International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

The Key Implications Toward the Future of Regenerative Medicine

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Abstract
For a long time, stem cell therapy has been utilized as a promising treatment for a variety of conditions, most notably those brought on by genetic disorders. A new generation of stem cells, known as mesenchymal stem/stromal cells (MSCs), and cutting-edge regenerative medicine and therapies technologies, like cellular immune therapy, have been made possible by the application of stem cells, tissue engineering, and gene editing. This review article provides a concise overview of the various types of stem cells, such as adult, embryonic, and pluripotent stem cells, in addition to discussing tissue engineering's methods and latest developments. The science of tissue engineering, gene editing, and upcoming prospects for regenerative medicine are the main topics of the review.

Keywords: Cell Therapy, Tissue engineering, Gene Editing, Cellular Immunotherapy

1.1 Introduction

One special quality of stem cells is their capacity to differentiate into a wide variety of cell lineages. Stem cells function as a tissue builder and stand-in for fully formed tissues. By restoring damaged adult tissues, adult progenitor cells and stem cells are essential for supporting the body's natural healing process. On the other hand, during the course of development, embryonic stem cells have the amazing capacity to differentiate into a variety of specialized cell types.

Within tissue engineering and molecular biology, the field of regenerative medicine focuses on the replacement, alteration, or regeneration of human cells, tissues, or organs. Restoring or returning these biological components to their regular functioning state is its main goal. This area demonstrates the ability to speed up the healing process of previously irreversible organs, hence promoting the regeneration of damaged tissues and organs inside the human body. [1]

For potential applications in osteochondral regeneration, bone tissue engineering, and myocardial tissue engineering, both the development of biomimetic scaffolds that mimic the structural and functional characteristics of native tissue and the potential of stem cells to enhance tissue regeneration have been successfully investigated [2,3]

Typically, these techniques rely on combinations of cells, genes, morphogens, or other biological building blocks with bioengineered materials and technologies to address tissue or organ.[4]

However, recent remarkable advancements in fundamental science have gradually shown that, under the right conditions, an injured spinal cord can recover. [5]

1.2 IMPORTANCE OF REGENRATIVE MEDICINE

People who had previously had limited access to therapeutic approaches now have hope due to recent developments in the field of stem cell research.

The importance of multipotent mesenchymal stem cells (MSCs) and human pluripotent stem cells (hPSCs) in the field of regenerative medicine has increased noticeably in recent years1. Many conditions, including but not limited to neurological illnesses, respiratory problems, metabolic and endocrine disorders, reproductive abnormalities, skin burns, and cardiovascular problems, may be helped by these treatments**.** [6] In brief, it can benefit those suffering from a range of ailments, such as aging, illness, damage, defects, chronic diseases, injuries, and congenital anomalies.

1.3 Scope and objectives

- By Regenerative therapies based on adult stem cells have demonstrated clinical benefits in the treatment of burn wounds, retinal degeneration, and hematological malignancies. Due to these To fix monogenic postsynaptic proteins, gene editing is also a viable therapeutic approach for these proteins. [8]
- Using cells as delivery systems: Genes and cytokines, among other therapeutic substances, can be delivered to cells through regenerative medicine.
- Stem cells are now used in medicine, with a focus on new techniques including chemical manipulation, CRISPR-Cas9 gene editing, and 3D bioprinting, and a discussion of immunological rejection, tumorigenicity, and ethical concerns.
- It offers a comprehensive analysis of the various types of stem cells, examining their genesis, traits, and ability to differentiate. [10]
- Substitution of organs or tissues: Age, illness, trauma, or congenital conditions can all cause damage to organs or tissues that can be repaired by regenerative medicine**.**

1.4 HISTORY OF REGENRATIVE MEDICINE

For thousands of years, the scientific community has been intrigued by regeneration, albeit not always referring to it by that name. Although not fully understood, records from the eighth century BC indicate to the recognition of the principle of regeneration. This is how Greek mythology portrays it. The Titan deity Prometheus's anguish is told in the legends of Homer and Hesiod. Prometheus was exiled to the Carpathian Mountains after defying Zeus by taking fire from Olympus to benefit humanity.

