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RECENT ADVANCEMENT IN BIOREACTOR: TISSUE CLONING AND INVIVO BIOREACTOR TRANSPLANTION

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ABSTRACT
The practice school report is entitled as "Recent advancement in bioreactor: tissue cloning and in vivo bioreactor transplantation". The main objective of this report is to assemble research data for tissue cloning and invivo bioreactor for further research and developments. Therapeutic cloning involves the introduction of a nucleus from a donor cell into an enucleated oocyte to generate embryonic stem cell lines whose genetic material is identical to that of its source. These autologous stem cells have the potential to become almost any type of cell in the adult body, and thus would be useful in tissue and organ replacement applications. This review considers the key elements necessary to enable bioreactors to address three main areas associated with biological systems. All entail recreation of the in vivo cell niche as faithfully as possible, so that they may be used to study molecular and cellular changes in normal physiology, with a view to creating tissue-engineered grafts for clinical use; understanding the pathophysiology of disease at the molecular level; defining possible therapeutic targets; and enabling appropriate pharmaceutical testing on a truly representative organoid, thus enabling better drug design, and simultaneously creating the potential to reduce the numbers of animals in research. The premise explored is that not only cellular signalling cues, but also mechano-transduction from mechanical cues, play an important role. Bioreactors are engineered systems capable of supporting a biologically active situation for conducting aerobic or anaerobic biochemical processes. Stability, operational ease, improved nutrient uptake capacity, time- and cost-effectiveness, and large quantities of biomass production, make bioreactors suitable alternatives to conventional plant tissue and cell culture (PTCC) methods. Bioreactors are employed in a wide range of plant research, and have evolved over time. Such technological progress, has led to remarkable achievements in the field of PTCC. Since the classification of bioreactors has been extensively reviewed in numerous reviews, the current article avoids repeating the same material. Alternatively, it aims to highlight the principal advances in the bioreactor hardware s used in PTCC rather than classical categorization. Furthermore, our review summarizes the most significant steps as well as current state-of-the-art of PTCC carried out in various types of bioreactor. This report documents the theoretical experience gained through the project work classes. The present report reviews recent advances and have occurred in therapeutic cloning and tissue engineering and describe applications of these new technologies that may offer novel therapies for patients with end stage organ failure.

KEYWORDS: Bioreactor, Tissue cloning, bioreactor transplantation, PTCC.

INTRODUCTION
Tissue cloning In biology, cloning is a asexual production of genetically identical cells, organisms, or copies of DNA. Identical gene and DNA fragment that are used in genetic engineering can be generated by gene cloning. In addition to gene cloning, there is also reproductive cloning which is defined as the ability to produce a new individual that have the same genetic information of the donor of the nucleus. Therapeutic cloning also enables scientists to rapidly generate a mature cell of a specific nature. Reproductive and therapeutic cloning is a direct outcome of recent research and discoveries on how the cell cycle is controlled**.** Cloning was once thought to be impossible. After doing a lot of research, scientists Have improved the technology used in the bioengineering field. This has allowed for The successful cloning of organisms. When the successful cloning of a sheep known as Dolly was achieved, animal cloning was revolutionized in 1997. The sheep Dolly was not the first cloned animal, but it was the most famous one. A variety of other animals Were cloned soon after the Dolly generation, including rabbits, cats, dogs, pigs, goats, Mules, and horses. Also, in 2006, Japanese scientists were able to create heart and

brain cells from adult stem cells. These advances demonstrate that scientists Are actively investigating methods of overcoming the current limitations and ethical Concerns of cloning.The objective of therapeutic cloning is to produce mature cells of various cell types and specialized tissue cells rather than an individual organism. The purpose of therapeutic cloning is to provide new cells and tissues that could be used by doctors to complete medical treatments. Therapeutic cloning can help in cultivation of several type of new cells and tissue such as nerve cells that could provide treatment or cure for Alzheimer's or Parkinson's disease patients, hematopoietic cells for leukemiapatients., or even for the cultivation of whole organs from embryonic stem cells The process of therapeutic cloning The therapeutic cloning is a technology used the somatic cell nuclear transfer (SCNT) method to provide new cells and tissue. It is a process of transferring the nucleus of a somatic cell into another enucleated cell called oocyte that considers an immature egg cell to produce stem cells.

Somatic cell nuclear transfer has three major steps

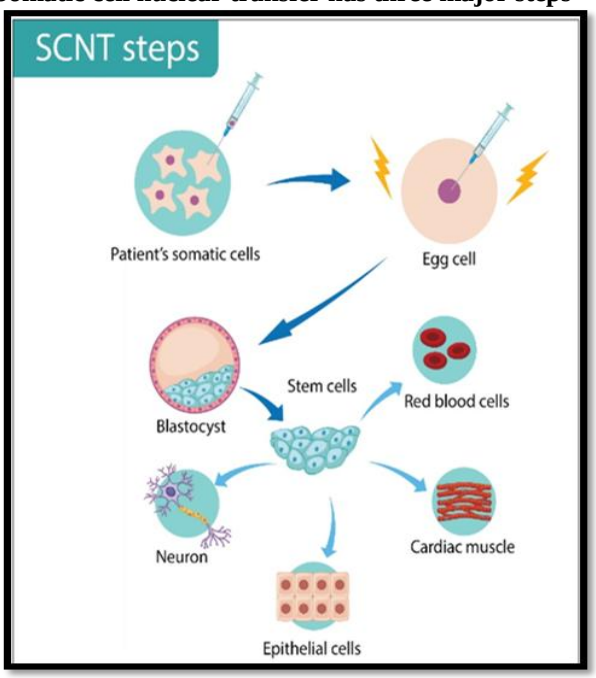

Figure no 1: Shows the process and the different types of cell that stem cell can turn into Stem cells can provide autologous cells that help in tissue and organ replacement.

1. Transferring a diploid nucleus of a somatic cell by using a small needle into an enucleated egg (oocyte) that had a haploid nucleus before removing it. The egg cell turns into a zygote which has the full number of chromosomes. This process differs from fertilization because no sperm is used to make the zygote

2. The activation of the zygote is done by using chemicals or zapping it with an electrical charge to stimulate cell division until it reaches the blastocyst stage Blastocyst is a very early embryo that contains a central fluid-filled cavity and it has two parts: the outer trophoblasts and the inner cell mass.

3. The inner mass of the blastocyst is isolated and cultured to form embryonic stem cells.[3,6] They are unspecialized and pluripotent cells that can turn into differently cell types.

Tissue cloning aims to make tissue cells instead of producing a Human baby. Despite popular belief, a tissue clone does not Contain any germ cells. In actuality, it is composed of Differentiated cells and yolk. Hence, the transferred differentiated somatic cell cannot be Compared with the seed of a plant, but can be likened to a Transplanted tree branch.) Tissue cloning requires no fertilization Step but reprogrammes the differentiated cells.

Each Chromosome is composing of DNA 'and some proteins. Half the chromosomes in any human are borrowed from the mother and half from the father. The Book of Life is written in the alphabet of DNA, comprising fours letter A. G. C, and I (Adenine, Guanine. Cyrosine and Thymine) which constitute the DNA molecule. Genes are words and sentences formed from these, the basic units of information and determined how a human being will be and how he will carry out essential functions"

Recently "Cloning" is used as the term in molecular biology for the insertion of another organism's gene into a target host organism (e.g. taking the Green Fluorescent Protein (głp) gene from the A. victoria jellyfish and putting it in E. coli to get E.coli to glow green).

TYPES OF CLONING

The following three types of cloning technologies will be discussed:

i. RECOMBINANT DNA OR DNA CLONING

The transfer of a DNA fragment of interest from one organism to a self-replicating genetic element such as a bacterial plasmid DNA cloning is used to produce many copies of a particular segment of DNA containing one or more genes, to be studied in the laboratory. The DNA fragment of interest from an organism such as a human is incorporated into the *plasmid DNA' of a bacterial cell. A plasmid is a circular self-replicating DNA molecule that is separate from the bacterial DNA. The plasmid containing the genes or DNA of interest is now a piece of recombinant DNA'made up of human and bacterial DNA. It is then put into a cell that will act as a host: as the host cell is copied over and over again, the recombinant DNA is copied as well. Bacteria are most often the host cells but yeast and mammalian cells can be used too. The end result is multiple identical copies of the same human DNA fragment or gene.

