS IN TISSUE ENGINEERING

Tissue engineering may be used for tissue regeneration such as bone, cartilage and neural tissues using degradable biomaterial scaffolds. For example, tubular collagen nerve guides (Neuragen from Integra Life Sciences) were used clinically to treat peripheral nerve injuries and the critical gap length treated by nerve guides was longer than 10 mm in primates and could be further increased by adding fibers or hydrogel with cells. In addition, tissue-engineered constructs for bone was osteoconductive to enhance bone cells to adhere, proliferate and migrate. For instance, PCL based scaffolds using fused deposition modeling was developed, approved by the FDA and used clinically as burr plugs and sheets for orbital floor reconstruction in more than 200 patients In addition, tissue engineering treatments for cartilage repair have been established clinically but are not widespread because of limitations in efficiency, consistency and applicabilityFurthermore, stem cells are used in in the field of various tissue engineering such as cardiac, neural, bone, liver tissue engineering, etc. and some examples of the use of scaffolds, biomaterials, and stem cells in tissue engineering were summarized in Table 1.

2.1. Cardiac Tissue Engineering

Congestive heart failure, resulting from myocardial infarction (MI) and ischemic loss of functional CMs, remains the leading cause of death in the United States. The complex events involved in ischemic myocardial cell loss, and the subsequent post-MI remodeling leading to heart failure are not efficiently addressed by existing therapies. Tissue engineering and stem celltherapy could be a promising approach for cardiac repair. Natural acellular scaffolds made of hydrogels have the mechanical structure to support the infarcted heart, reducing wall stress, compensating for contraction function, and inhibiting ventricle remodeling. In vivo study has shown that hydrogels alone can provide mechanical support to the infarcted heart by attenuating

wall stress, compensating for contraction function and preventing ventricle remodeling. Basic fibroblast growth factor (bFGF) plays an important role in angiogenesis and bFGF encapsulated in heparin-alginate microspheres, within a pig model of chronic MI, demonstrated significant enhancement in myocardial function in vivo. Hydrogel scaffolds have also been used in vitro for cell expansion and the induction of cardiogenic differentiation. For example, cell-cell interactions of aggregates of CMs, derived from skeletal muscle-derived stem cells (MDSCs), were enhanced in collagen scaffolds. The expression of cardiac genes, including connexin 43 andcardiac troponin-T were also enhanced, suggesting that MDSCs within collagen scaffolds is a useful 3D culture system to directly assess the contractile properties of differentiated CMs in vitro. In addition, evaluation of a composite scaffold made of the natural and synthetic biomaterials, collagen and PGA, in a perfusion bioreactor demonstrated enhanced attachment of cardiac stem cells (CSCs). Moreover, physical stimuli such as mechanical stress promoted 2-foldincreases in CMs, in addition to matrix fiber alignment, myofibrillogenesis and sarcomeric banding, while cyclic mechanical stress increased CM hypertrophy (2.2-fold) and proliferation rates (21%) when compared to controls with no mechanical stress.

2.2. Neural Tissue Engineering

The central nervous system (CNS), consisting of the spinal cord and the brain, is a very unique tissue network with an unusual ECM structure and characteristic soft physical properties (elastic modulus of natural brain tissue is around 500 Pa) when compared to muscle (10^4 Pa) and bone (10^9 - 10^{10} Pa), which is susceptible to damage, illnesses, and injuries, including traumatic brain injury, spinal cord injury, stroke, Parkinson's disease, and multiple sclerosis. The mechanical properties, structure, and composition of the ECM are effectors of cell function, thus, soft hydrogel scaffolds are utilized for CNS applications to mimic the biochemical and mechanical properties of the CNS. For instance, hydrogel scaffolds made of acrylamide and PEG with argininеglycine-aspartic acid (RGD) can regulate cell behaviors, such as adhesion, cell renewal, and differentiation of neural stem cells (NSCs). Platelet-derived growth factor (PDGF)-AAimmobilized agarose scaffolds have been reported to support differentiation of NSCs and neural progenitor cells (NPCs) to oligodendrocytes. Hydrogel scaffolds made of AcN- RADARADARADARADAIKVAV-$CONH_2$ (RADA16-IKVAV) have been shown to serve as a guiding cue to direct NSC adhesion and neural