The eagle Ethos visited him every night to pick at his liver while he was shackled to a rock and subjected to 30,000 years of misery. Doomed to immortality, his liver would regrow every day, only to meet the same end that night. Having named the liver ἦπαρ (hepar) after the verb ἠπάομαι, which literally translates to "to repair oneself," the Ancient Greeks were obviously aware of the liver's capacity for regeneration. As of right now, we understand that the liver is the only organ in the body capable of healing itself on its own following damage.[12]

From earlier practices such as surgery, surgical implants (artificial hips), and increasingly complex bio-material scaffolds (skin grafts), regenerative medicine has grown. Cell therapy was the initial research that really helped regenerative medicine become a real field of study. Some of the first therapeutic procedures

in medicine were the result of work done in the field of transplantation in the mid-1950s. The first kidney transplant was done in 1954 on identical twins. The first liver and lung transplants were done in 1963, the first pancreatic transplant was done in 1966, and the first heart transplant was done in 1967.
Bone marrow transplants for leukemia patients have caused great excitement in both the scientific and general public populations. Following this surge of excitement, cell biologists started to doubt the ability of the transplanted tissues to maintain their integrity and started to wonder if it would be feasible to produce, cultivate, and harvest these tissues in the lab. This marked the start of the tissue engineering period, which eventually led to the development of regenerative medicine. [13]

1.5 THE REGENERATIVE POTENTIAL OF CELLS

Regenerative medicine may find inspiration from natural biological processes. Stem and progenitor cells produce specialized progeny during embryogenesis and postnatal growth that construct and sustain tissues, including ordered multicellular structures and the extracellular matrix that surrounds them. Coordinated cellular activities also control normal wound healing. Platelets and neutrophils are the first to respond to damage; they gather at the wound site and produce growth factors and chemoattractants, which cause macrophages, lymphocytes, fibroblasts, and endothelial cells to be drawn in.
These cells eliminate injured tissue and cover the wound with granulation tissue rich in collagen, which progressively transforms into an extracellular matrix with vascularization**.** [14]
These natural examples give us ideas about how to fix damaged tissues and justify treatments that aim to replace damaged cells or alter the host environment to encourage endogenous regeneration.
Hematopoietic stem cells, neural stem cells, and mesenchymal stem cells are a few types of cells that possess multipotency, or the ability to differentiate into a limited number of distinct cell lines.[15] Using stem cells with pluripotency—the capacity to differentiate into any of the three germ layers' cells—is a popular substitute (endoderm, mesoderm, and ectoderm). Although embryonic stem cells (ESCs) are naturally pluripotent, their allogeneic and morally dubious source limits the applications of these cells. In the meantime, autologous somatic cells, such as fibroblasts [16] or other prevalent cell types, can be reprogrammed to produce induced pluripotent stem cells (iPSCs). [17]

1.6 Principles of regenerative medicine

The technology and advancements used in recent years to approaches to tissue regeneration and healing. The scientific and molecular underpinnings of regenerative medicine are covered in contributions from an amazing group of researchers, with a focus on stem cells, wound healing, and cell and tissue development. To provide a glimpse into the future and a framework for further research, advances in cell and tissue therapy are also included. These include the replacement of damaged tissues and organs caused by disease and previously incurable disorders like diabetes, heart disease, liver disease, and renal failure**.** [18]

1.7 Cell Therapy (Stem cells)

Stem cells are used in stem-cell therapy to treat or prevent certain illnesses. [19] Hematopoietic stem cell transplantation is the sole FDA-approved stem cell therapy as of 2024.[20, 21] Usually, this is done by transplanting bone marrow or peripheral blood stem cells, however umbilical cord blood can also provide the cells. Research is being done to create new stem cell sources and to use stem cells as treatments for disorders like diabetes and heart disease, as well as neurodegenerative diseases. [22] Since scientists have been able to identify and cultivate embryonic stem cells, generate stem cells using somatic cell nuclear

transfer, and produce induced pluripotent stem cells, stem-cell treatment has gained controversy. This debate frequently touches on issues of human cloning and abortion politics. Furthermore, there has been controversy around attempts to commercialize therapies based on the transplant of preserved umbilical cord blood. [23]