Benefits: Cloning is essential to enable enough copies of a gene or DNA segment to be analysed for human

genetic testing to diagnose genetic conditions and enable predictive or presymptomatic genetic testing for genetic conditions in symptomatic individuals or prenatal.

ii. REPRODUCTIVE CLONING

Reproductive cloning is a technology used to generate an animal that has the same nuclear DNA as another currently or previously existing animal. Dolly was created by reproductive cloning technology. E.g. Dolly (1996-2003). The purpose of this type of cloning is to produce a genetic duplicate of an existing or previously existing organism.

Benefits: Reproductive cloning can have many uses:

- If the low success rates and issues of safety could be improved as discussed below, the technology can be used to mass produce animals with special qualities, such as animals that are important agriculturally or are able to produce helpful drugs for human use.
- SCNT" has environmental uses in that it can be used to repopulate endangered species, as has been shown with the wild ox and the gaur.
- Some supporters of human reproductive cloning also see it as a way of overcoming male infertility, where other methods of ass.

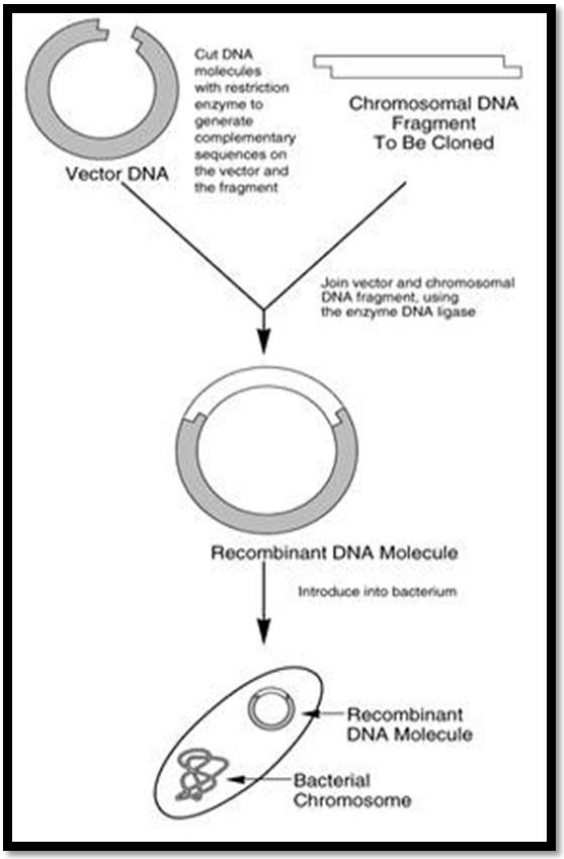

Figure no 2: Reproductive cloning.

iii. THERAPEUTIC CLONING

Therapeutic cloning, also called "embryo cloning," is the production of human embryos for use in research. The goal of this process is not to create cloned human beings, but rather to harvest stem cells" that can be used to study human development and to treat disease.

The purpose of this method of cloning is reflected in its name: to enable the correction of health problems. The aim is to create stem cells containing the person's own DNA that could be grown in the laboratory and then transplanted into them without the risk of tissue rejection.

INVIVO BIOREACTOR

Tissue engineering aims to realize biological functional substitutes to be used to repair or regenerate damaged tissues. To achieve tissue remodeling, cell culture must tangibly differ from the traditional static 2D culture. In fact, traditional cell cultures are usually carried out on multi-well polystyrene plate, containing a specific treated surface to promote cell adhesion. Cells are seeded with medium and then plates are placed in an humified incubator where the environmental conditions of 37°C and 5% CO2 are maintained. We will view more on requirements and working of bioreactor.

A bioreactor can be defined as any apparatus that attempts to mimic physiological conditions in order to maintain and encourage tissue regeneration, simulating the living organism. In a bioreactor, tissue culture is a non-steady state process in which all parameters can be measured and controlled. Precisely, temperature, pH of the medium, gas exchange, O2 and CO2 level, humidity, nutrient flow and waste removal. Moreover, mechanical-biochemical stimuli can also be tuned.

In vivo bioreactors approach is an emerging strategy for bridging the in vitro gap between experimental successes and clinical translation in managing scaffolds manipulation, seed cells seeding and growth factors delivery for bone defect reconstruction. Bones is a highly vascularized tissue, with an intricate cellular architecture that continues to remodel throughout the lifetime of an individual. Despite the regenerative capacity of bone, large bone defects, as observed after bone tumor resections and severe fractures, lack the template for an orchestrated regeneration and require bone grafting Furthermore, annually 200,000 spinal fusions are performed in the U.S.alone that also require massive bone grafting In spinal fusion and long-bone fractures, autologous bone is considered the gold standard because of its ability to integrate with the host bone and its lack of immune-related complications.

Despite numerous attempts, in vitro engineering of functional bone tissue using principles of tissue engineering has proven elusive because of the challenges involved in the differentiation and sustenance of different cell types in a concomitant fashion and in achieving a vascular network in vitro. Recently, in vivo engineering of bone has been demonstrated by combining porous ceramic or demineralized bone matrix supports with mesenchymal (marrow-derived) cells and or bone morphogenetic prot.

NEED OF STUDY

- Introducing the concept of molecular cloning.
- Presenting the various molecular cloning techniques and their relative strengths and weaknesses.
- Describing step-by-step the methods involved in an experiment of traditional" molecular cloning.
- Providing a broad outline of the applications of molecular cloning to address biomedical research questions
- Exemplifying the use of molecular cloning to address a specific research question
- To review the application and research progress of in vivo bioreactor as vascularization strategies in bone tissue engineering.
- The in vivo bioreactor principle focuses on using the body's self-regenerative capacity to regenerate new tissue.
- This strategy has been successfully used to reconstruct critical-sized bone defects in humans
- The overarching goal of in vitro tissue engineering is to create a functional tissue that is equivalent to native tissue in terms of composition, biomechanical properties, and physiological performance.
- However, in vitro tissue engineering suffers from a limited ability to mimic in vitro conditions, often leading to inadequate tissue substitutes
- Therefore, in vivo tissue engineering has been suggested as a method to circumvent the tedium of environmental manipulation and use native in vivo stimuli to direct cell growth.
- To achieve in vivo tissue growth, an artificial bioreactor space must be established in which cells may grow. The in vivo bioreactor depends on harnessing the reparative qualities of the body to recruit stem cells into an implanted scaffold, and utilize vasculature to supply all necessary growth components.
- To know an approach to generate prefabricated tissue flaps
- To create a functional tissue that is equivalent to native tissue
- To produce a human being but to create embryonic stem cells that are genetically compatible with that of the recipient.

PRINCIPLE AND WORKING

Principle

Molecular cloning is the collection of experimental procedures required to isolate and expand a specific fragment of DNA into a host organism in order to create a large number of identical copies. On top of allowing the study of a single DNA sequence of interest, molecular cloning is a powerful technique that permits the generation of complex combinations of DNA fragments for the most disparate downstream applications. As such, this process is key to most modern biomedical basic research studies and translational applications.

The general principles of tissue engineering involve combining living cells with a natural/synthetic support or scaffold to build a three dimensional (3D) living construct that is functionally, structurally and mechanically equal to "or better" than the tissue that is to be replaced. The development of such a construct requires a careful selection of four key materials:

1) Scaffold,
2) Growth factors,
3) Extracellular matrix,
4) Cells

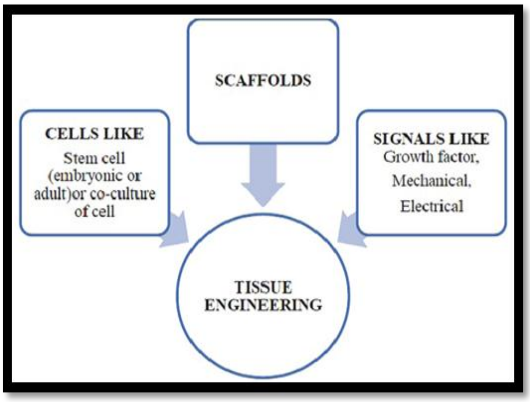

Figure no 3: Tissue engineering.