differentiation with in vitro and in vivo to direct stem cell differentiation toward neural lineages and to promote the signal transmission among neurons because of electrical conductivity. The hydrogel in a rat brain surgery model enhanced survival of NSCs, reduced the formation of glial astrocytes, and improved brain tissue regeneration after 6 weeks post-transplantation. In addition, CNT is used. For example, electricalstimulation was shown to enhance the proliferation and differentiation of NSCs on thin film scaffolds made of laminin and single-wall carbon nanotubes (SWCNT). Bioelectricity also has been shown to affect intercellular signaling of the nervous system, as fibrous scaffolds made of poly-l-lactide/polyaniline (PLLA/PANi), applied with an electric field of 100 mV/mm for a period of 60 minutes, showed extended neurite outgrowth compared to cells grown on non- stimulated scaffolds.

2.3. Bone Tissue Engineering

Bone is a connective tissue consisting of a collagenous ECM that is extensively mineralized with hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) and other ions that contribute to the high density and strength of bone, as well as its homeostatic regulation and metabolic function. Collagen, however, has poor structural stability, thus, scaffolds made of hybrid materials, consisting of natural and synthetic components, are utilized in bone tissue engineering. For example, fibrous scaffolds made of PLGA/collagen/hydroxyapatite were shown to provide structural stability and mechanical integrity, as well as improve human MSC (hMSC) binding. Inorganic materials such as calcium phosphate or amorphous calcium phosphate (ACP) may enhance the structural stability of collagen scaffolds. Scaffolds made of a composite of collagen and ACP, for example,have been shown to support the proliferation and osteogenic differentiation of MSCs [67], while rat MSCs (rMSCs) on carboxyl-functionalized MWCNT (cMWCNT)/PLGA composites showed enhanced levels of alkaline phosphatase produced by osteoblasts. In addition, bone is a highly metabolic tissue requiring an abundant vascular supply throughout its structure for homeostasis, growth, and remodeling. As a result, 3D co-culture systems based on biomaterials have been studied for concurrent angiogenesis/vasculogenesis and osteogenesis. A spheroid co-culture system of bone marrow derived mesenchymal stromal cells (bmMSC) and human dermal microvascular endothelial cells (HUVECs) produced well-organized 3D vascular structures in vitro and resulted in increased alkaline phosphatase expression when compared to a control culture system of bmMSCs and fibroblasts.

2.4. Liver Tissue Engineering

The liver is the largest organ in the human body and has major roles in metabolism, detoxification, and protein synthesis. Hepatocytes, the major cell type in the liver, execute most of the metabolic, synthetic and storage functions of the liver. The interactions between hepatocytes and non-parenchymal cells also affect the function of the liver. Within in vitro culture environments, hepatocytes tend to lose their function, suggesting that stem cells could be an alternative cell source in combination with supplementary factors, such as differentiation- inducting factors, for liver tissue engineering. Evaluation of rat ESCs (rESCs) cultured within a 3D culture system were shown to differentiate into hepatic-like cells with morphological characteristics of typical mature hepatocytes in the presence of supplementary factors, such as recombinant mouse hepatic growth factor, fibroblast growth factor, insulin, transferrin, selenium,oncostatin, and dexamethasone. Additionally, when these stem cellbearing scaffolds were transplanted into severe combined immunodeficient mice, the rESCs remained viable,undergoing further differentiation and maturation of hepatic-like cells in vivo. Studies of PCL/collagen/polyethersulfone composite scaffolds showed that these scaffolds promoted hMSCdifferentiation to hepatocytelike cells and the expression of hepatocyte-specific markers, such as albumin, α-fetoprotein, cytokeratin-18, cytokeratin-19, and cytochrome P450 3A4 at mRNA levels, where the number of albumin-positive cells cultured on the scaffold (47% ± 4%) was higher than that in the two-dimensional culture system (28% ± 6%) in vitro. However, additional functional assessment of hepatocyte like cells was needed because of the uncertainty of their functionality when compared to adult hepatocytes.

2.5. Other Applications

In addition to cardiac muscle, nerve, bone, and liver applications as described above, the combination of stem cells and tissue engineering could apply to regenerate other tissue types, such as the eyes, cartilage, skin, bladder, and tendon. Clinical trials of strategies using a combination of tissue engineering and stem cells to regenerate bladder, kidney, and urethra tissue are already underway.The stem cell theory of aging postulates that the aging process is the result of the inability of various types of stem cells to continue to replenish the tissues of an organism with functional differentiated cells capable of maintaining that tissues (or organ's) original function.