Hematopoietic stem cell transplantation (HSCT), the only extensively utilized type of stem-cell treatment, has been used to treat patients with diseases including leukemia and lymphoma for more than 90 years. [24,25]

The majority of growing cells are destroyed by the cytotoxic chemicals during chemotherapy. However, these substances are unable to distinguish between the bone marrow's hematopoietic stem cells and leukemia or cancerous cells. The goal of a stem-cell transplant is to counteract this side effect of traditional chemotherapy methods by replacing the cells lost in the host's body during treatment with functional stem cells from a donor's healthy bone marrow. The most dangerous adverse effect of this treatment, graft versus host disease, can result from the transplanted cells' induction of an immune response that aids in the destruction of the cancerous cells. [26]

In 2012, Prococvhymal, an additional stem-cell treatment, received provisional approval in Canada to treat acute graft-versus-host disease in children who do not respond to steroids.
[27]

Based on mesenchymal stem cells (MSCs) obtained from adult donors' bone marrow, it is an allogenic stem treatment. MSCs are isolated from bone marrow, grown, and packed; a single donor can yield up to 10,000 doses of MSCs. Until they are needed, the doses are kept frozen.[28]

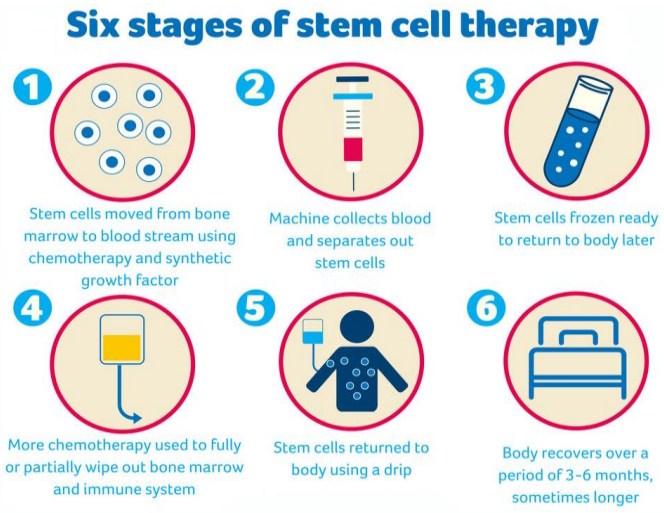

Figure: -1 This figure shows the stages of stem cell therapy. [7]

1.7.1 Human stem cell sources

The majority of stem cells used in regenerative medicine are typically separated from either adipose tissue or the patient's bone marrow. [29,30]

In addition to muscle, neural, and other progenitor tissues, mesenchymal stem cells can differentiate into the cells that make up bone, cartilage, tendons, and ligaments. They are the primary stem cell type that has been researched for the therapy of illnesses affecting various tissues. [31,32]

Researchers are looking at new sources of mesenchymal stem cells, such as stem cells found in the epidermis and dermis, which are interesting since they can be easily extracted from an animal with little

danger to them. [33]

It has also been found that hematopoietic stem cells, which may be harvested in a highly non-invasive manner, are circulating in the bloodstream and have the same capacity to differentiate as other mesenchymal stem cells. [34]

1.7.2 Types of Stem Cells

- Embryonic stem cells (ESCs)
- Adult stem cells (ASCs)
- Induced pluripotent stem cells (iPSCs)

1.7.2.1 Embryonic stem cells (ESCs)

The inner cell mass of the blastocyst is where pluripotent embryonic stem cells (ESCs) are extracted. The cells' capacity to develop into cells of the three germ layers gives them potential applications in tissue engineering and regenerative medicine. Recent research has demonstrated that embryonic stem cells (ESCs) are capable of controlled differentiation, which results in the creation of numerous cell lineages, including neurones. Based on the research conducted by Maassen and colleagues, [35] Research has demonstrated that under specific in vitro conditions, stem cells produced from Wharton's Jelly, a gelatinous substance found in the umbilical cord, can develop into fully functioning neurones.