After an adequate amount of cells are expanded from a cloned source, tissue engineering will be required to produce transplantable tissue or organs. The field of tissue engineering has emerged over the past 40 years to address the shortcomings of previous replacement therapies. Scientists in this relatively new field aim to combine the principles of cell transplantation, material science, and engineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissues. Over the past two decades, scientists have attempted to engineer tissue replacements for virtually every tissue and body part of the human body. Matrices (50–53). These matrices tend to slowly degrade on implantation and are generally replaced by the extracellular matrix (ECM) proteins that are secreted by the ingrowing cells.

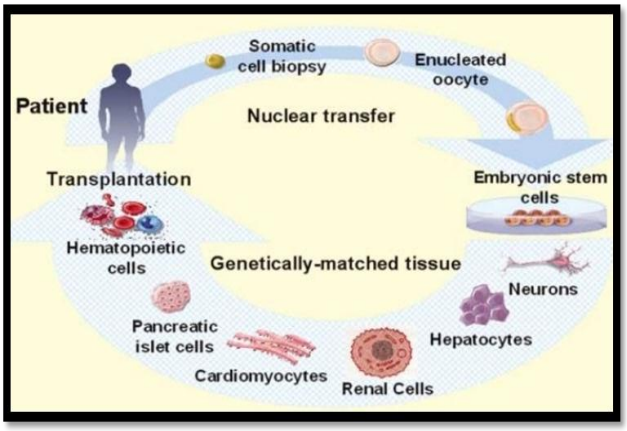

Figure no 4: Strategies for therapeutic cloning.

When cells are used for tissue engineering, a small piece of donor tissue or cloned tissue is dissociated into individual cells. These cells are either implanted directly into the host or are expanded in culture, attached to a support matrix, and then reimplanted into the host after expansion. The source of donor tissue can be heterologous (such as bovine), allogeneic (same species, different individual), or autologous. Ideally, both structural and functional tissue replacement will occur with minimal complications. The most preferred cells to use are autologous cells, where a biopsy of tissue is obtained from the host, the cells are dissociated and expanded in culture, and the expanded cells are implanted into the same host. The use of autologous cells or cloned cells avoids rejection and, thus, the deleterious side effects of immunosuppressive medications can be avoided. Tissue engineering strategies generally fall into two categories: acellular matrices, where matrices are used alone and depend on the body's natural ability to regenerate new tissue, and matrices with cells. Acellular tissue matrices are usually prepared by removing cellular components from tissues via mechanical and chemical manipulation to produce collagen-rich. One promising approach for bone defect reconstruction is using in tow bioreactor principle to generate autologous bone tissue. The term 'in vivo' bioreactor was first coined in 2005 by two independent studies 1 in which the periosteum or vascular pedicle were demonstrated to act as bioreactors to successfully induce new bone formation 14. Inspired by the body's self regeneration phenomena, such as nerve regeneration after peripheral nerve injury, liver regeneration after 1liver resection and scarless fetal skin healing, the main bioreactor strategy focuses on taking the body as a bio-1reactor to cultivate the traditional triad (scaffolds, cells,growth factors) or a combination thereof and leveraging the body's self-regenerative capacity to regenerate new tissue.

The in vivo bioreactor principle has been successfully used to create a series of complex tissues, including whole human organs such as cartilage 11, fat (S), muscle 19 and mandible 10.11]. For regeneration of functional bone tissue, a key advantage of following this principle is that the body can offer a Constant stream of different stem/osteogenic cells to create a regenerative niche and native signals for bone tissue.growth and development, which makes it possible to bypass excessive manipulation of cells, scaffolds and growth factors during ex into culture.Anatomically, the in o bioreactor is not only a vascular territory but also a regenerative niche for vascularization, regeneration and remodeling of the regenerated bone tissue.

The most important consideration for an in www bioreactor strategy is the tissue type surrounding the bioreactor that may directly affect the interaction with the implanted construct, the recruitment of autologous cells, the reestablishment of a functional neurovascular network and finally the results of bone regeneration. To date, different in vivo bioreactor strategies for bone regeneration have been extensively investigated and yielded promising results. Examples i of such strategies include subcutaneous pouch 12)muscular pouch/flap m), abdominal cavity 13), periosteal flap 114, axial vascular bundles), arteriovenous loop 116) and omentum (11). Although the study models and designs entirely differ from each other, the basic principles of an in vivo bioreactor strategy for bone regeneration are similar choose a right anatomical site for providing a regenerative microenvironment, and seek an optimal combination of the traditional for serving as a structural and logistical template for bone formation. Nowadays, research is ongoing to develop an ideal in dit bioreactor strategy for clinical application of the in vivo BTE approach

WORKING AND COMPONENTS:

The working of Tissue cloning is as follows:

Indeed, genetically identical copies of whole organisms are commonplace in the plant breeding world and are commonly referred to as "varieties" rather than clones. Many valuable horticultural or agricultural strains are maintained solely by vegetative propagation from an original plant, reflecting the ease with which it is possible to regenerate a complete plant from a small cutting. The developmental process in animals does not usually permit cloning as easily as in plants. Many simpler invertebrate species, however, such as certain kinds of worms, are capable of regenerating a whole organism from a small piece, even though this is not necessarily their usual mode of reproduction. Vertebrates have lost this ability entirely, although regeneration of certain limbs, organs, or tissues can occur to varying degrees in some animals. At the molecular and cellular level, scientists have been cloning human and animal cells and genes for several decades. The scientific justification for such cloning is that it provides greater quantities of identical cells or genes for study; each cell or molecule is identical to the others.At the simplest level, molecular biologists routinely make clones of deoxyribonucleic acid (DNA), the molecular basis of genes. DNA fragments containing genes are copied and amplified in a host cell, usually a bacterium. The availability of large quantities of identical. Another type of cloning is conducted at the cellular level. In cellular cloning copies are made of cells derived from the soma, or body, by growing these cells in culture in a laboratory.

The third type of cloning aims to reproduce genetically identical animals. Cloning of animals can typically be divided into two distinct processes, blastomere separation and nuclear transplantation cloning.

In blastomere separation, the developing embryo is split very soon after fertilization when it is composed of two to eight cells. Each cell, called a blastomere, is able to produce a new individual organism. These blastomeres are considered to be totipotent, that is they possess the total potential to make an entire new organism. This totipotency allows scientists to split animal embryos into

several cells to produce multiple organisms that are genetically identical. This capability has tremendous relevance to breeding cattle and other livestock

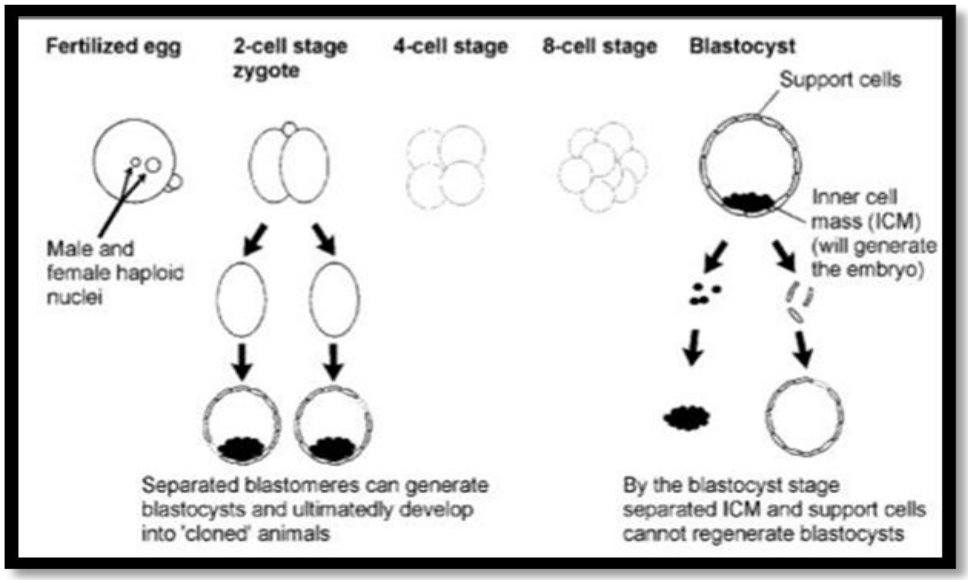

Figure no 5: - Preimplantation embryo development in mammals.