All tissue is composed of parenchymal (from Greek, that poured in beside) and stromal (Greek,

framework or foundation) cells. Parenchyma are the functional cells of a tissue (e.g., for liver, hepatic parenchymal cells or hepatocytes; for bone marrow, hematopoietic cells), where stroma comprises primarily connective tissue elements which, together with their products, form the structural framework of tissue. Parenchymal cells can be derivatives of any of the three germ layers, and during development they usually grow into areas populated by stromal cells or their progenitors. Under the strictest definition, stromal cells are derivatives of mesenchyme and include fibroblasts, osteogenic cells, myofibroblasts, and fat cells which appear to arise from a common stem/progenitor cell. Some investigators apply the term stromal cell to all the nonparenchymal cells that contribute to the microenvironment of a tissue and include endothelialcells and macrophages (histiocytes) in this classification as well. However, the ontogency of bothendothelial cells and macrophages is distinct from that of mesenchymal tissue-derived cells. A partial listing of tissue cells that may influence the function of organ parenchyma. For the sake of brevity, migrating cells of bone marrow origin will not be discussed in the text (e.g., mast cells, B lymphocytes, natural killer cells), although these cells can influence parenchyma either directly or via cytokine-mediated modulation of stromal cell function.

One key component of the in vitro bone model is the scaffold, which provides a structural and logistic template for the developing tissue, and can markedly affect cell behavior. Several typesof porous scaffolds have been shown to support in vitro bone formation by human cells, including those made of ceramics, native and synthetic polymers and composite materials. Scaff old properties important for bone formation include: the size, distribution and shape of the pores , surface roughness ; the presence of cell attachment sites and the biomechanics of both the material and the scaff old structure. In general, the most suitable scaff olds for bone formation are those with large and interconnected pores (which facilitate cell infi ltration and matrix deposition) and rough inner surfaces (which facilitate cell attachment), made of osteoconductive materials (such as bone protein and hydroxyapatite), and with mechanical properties similar to those of native bone (both to enable load-bearing and stimulate osteo genesis). Additional features of interest include aniso tropic structure, capacity for vascularization, and process ability into anatomically correct shapes. Scaffolds can also incorporate and modulate delivery of molecular signals controlling cellular functions. Another key component of bone tissue engineering is the culture system or bioreactor. Bioreactor systems

can be designed to control transport of nutrients and oxygen to cells in clinically sized constructs and provide lineage specifi c biological stimuli in various regions of the graft. Additionally, the development of functional, load bearing characteristics of the graft would be enhanced by the application of biophysical stimulation in order to attain mechanical competence in both the cartilage and bone regions. Advanced bioreactor designs maintain the physiological milieu in the cell microenvironment (pH, temperature, oxygen and nutrient delivery) by perfusion and conditioning of culture medium . Bioreactors can also be designed to recapitulate one or more of the developmentally relevant biophysical signals in a time-controlled manner. For example, increased mass transport and fluid shear by medium perfusion, and cyclic loading have been shown to improve osteogenesis and enable formation of homogenous bone constructs. Ideally, a bioreactor system should be capable of coordinating biological, physiological and mechanical stimuli, and applying them in a spatially and temporally controlled manner to provide lineage- specific stimulation within clinically sized grafts. The clinical and scientific utility of tissue engineering largely depends on our ability to predictably direct cells to differentiate into the right phenotypes in a spatially and temporally defined pattern. The control of environmental conditions provided through the design of bioreactors - in conjunction with scaffolds - can help gain more insight into the interplay of molecular and physical factors that guide the development of bone from various types of osteogenic cells. Understanding of the developmental process may then serve as feedback to the optimization of engineering parameters toward better graft designs, and towards the use of engineered grafts as models of development and disease. Sources of human osteogenic cells, ` there are several basic considerations when choosing a cell source for bone tissue engineering: the choice. Tissue-engineered bone constructs have the potential to alleviate the demand arising from the shortage of suitable autograft and allograft materials for augmenting bone healing. They also can serve as controllable in vitro models of high biological fidelity for studies of bone development, disease or regeneration. Each of the sources of osteogenic human cells - primary cells, MSCs, ESCs and induced pluripotent stem cells - has distinct advantages when used for bone tissue engineering, and the quest for an ‘ideal’ cell source is still in progress.