1.7.2.2 Adult stem cells (ASCs)

Adult stem cells are also known as mesenchymal stem/stromal cells (MSCs), and they can be taken from several bodily locations. Stem cells produced from Adipose Stem Cells (ASCs) are frequently obtained from adipose tissue. These cells are easily accessible and widely dispersed. Although there are numerous techniques for eliminating these cells, the most popular ones include the digestion and explanation of collagenase. Stage and the research team assessed the capacity of equine ASCs obtained from various adipose tissues to differentiate into many lineages using these techniques. The process of tissue localisation and separation has a major influence on ASCs' capacity to undergo adipogenic and osteogenic differentiation. [119]

1.7.2.3 Induced pluripotent stem cells (iPSCs)

The capacity of induced pluripotent stem cells (iPSCs) to self-renew and differentiate into a variety of cell types is one of their defining characteristics. A great deal of attention has been paid to the process of creating induced pluripotent stem cells, or iPSCs, especially in the field of regenerative biology. Pluripotent stem cells can be induced to reprogramme adult differentiated cells using a variety of transcription factors. Through genetic modification and specialisation into certain cell types, this technique provides a pool of pluripotent cells that may be used for a variety of therapeutic applications. Numerous publications, most notably this one, have highlighted the importance of therapeutic research employing CRISPR/Cas9 gene editing technologies and induced pluripotent stem cells (iPSCs).[36]

1.7.3 Ethics-related issues and disputes:

Due to the fact that embryonic stem cells (ESCs) are derived from human embryos, their use in scientific research and medical therapy has generated significant ethical debate. Nevertheless, there are moral questions with the possible loss of embryos while using these cells for medicinal purposes. Numerous sources of pluripotent stem cells (PSCs) have been studied as potential remedies for these ethical issues. These sources include adult mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs). Very Small Embryonic-Like Stem Cells (VSELs), a unique population of adult stem cells with the capacity

to differentiate into cardiomyocytes, have been demonstrated to exist by Sun and colleagues [37]
It has been shown that ASCs can differentiate into a wide range of unique cell types. Horse ASCs, for example, have demonstrated exceptional capacity for chondrogenic, osteogenic, and adipogenic growth. [38] Stage's group did, however, show that they were resistant to a few inducers of cardiomyogenic differentiation. It was demonstrated in a different study that hPSCs may develop into MSCs with the ability to treat. These cells are characterised by reduced cellular senescence, enhanced cytokine release, effective tri-lineage differentiation, and higher proliferative potential. [39]
The main advantage of iPSCs is patient-specificity, which reduces the risk of graft-versus-host disease following transplant.
However, because of their immunogenic and tumorigenic qualities, questions have been raised about their safety following transplantation. The prospective application of iPSCs as a promising cancer vaccine in cancer immunotherapy, while acknowledging the drawbacks of their effective use, such as their propensity to develop tumours. [40] Martin draws attention to additional issues, such as the low success rate and insufficient functional integration of transplanted cells. [41]

1.7.4 Present Stem Cell Therapy Uses

Transplantation of haematopoietic stem cells (HSCT)

- Treatment for cancers and blood problems [42]
  Transplanting organs and tissue engineering
- Regeneration of the skin and bones [43]
  Neurological disorders
- Treatment for Alzheimer's and Parkinson's diseases [44]
  Immunomodulation and autoimmune disorders
- Treatments for Type 1 diabetes and multiple sclerosis [45]