In such nuclear transplantation cloning there is a single genetic "parent," unlike sexual reproduction where a new organism is formed when the genetic material of the egg and sperm fuse. The first experiments of this type were successful only when the donor cell was derived from an early embryo. In theory, large numbers of genetically identical animals could be produced through such nuclear transplantation cloning. In practice, the nuclei from embryos which have developed beyond a certain number of cells seem to lose their totipotency, limiting the number of animals that can be produced in a given period of time from a single, originating embryo.

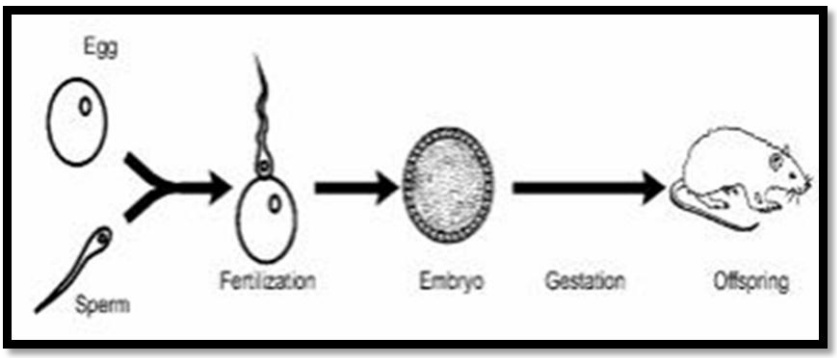

Figure no 6: - sexual reproduction.

Given the fact that cells develop and divide after fertilization and differentiate into specific tissue, the development of a viable adult sheep from a differentiated adult cell nucleus provided surprising evidence that the pattern of gene expression can be reprogrammed. Until this experiment many biologists believed that reactivation of the genetic material of mammalian somatic cells would not be complete enough to allow for the production of a viable adult mammal from nuclear transfer cloning.

The classical TE strategy consists of:

1) Isolating specific cells through a biopsy from a patient, growing them on a biomimetic scaffold under controlled culture conditions.
2) Delivering the resulting construct to the desired site in the patient's body.
3) Directing the new tissue formation into the scaffold that can be degraded over time.

REQUIREMENTS AND WORKING

The working of Invivobioreactor are as follows:

1) CELLS

TIssue engineering utilizes living cells as engineering materials. Examples include using living fibroblasts in skin replacement or repair, cartilage repaired with living chondrocytes.

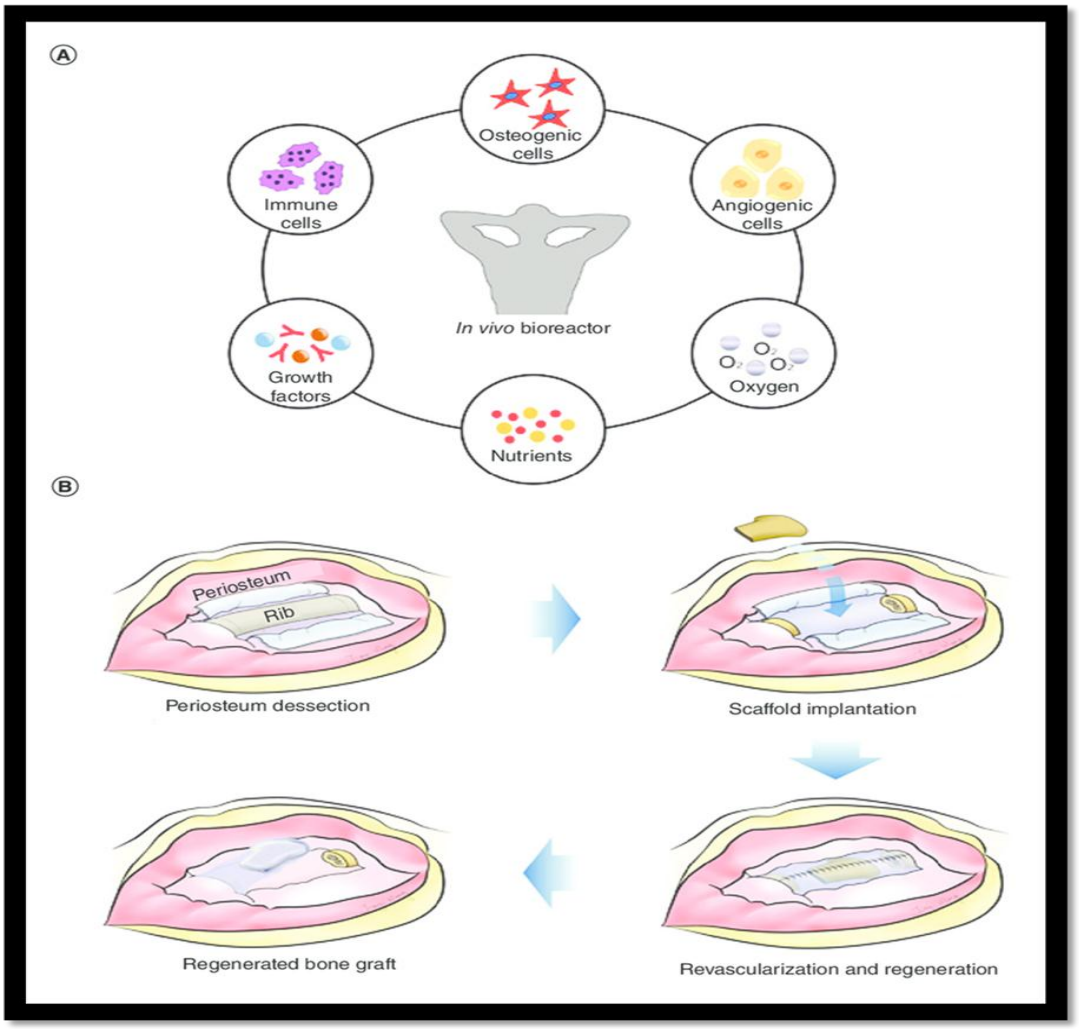

Figure no 7: Paradiagarm of a bioreactor.

Tissue engineering done in vivo is capable of recruiting local cellular populations into a bioreactor space. Indeed, a range of neotissue growth has been shown: bone, cartilage, fat, and muscle. In theory, any tissue type could be grown in this manner if all necessary components (growth factors, environmental and physical ques) are met. Recruitment of stem cells require a complex process of mobilization from their niche, though research suggests that mature cells transplanted upon the bioreactor scaffold can improve stem cell recruitment. These cells secrete growth factors that promote repair and can be co-cultured with stem cells to improve tissue formation. Cells are often categorized by their source: Some of cells used in tissue engineering are Autologous cells, Autologous cells, Allogeneic cells, Xenogenic cells, Isogenic cells etc, Stem cells are undifferentiated cells with the ability to divide in culture and give rise to different forms of specialized cells.

2) SCAFFOLDS

Scaffolds serve as temporary or permanent artificial Extracellular Matrices (ECM) to accommodate cells and support 3D tissue regenerations. ECM is a blend of macromolecules (proteins and carbohydrates) around cells—as space fillers. Scaffold materials are designed to enhance tissue formation through control of the local and surrounding environments. Scaffolds are critical in regulating cellular growth and provide a volume in which vascularization and stem cell differentiation can occur.

Scaffold geometry significantly affects tissue differentiation through physical growth ques. predicting tissue formation computationally requires theories that link physical growth ques to cell differentiation. Current models rely on mechanic regulation theory, widely shaped by Prendergast et al. for predicting cell growth.

Such materials include: Porous ceramic and demineralized bone matrix supports, Coralline cylinders, Biodegradable material such as poly(α-hydroxyl esters), Decellularised tissue matrices, Fibrin, Sponges made from collagen etc,.

Requirements of scaffolds

i. Three-dimensional and highly porous with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste.
ii. Biocompatible and bioresorbable with a controllable degradation and resorption rate to match cell/tissue

growth in vitro and/or in vivo.
iii. Suitable surface chemistry for cell attachment, proliferation, and differentation.
iv. Mechanical properties to match those of the tissues at the site of implantation.