Tissue engineering of Bone marrow

The bone marrow (BM) tissue is the main physiological site for adult hematopoiesis. In recent years, the cellular and matrix components composing the BM have been defined with unprecedent resolution, both at the molecular and structural levels. With the expansion of this knowledge, the possibility of reproducing a BM-like structure, to ectopically support and study hematopoiesis, becomes a reality. A number of experimental systems have been implemented and have displayed

the feasibility of bioengineering BM tissues, supported by cells of mesenchymal origin. Despite being known as an abundant component of the BM, the vasculature has been largely disregarded for its role in regulating tissue formation, organization and determination. Recent reports have highlighted the crucial role for vascular endothelial cells in shaping tissue development and supporting steady state, emergency and malignant hematopoiesis, both pre- and postnatally. Herein, we review the field of BM-tissue bioengineering with a particular focus on vascular system implementation and integration, starting from describing a variety of applicable *in vitro* models, ending up with *in vivo* preclinical models. Additionally, we highlight the challenges of the field and discuss the clinical perspectives in terms of adoptive transfer of vascularized BM-niche grafts in patients to support recovering hematopoiesis.

Recent advances in bioengineering have dramatically improved the ability of tissue reconstitution with partially restored functions, for both fundamental research and regenerative medicine. In the specific case of the bone marrow (BM), the reconstitution of a functional multicellular unit is of therapeutic interest both for BM grafts and for bone repair. Besides the generation of organ sized tissue substitutes, generating a BM-on-a-chip also have multiple interests. Indeed, there is a need of *ex vivo* cultures for stem cell clinical expansion, but it is also a necessity to have strong physiological-like models for fundamental research, that for ethical reasons might replace in the future the necessity for *in vivo* studies, also providing physiologically relevant human model systems. However, as of today the human hematopoietic niche complexity is still not fully understood, and despite the recent advances in murine studies, the function and cellular organization/composition in humans is vastly missing. As human genetic *in vivo* manipulation studies are not feasible experimentally and ethically, tissue bioengineering and modeling represents a straightforward approach to progress our understanding of human hematopoiesis. Mimicking tissue function “on a chip” can help to grasp which are the minimal components necessary for proper hematopoietic niche homeostasis or for instigation and propagation of a malignant phenotype. “Niche-chips” can complement drug tests, which are not always translatable with 1:1 accuracy to human from rodent models, to assess a specific compound impact on multiple types of human cells and to decipher the mechanisms of action. “Niche chips” can be used for the comprehension of radio-chemoresistance in investigation of cancer behavior without inducing diseased animal models, which represents an ethical step forward. All in all, the increase in bioengineered chips in biology reflects an overall need and scientific will to move forward to superior physiological and ethical models relevant for the design of new clinical therapies.

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UNIT V

PART A

S.No	Questions
1	What are stem cells?
2	Differentiate between embryonic stem cell and adult stem cells.
3	What are the different kinds of matrix materials used in tissue engineering?
4	How sterile condition is maintained in the cell culture laboratory?
5	What is the significance of autoclave in cell culture?
6	What is scaffold?
7	Name the engineering materials used for scaffold fabrication.
8	What are adult stem cells?
9	What are embryonic stem cells?
10	What are growth factors?
11	Mention one ethical problem in relation to TE.
12	Mention one commercial problem associated with the development of TE.

PART B

S.No	Questions
1	Define stem cell. Classify them based on their functioning. Add a note on their applications.
2	Write short notes on the following a) cells for tissue engineering b) cell preservation and storage.
3	With the help of suitable diagram explain the process of differentiation of stem cells into cell lines
4	What do you understand by cell-cell interaction? Briefly explain the different types of stem cells.
5	What are stem cells? Differentiate embryonic from adult stem cells. Mention the applications of the same.
6	Narrate the importance of embryonic stem cells. What are the sources of embryonic stem cells?
7	What are the ethical debates from stem cell research? Explain the future scope of stem cell research.
8	Describe the advantages and disadvantages of using embryonic stem cells in tissue engineering.
9	Describe a method to promote or increase cell adhesion on scaffolds