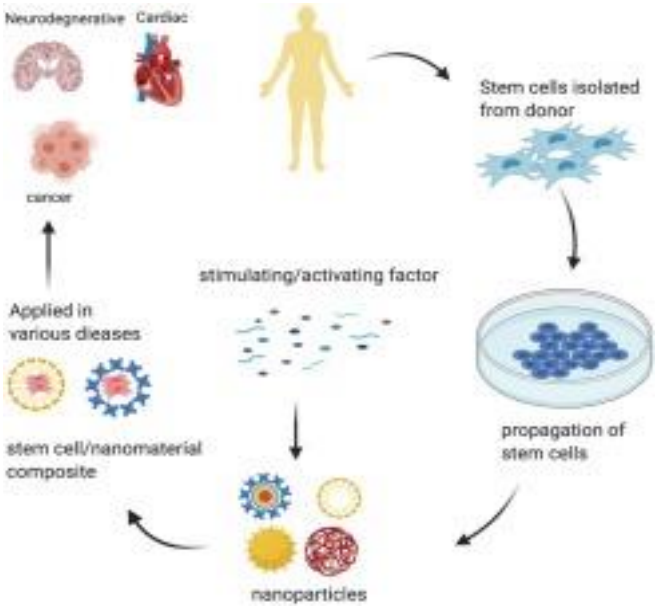

Figure: - 2 Currently, stem cell nanotechnology is one of the novel and exciting fields. Certain experimental studies conducted on the interaction of stem cells with nanostructures or nanomaterials have made significant progress. The significance of nanostructures, nanotechnology, and nanomaterials in the development of stem cell-based therapies for degenerative diseases and injuries has been well established.

Specifically, the structure and properties of nanomaterials affecting the propagation and differentiation of stem cells have become a new interdisciplinary frontier in material science and regeneration medicines. In the current review, we highlight the recent major progress in this field, explore the application prospects, and discuss the issues, approaches, and challenges, to improve the applications of nanotechnology in the research and development of stem cells.[9]

17.5 Development of stem cells

Large amounts of superior stem cells are required for research or therapeutic uses. Therefore, techniques for cultivating pure populations of tissue-specific stem cells in vitro without sacrificing stem-cell potential must be developed. For this, two- and three-dimensional cell culturing are the two main methods used. [46]

1.7.5.1 Two-dimensional cell culture

For the past forty years, two-dimensional cell culture has been conducted on a regular basis in thousands of laboratories across the globe. Cells are usually exposed to a liquid at the apical surface of two-dimensional platforms, and to a solid, inflexible flat surface on the basal side. The surviving cells must drastically adjust to live on such a two-dimensional stiff substrate since they lack the extracellular matrix specific to each type of cell, which can change cell metabolism and diminish functionality. [46]

1.7.5.2 Three-dimensional cell culture

Three-dimensional cell culture systems have the potential to provide stem cells with a microenvironment that mimics their original three-dimensional extracellular matrix (ECM), a process known as biomimicking. In recent decades, three-dimensional cell culture systems have benefited greatly from advanced biomaterials, and more intricate and novel biomaterials have been suggested to enhance stem-cell proliferation and regulated differentiation. Among these, nanostructured biomaterials are particularly intriguing due to their high surface-to-volume ratio and ability to replicate the biological and physical characteristics of natural extracellular matrix at the nanoscale. [46]

1.8 Tissue engineering

In order to create tissue-like structures, the multidisciplinary discipline of tissue engineering combines biomaterials, cells, biochemical (such as growth factors) and physical (such as mechanical loading) signals. [47]

The aim of tissue engineering is to develop biological replacements that can preserve, repair, or enhance the functionality of injured tissues. [48]

The phrase "tissue engineering" was not used until 1987, despite the fact that the first tissue-engineered skin products were produced in the late 1970s and early 1980s, giving rise to modern tissue engineering. [49]

In real life, prosthetics—such as gold for replacing teeth and wood for limbs and toes—were used as early as by the ancient Egyptians. Though they were far removed from the original tissue, these treatments were all based on inanimate materials that offered some shape and function. Around the middle of the 20th century, advances in medicine made it possible to replace a whole organ with an organ from a donor; this procedure is now known as organ transplantation. [50]

The total number of accessible donated organs is always less than the demand for organs, despite the fact that this is a commonly used procedure that is recognized as the best treatment for organ failure. [51] The success of skin graft tissue engineering increased interest in using related ideas to other tissues and organs.

[52]

1.8.1 Techniques for Tissue Engineering

Utilising the three components of tissue engineering requires a skilful scaffolding method. Many techniques have been developed over time, leading to the creation of scaffolds that support cells and promote tissue growth following implantation.