MATERIALS AND METHODS

Initially, focusing on bone growth, subcutaneous pockets were used for bone prefabrication as a simple in vivo bioreactor model. The pocket is an artificially created space between varying levels of subcutaneous fascia. The location provides regenerative ques to the bioreactor implant but does not rely on pre-existing bone tissue as a substrate. Furthermore, these bioreactors may be wrapped with muscle tissue to encourage vascularization and bone growth. Another strategy is through the use of a periosteal flap wrapped around the bioreactor, or the scaffold itself to create an in vivo bioreactor. This strategy utilizes the guided bone regeneration treatment scheme, and is a safe method for bone prefabrication. These 'flap' methods of packing the bioreactor within fascia, or wrapping it in tissue is effective, though somewhat random due to the non-directed vascularization these methods incur.

The axial vascular bundle (AVB) strategy requires that an artery and vein are inserted in an in vitro bioreactor to transport growth factors, cells, and remove waste. This ultimately results in extensive vascularization of the bioreactor space and a vast improvement in growth capability. This vascularization, though effective, is limited by the surface contact that it can achieve between the scaffold and the capillaries filling the bioreactor space. Thus, a combination of the flap and AVB techniques can maximize the growth rate and vascular contact of the bioreactor as suggested by Han and Dai, by inserting a vascular bundle into a scaffold wrapped in either musculature or periosteum.

MATERIALS

Materials used in the construction of an in vivo bioreactor space vary widely depending on the type of substrate, type of tissue, and mechanical demands of said tissue being grown. At its simplest, a bioreactor space will be created between tissue layers through the use of hydrogel injections to create a bioreactor space.Early models used an impermeable silicone shroud to encase a scaffold, though more recent studies have begun 3D printing custom bioreactor molds to further enhance the mechanical growth properties of the bioreactors. The choice of bioreactor chamber material generally requires that it is nontoxic and medical grade, examples include: "silicon, polycarbonate, and acrylic polymer". Recently both Teflon and titanium have been used in the growth of bone, One study utilized Poly methyl methacrylate as a chamber material and 3D printed hollow rectangular blocks. Yet another study pushed the limits of the in vivo bioreactor by proving that the omentum is suitable as a bioreactor space and chamber. Specifically, highly vascularized and functional bladder tissue was grown within the omentum space.

The requirements of In Vivo Bioreactors are

- The concept of "in vivo" bioreactors is inherent in the body self regenerative capacity. However, mixing bone tissue engineering with body's self-regenerative capacity to regenerate new tissue is a relatively new approach in critical size skeletal defects and, despite numerous different techniques and studies in small and large animal models, only a few clinical applications with successful results are reported in medical literature.
- The core of this novel approach is microsurgery with flap prefabrication and other techniques to vascularized artificial bone scaffolds. In vivo bioreactors approach is an emerging strategy for bridging the in vitro gap between experimental successes and clinical translation in managing scaffolds manipulation
- n, seed cells seeding and growth factors delivery for bone defect reconstruction. This principle focuses on using the body itself as a bioreactor, hosting the traditional triad (scaffold, seed cells, and growth factors). Prefabrication of vascularized bio artificial bone grafts in vivo might be an alternative to in vitro tissue engineering techniques.
- Combining tissue engineering approaches with flap prefabrication techniques may allow the application of vascularized bio artificial bone grafts grown in vivo with the advantage of minimal donor site morbidity, if compared with conventional vascularized bone grafts. Prefabrication is a surgical term first introduced by Yao in 1982 and that describes the implantation of a vascular pedicle into a new territory, followed by a neovascularization period and subsequent tissue transfer based on its implanted pedicle.
- Tissue prefabrication is commonly realized in two steps. Initially, the selected tissue, is designed into the required configuration and is then implanted in the convenient body area for the introduction of a vascular pedicle. Subsequently, during the second step, the autologous implant is harvested with the surrounding tissue and the vascular pedicle as a free flap.
- While the flap is connected to the local circulation by means of a microvascular anastomosis, the implant acquires its vascularization from the tissue block.
- Flap are prefabricated basically using two strategies: wrapping the bone graft in axially vascularized tissues (cutaneous, fasciocutaneous or muscle flaps) or implanting a vascular axis into the bone graft itself (intrinsic mode vascularization).
- With the intrinsic mode, the construct acquires naturally a native perfusion, without relying on favorable local environmental conditions. This can be fundamental in case of trauma or tumor.
- The recent induction of intrinsic vascularization techniques for scaffolds maturation allowed

obtaining, for several animal models, vascularized tissues directly transferable as pedicles or free flaps.

- Four different techniques are, at present, the most interesting approaches proposed in microsurgery for vascularizing an artificial tissue: muscular flap prefabrication, periosteal flap prefabrication, axial vascular bundle prefabrication, arteriovenous. loop prefabrication.

a. MUSCULAR FLAP OR POUCH

A perfused muscle is a poor bed for cancellous bone graft but local condition of good perfusion which are likely to be obtained with fascial or muscular flap wrapped around an autologous bone graft could promote bone tissue regeneration.

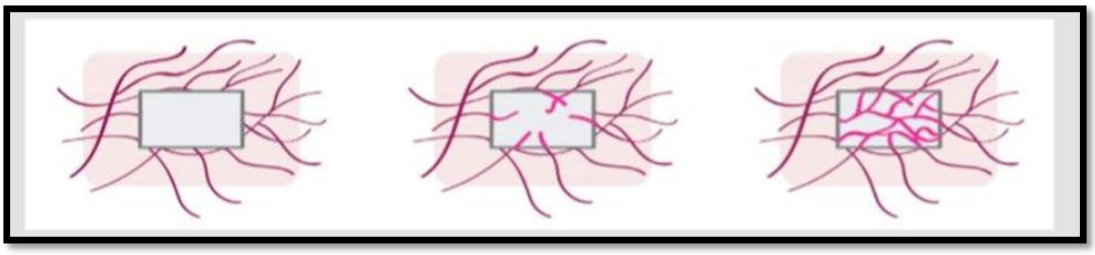

Figure no 8: - Muscular flap or pouch.

Vascularization induced by this method is called "inosculation", in which blood vessel already present in the graft rapidly connect with the vascularized muscular flap. The preformed In the clinical setting, a large mandibular defect was successfully reconstructed using custom made titanium cage, realized according to CT scan followed by 3D reconstruction and filled with bone marrow aspirate, xenogeneic bone minerals and OP-1, after a prefabrication period of 7 weeks.

b. PERIOSTEAL FLAP

In the periosteal flap strategy, a periosteal flap is used to wrap the tissue-engineered construct or to cover the chamber containing the tissue-engineered construct. Therefore, the periosteum-construct combination forms an in vivo bioreactor capable of providing pluripotent cells and molecular signals, actively stimulating the bone formation process. This results in a wound-in by healing response within the space, leading to new bone formation, micro-vessels simply must develop interconnections to the host microvasculature to get fully blood perfused within a short period of time.

Although inosculation of preformed micro-vessels is a very promising strategy in tissue engineering, some studies have shown that adequate blood perfusion of artificial bone graft is not guaranteed during the very first days after implantation of the tissue construct and much of the inosculation process has to rely on favorable local conditions. Bone formation beneath "standard muscular flaps" has been successfully induced using bio ceramics scaffolds seeded with autologous bone marrow stromal cells. Prefabricated vascularized bone grafts have also been tested for jaw reconstruction with a thorough in vivo evaluation in a pig model instead of fibrotic scar.

c. AXIAL VASCULAR BUNDLE (AVB)

The Axial Vascular Bundle strategy unlike the random blood vessel pattern in the subcutaneous pockets and tissue flaps, is an inherent model of axial osteogenesis and vascularization for prefabrication of bone grafts. In this methodology, an artery and a vein are inserted centrally inside a scaffold and are supposed to transport progenitor/stem cells, cytokines, oxygen, and nutrients and to remove waste products. Therefore, an extensive vascularization and osteogenesis of the scaffold should be obtained.

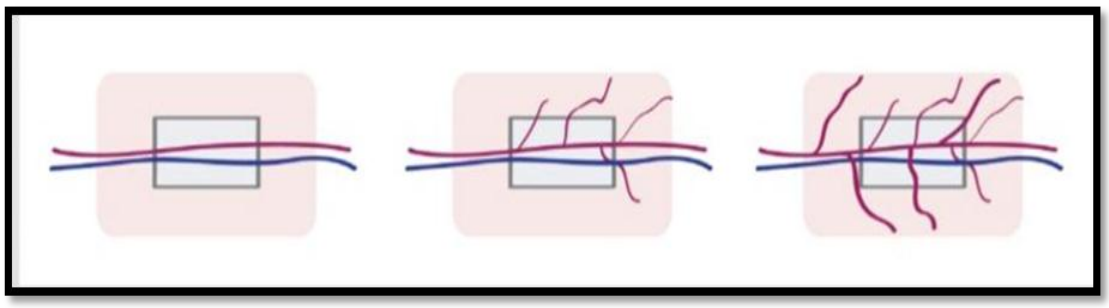

Figure no 9: Axial Vascular Bundle.

The main limitation of the AVB model is that it can provide only a small contact surface within the scaffold and, for this reason, it is difficult to provide a short pre-fabrication period. As an alternative approach, a hybrid solution, like the envelopment of the scaffold with a muscular flap or a periosteal flap, is possible. The

combined use of the AVB and tissue flap to form an IVB is advantageous, due to the utilization of two well-established bone graft prefabrication strategies, which makes it the most frequently applied model in small and large animal studies.

d. ARTERIOVENOUS LOOP (AV LOOP)

Erol and Spira in 1980 developed in a rat an arterio-venous loop model interposing a venous graft between the femoral artery and vein in the thigh, to create a prefabricated full thickness skin graft. with this they also described the first AV-loop model.

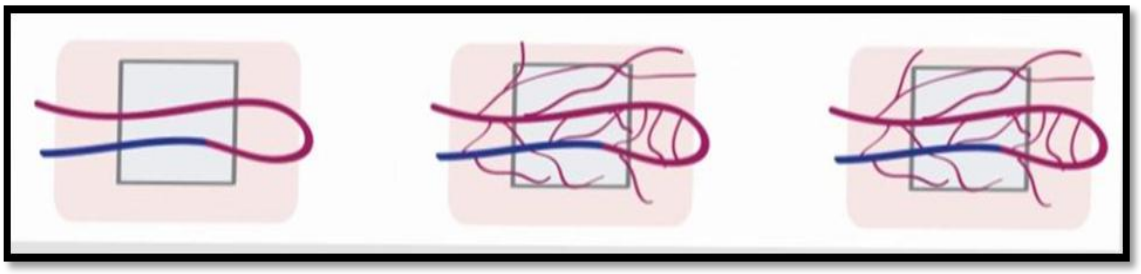

Figure no 10: Arteriovenous fistula.

In principle, three mechanisms are considered responsible for the accelerated angiogenesis: the local inflammation due to the surgical trauma on the vessels which bundle as an in vivo bioreactor in the skeletal defect site. In these cases, arteriovenous loop can be a good solution for bone graft prefabrication. Normally, an arteriovenous loop is realized by a direct microsurgical anastomosis of an artery and a vein or by the interposition of a venous graft between an artery and a vein, to form an arteriovenous fistula (Figure 10)

Applications, recent developments and future aspects

Some applications of tissue cloning are discussed below

- The patient's immune system will not reject them since the nucleus of the somatic cell was for the patient.
- Also, there will be no need for immunosuppressant's to prevent rejection because the cells will match the immune system.
- Therapeutic cloning through somatic cell nuclear transfer enables the development of specialized cells that could be used in tissue replacement and organ transplants.
- In the USA, every day 3,000 people die due to diseases that could be treated by using therapeutic cloning.

"In 2002, cardiovascular disease (CVD) claimed roughly as many lives as cancer, Chronic lower respiratory diseases, accidents, diabetes mellitus, influenza, and Pneumonia combined."

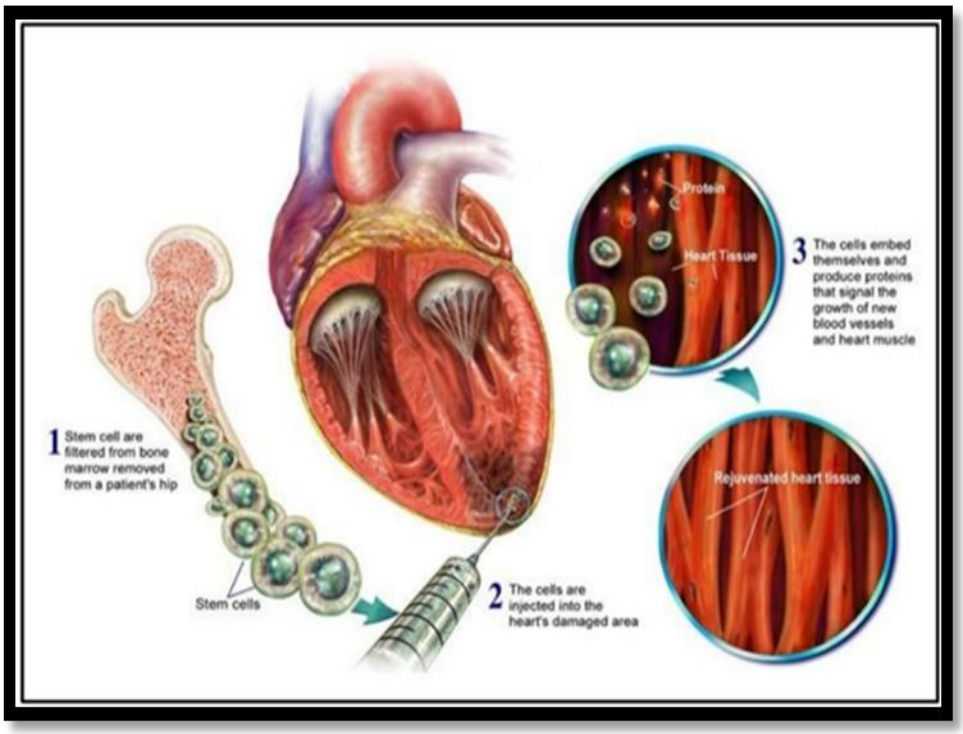

Figure no 11:- Steps in improving cardiac health by using stem cells.

There are two ways to improve cardiac Health by using stem cells that have been Found by researchers. First, grow the stem Cells in a culture dish to develop into the Heart muscle. Discover new drugs by using The stem cell-derived heart muscle of Patients who suffer from inherited cardiac Conditions in experiments. Second, Damaged heart tissue can be replaced by stem cells.

- Cloning is most likely to serve as a new unusual but perhaps efficacious treatment for infertility or sought by couples, who because of a high risk of genetic disease or other factors cannot or do not wish to conceive a child.
- Researchers believe that stem cells have a potential to serve as replacement cells in treatment of degenerative diseases such as Alzheimer's disease, Parkinson's disease, spinal cord damage, diabetes, cardiovascular disorders, cancer research and therapy etc.
- Cloning technology can also be used to generate tissues and organs for transplants. The cloned tissue or organ is a genetic match to the recipient thus risk of tissue rejection would be eliminated.
- This technology might be helpful in preservation and repopulation of the endangered species and genetic improvements etc.

There are some uses of Invivo bioreactor

- The in vivo bioreactor strategy has been applied for the synthesis ofbone to repair musculoskeletal defects. For example, Stevens et al. created an injectable hydrogel capable of functioning as a bioreactor supporting new bone growth when injected underneath the periosteum in a rabbit model.
- The in vivo approach permits to implant a tissue ready to his function; provides a tissue with a vascular network through microsurgery techniques; allows to implant a bone graft that doesn't undergo creeping substitution; permits the reconstructing of tissue loss in one procedure.
- An example of the implementation of the IVB approach was in the engineering of autologous bone by injecting calcium alginate in a sub-periosteal location.

RECENT DEVELOPMENT

Skeletal Muscle Bioreactors

Skeletal muscle TE is a promising interdisciplinary field which aims at the reconstruction of skeletal muscle loss. Tissue-engineered muscle constructs require an adequate connection to the vascular system for efficient transport of oxygen, carbon dioxide, nutrients and waste products.

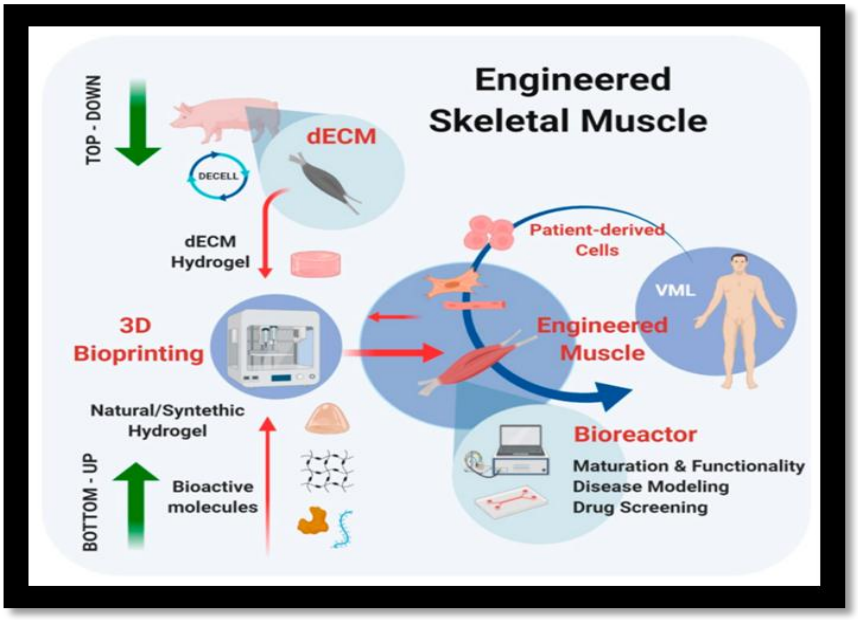

Figure no 12: Engineered skeletal muscle.

Moreover, functional and clinically applicable muscle constructs depend on adequate neuromuscular junctions with neural cells. In order to engineer muscle tissue successfully, it may be beneficial to mimic the in vivo environment of muscle through association with adequate stimuli from bioreactors. A very recent study has shown the electrical excitability of different muscle cells, from adult and neonatal, to primary and cultured cell lines as well as adult denervated cells.These studies have shown that adult myocytes are the most exciteable, but that denervated cells, while originally having a level of exciteability close to that of 4 day neonatal myocytes, regain excitability after electrical stimulation.

VASCULAR BIOREACTORS

It is estimated that in 2005, 17.5 million deaths were due to cardiovascular disease. Due to the large incidence of cardiovascular disease in the United States alone, the amount of research being employed to prevent, treat, and cure cardiovascular disease is predominantly large. This is true also of the amount of research working towards engineering constructs to replace cardiovascular tissue.

Because of this, our review breaks the cardiovascular research into two areas cardiac and vascular-in order to review this research in more detail.

HEART VALVE

The field of vascular tissue engineering mainly involves the engineering of constructs for the replacement of heart valves or vasculature. It is estimated that every year, more than 100,000 US patients need their dysfunctional or diseased valves replaced with a prosthetic or replacement valve. Because of this, there is a large market for the development of suitable replacements. The development, implementations, and success of various strategies for heart valve replacement have previously been reviewed in great detail. Here however, we explore those methods that explicitly involve the use of some bioreactor for the preconditioning or fabrication for said heart valve replacements.

The perfusion bioreactor is the most common bioreactor employed in the field of heart valve engineering, as it provides easy loading and preconditioning of the valve in physiological conditions. It also allows the researchers to test the valve's efficacy under a variety of conditions, such as hypo and hypertension, prior to implantation.To date, many different types of perfusion bioreactors have been designed for this purpose. While most of the studies claim these bioreactors to be “special” or “novel”, all are slightly different designs to the classic perfusion pump bioreactor. One of these bioreactors, for example, was designed for real time measuring of the heart valve's compliance during perfusion

VASCULATURE

Due to the almost identical physiological conditions that both vasculature and heart valves are exposed to, research pertaining to these two fields is somewhat similar. Like replacement heart valve constructs, replacement vascular constructs need to bypass the issues of thrombosis and intimate hyperplasia, as well as have appropriate mechanical properties to avoid rupture, failure, and mechanical mismatch at the site of implantation. Because of these similarities, the research that has arisen involving bioreactors for the preconditioning of vascular grafts mainly involves either mechanical stretching bioreactors and perfusion bioreactors, as with heart valve research.

Unlike those bioreactors employed for heart valve research however, much of the bioreactors employed for mechanical stretching of tissue engineered vasculature include some sort of perfusion mechanism which in turn distended the constructs, providing the mechanical stretching as well as all of the advantages of regular perfusion bioreactors. Some of these studies designed their bioreactors to cater to the perfusion of small vessel constructs.

Of these, one study was equipped to provide real-time data on the distension of the vessel constructs using a built-in LED system, while another seeded Endothelial Cells through perfusion, but only 13 days after the construct had been seeded with Smooth Muscle Cells, providing a layered construct similar to blood vessels Of the bioreactors designed for the larger constructs, one is worth noting in that it also incorporates rotation to minimize the effect of gravity on the cell growth and matrix deposition of the construct. This additional feature ensured a more uniform distribution of cells and matrix throughout the construct.

CARDIAC BIOREACTORS

The main focus of CTE is to ameliorate the complications that arise following myocardial infarction (MI). The first endeavors regarding CTE were made using direct cell transplantation at the infarcted site. Several cell candidates have been studied for this purpose, and each of them has shown a particular set of advantages and disadvantages. For example, skeletal myoblasts (SMs) and mesenchymal stem cells (MSCs) are the two most widely studied cell types.

Nevertheless, a suitable scaffolding that allows cells to attach and reside in the targeting tissue greatly limits the success of this approach.This technique is probably best suited for simple tissues with smalfigure

The field of cardiac tissue engineering is one of the faster-growing fields in tissue engineering due to the preva- lence of cardiovascular disease around the world. This extensive field and the strides that have been made in it have been previously reviewed. Due the abilities of cardiac muscle to become excited through both electrical and mechanical stimulation, similar to skeletal muscle, the bioreactors that have arisen for the stimulation

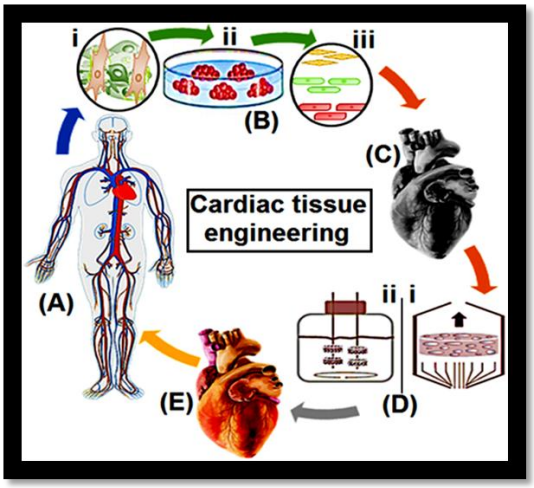

Figure no 13: Cardiac tissue engineering.

BONE BIOREACTORS

The discipline of bone TE involves the combined use of osteoconductive matrices, bone-forming cells, and osteogenic growth factors. the tissue constructs need to be maintained in a suitable cultivation environment. Osteoblasts have been widely used for generating

mineralized cell/scaffold constructs in Mesenchymal stromal cells (MSCs) represent a proliferating and undifferentiated cell source. MSCs are mostly isolated from bone marrow aspirates, but can also be obtained from other tissues, for example, adipose tissue or cord blood.MSCs have the potential to differentiate toward diverse mesenchymal lineages, including osteoblasts, chondrocytes, adipocytes, and myocytes.

The field of bone tissue is similar to other fields in tissue engineering in that it involves the in vitro expansion of cells on a scaffold followed by implantation into the bone. The use of bioreactors in this field is varied.

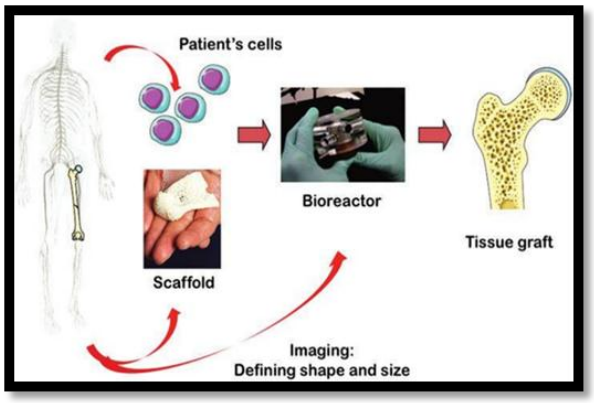

Figure no 14: - bone reactors.

Cartilage Bioreactors

The regeneration of articular cartilage (AC) is one of the challenges in regenerative medicine due to its poor regenerative capacity. Natural AC is a complex hierarchical structure that is avascular with four layers: the surface zone, middle zone, deep zone, and calcified zone. These zones have different biochemical compositions, chondrocyte phenotypes, and physiological characteristics tied directly to the effects of mechanical loading and the physiological environment. The current clinical treatment strategies mainly include arthroscopic debridement, microfracture, and autogenous osteochondral transplantation, which can promote tissue recovery to some extent, but the quality and long-term repair effect of regenerated tissue are not satisfactory Adult cartilage has a limited healing capacity. Damages resulting from disease or injury increase over time and cause severe pain Currently there is no surgical procedure available to treat large and deep cartilage defects associated with advanced diseases such as osteoarthritis. For this reason cartilage tissue bioreactors serve as a promising means for the successful regeneration of damaged or diseased cartilage. Studies on cyclic compression bioreactors have shown that they can be used for the purpose of cell proliferation for chondrogenic differentiation. Our group studied the effects of cyclic compression on these very properties.

Neural Bioreactors

He field of Neural TE is different to that of other TE fields in that the complexity and delicacy of the neural connections of the central and peripheral nervous systems (CNS, PNS) make constructs and transplant of those constructs a bigger challenge. The application of bioreactors to this field reflects this as well. While bioreactors in other TE fields stimulate and preload constructs, neural TE bioreactors focus mainly on the culturing and proliferation of Neural Stem Cells (NSCs) and Neural Progenitors (NPs) to produce enough cells to be clinically applicable and relevant.

The culture of NSCs and NPs has been developed for quite some time, and it is now known that they grow best in 3D cultures. When grown in suspension, both cell types have a tendency to aggregate into large cell bodies known as neural spheres, or aerosphere. Bioreactors in the field are used to control the growth, proliferation, size, and phenotype of these neurospheres, and are mostly either spinner-flask bioreactors-also known as suspension bioreactors in this field-or rotating wall vessel (RWV) bioreactors.

FUTURE PERSPECTIVE

Based on the understanding of core elements of bone regeneration, basic principle of in vivo bioreactor strategy and anatomy characteristics of periosteum, we hypothesize that large volume and functional bone tissues may be eventually generated in vivo only using costal periosteum as an in vivo bioreactor without participation of any exogenous elements. Rib and costal cartilage is a widely-used donor site of bone and cartilage tissue for plastic and reconstructive surgery.

Current techniques for rib or costal cartilage harvest can minimize the surgical scar and do not cause severe functional problems. The protocol to test this hypothesis is to dissect a costal periosteal flap and create an enclosed space with the help of a special device. This artificial space under the cambium layer of costal periosteum will serve as an in vivo bioreactor and reimplanted in situ to promote vascularization and osteogenesis.

After a period of in vivo cultivation, large volume of functional bone tissues will be observed in this space and can be transferred as a pedicled bone flap. This novel strategy will demonstrate the possibility of bone regeneration by harnessing the body's self-regenerative capacity and will likely open new options for reconstructing large bone defects and facilitate clinical translation if the hypothesis proves to be practical As further research and clinical experiences are gained within bone regeneration following the in vivo bioreactor principle, the potential for regenerative techniques to functionally replace vascularized and composed tissues will be transformative to clinical conditions. Such future work will lead to better options for regeneration of complex tissues, even whole organs.

Despite this, further investigation is needed to elucidate the specific biochemical and biomechanical factors

required for the development of cells, tissues, or organs. Results and parameters of theoretical research are indispensable for the design of bioreactors, which is beneficial to fully understand the regulatory mechanisms of cartilage growth and differentiation in order to produce successfully engineered tissues with the best characteristics. Most importantly, the basic mechanical biology should also be explored to enhance this achievement. In addition, there are also some new ideas for bioreactors, such as the proposal of the in vivo bioreactor, which provides a promising approach to provide in vivo conditions for cartilage engineering.

Finally, the combination of tissue engineering and advanced technologies is not only the development trend of tissue engineering but also the development direction of bioreactors. We believe that the application of bioreactors in cartilage tissue engineering, especially the customized construction of in vitro engineered cartilage, will play a significant role in the personalized treatment and prognosis of clinical cartilage damage repair in the future.

CONCLUSION

Over the past decade, significant improvements in design and construction of bioreactors have been made. Systems have been developed that allow robust and reproducible culture conditions to be maintained. Specific bioreactor design is critical to the production of useful systems that can predict performance if based on a natural cell niche from in vivo physiology. Whilst the more sophisticated the bioreactor approach, the more likely it is to reflect the natural physiological state, simpler designs are likely to be more operationally robust, so a compromise based on bioreactor complexity versus the essential functional parameters of the desired end-product will always be necessary.

The combining between therapeutic cloning and Tissue engineering can lead to enhance the developing of tissues and organs that match. The patient's immune system. Furthermore, researchers keep trying to treat heart Attacks by cellular therapies using one of the next improving stem cell patches, combining different types of stem cells, or repeating transplantations. Clinical trials Will begin around 2020 using these improved methods. A human is composed of organs and tissues. But a human being Is not equivalent to an organ, and similarly an organ is not Equivalent to tissue cells.

Human cloning, organ cloning and Tissue cloning are various types of cloning that occur at three Different levels. They are based on three distinctive Concepts according to different aims, methodologies, developing Courses, and results, thus they should remain differentiated. Of These three types of cloning, non-germinal tissue cloning and organ cloning are beyond the ethical question, and should be Advocated and further encouraged. Using cloned tissues to make. New tissues or organs is promising for the future of medicine.

On the other hand, "in vitro" bioreactors designed for bone tissue engineering, in some of their interpretation seem to suffer of excessive simplification, reducing to standard perfusion enhancement of 3D constructs, without introducing controllable mechanical stimulation and allowing adequate constructs evolution monitoring. In some others, because of their complexity, for their optimal setup they require specialized operators, limiting their wide diffusion. In all the cases they still have not been yet capable to resolve the problem of vascularization. Until now, only muscular pouch prefabrication has emerged as the only "in vivo" bioreactors strategy that has been applied with success in clinical setting.

It was of significance to have found new "in vitro" techniques adapted to the muscular pouch prefabrication process for increasing the artificial bone graft vascularization. Microsurgery through the concept of "in vivo bioreactors" needs in vitro techniques to find a solution for critical size skeletal defects. The union between clinician and scientist point of view is fundamental in the difficult process of transforming engineered bone tissue into limitless vascularized bone graft.

In the present literature, it was hard to find a single winning approach to bone reconstruction: in most cases different strategies were applied together to create engineered bone tissue, as evidenced both in small and large animal studies. Even if the body antigenicity capacity, emblematic in pathologic heterotopic bone formation, it is well known; the new bone formation needs artificial scaffolds and growth factors in the case of critical size defects. Considering that neither scientists neither the microsurgeons could solve alone the problem of critical size bone reconstruction in trauma, the most promising approach is probably integration of the different approaches.

The route to a vascularized bone graft could, as a principle, starts from the "in lab" realization of a cell constructs with osteocyte phenotype expressed and bone synthesis genes activated taking full advantage of the in vitro bioreactor approach. Only at this time, the alive, but non-vascularized cell construct could undergo to "in vivo" bioreactor maturation for vascularization development. Such a multidisciplinary approach would inevitably imply the integration of knowledge and side-by-side collaboration of clinicians, engineers, biologists and physicists, the only key for a successful ending.

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