The most popular method is using a prefabricated porous scaffold. One of the various fabrication procedures currently in use creates a porous scaffold using raw materials, which can be synthetic or natural. This approach is particularly beneficial because of the wide range of biomaterial options and the capacity to manage the scaffold's physicochemical characteristics through scaffold design. The utilisation of fiber-based or porogen-based manufacturing processes, as well as novel solid free-form technologies, are a few examples. Cells can be sown inside or on top of the supporting scaffold once it is ready. This method's post-fabrication cell seeding is time-consuming and inefficient, which is a drawback. [53] The extracellular matrix (ECM) of either xenogenic or allogeneic tissues can be decellularized as a further scaffolding technique. A natural scaffold that promotes cell attachment, growth, and differentiation is the extracellular matrix (ECM).It can create an autologous construct when seeded with the right cells, negating the need to remove tissues from the patient. [54] This approach has the advantages of being biocompatible and exhibiting the closest natural mechanical and biological characteristics required by the body. The primary drawbacks of these systems are the immune system's reaction to non-autologous tissues and the restricted availability of autologous tissues. Furthermore, a few little issues remain, like the uneven dispersion of the seeded cells and the challenge of eliminating all immune-stimulating substances. [53] This method has shown benefits for the restoration of heart valves, bladders, and skin. Additionally, it has created a large number of commercialised decellularized scaffolds that have been approved for use in humans by the US Food and Drug Administration (FDA). [54]

1.8.2 Advances in Tissue Engineering Nowadays

1.8.2.1 Innovations in Cell Manipulation and Cell Sourcing

Although cells are the fundamental units of all living tissues, they serve as the foundation for the creation of tissue substitutes. Larger cell pools are now available for all tissue engineering applications thanks to advances in the field's understanding of stem cell development and cell manipulation. Since auto logous cells do not trigger immunological responses, they are preferred for tissue engineering applications because they reduce the requirement for immune suppressants and their associated adverse effects. [55] On the other hand, autologous cells are scarce and need a lengthy culture time to create the correct tissues. Nowadays, a lot of research attempts to employ allogeneic [56,57] approaches to get around the lack of autologous cell availability. However, there are still significant barriers involved with using allogeneic or xenogeneic sources in clinical applications, including immunological rejection, disease transmission, mismatches between the cellular environments of donors and recipients, and ethical issues. [58]

Stem cell applications in tissue engineering are expanding, and they are now being used in clinics. While the predominant stem cell type utilised in tissue engineering is still adult mesenchymal stem cells, embryonic stem cells are also being utilised and are beginning to make their way into the market. [59] The recent discovery by Shinya Yamanaka that adult differentiated cells might be stimulated to become pluripotent stem cells was a significant advancement in cell sourcing. [60]

1.8.2.2 Innovations in the Production of Scaffolds and Biomaterials

Numerous biomaterials with synthetic and natural origins, together with innovative production techniques,

have been presented in the last few decades. The goal of current research is to create "smart biomaterials" that can control cell processes and/or improve cellular performance. [61]

The scaffold's function is to give cells the necessary signalling cues and structural support so they can replace the scaffold with their own synthesised matrix.

Creating a scaffold that closely resembles the composition and structure of the target tissue is usually the aim. It is frequently impossible to completely recreate real tissues in vitro due to their intricate chemical makeup. Techniques like bio-printing have been established as a result of recent developments. [62-65] facilitating the synthesis of accurate three-dimensional structures for tissue engineering uses. Additionally, these methods enable the precise placement of recognition sequences and growth hormones for regulated cell behaviour. [66,67]

Chemical composition of scaffolds can be carefully controlled to either promote or hinder cell proliferation (e.g., collagen, gelatin) or allow cells to disseminate and proliferate (e.g., alginate, polyethylene glycol)). It is possible to create scaffolds that give cells adhesion sequences (such RGD, GFOGER, and IKVAV) for cell attachment and matrix metalloproteinase (MMP)-sensitive sequences for scaffold destruction. [68-73]

1.8.2.3 Research on Cell Signalling and Bioreactor Development Advances

In the provision of a developing substrate, or scaffold, cells need specific signals in order to endure and begin the process of synthesising their own matrix, which will ultimately take the place of the scaffold. Cell signalling has been extensively studied, and even more is still being clarified at this time. Normally, signals are produced by the surrounding cell microenvironment, detected by receptors inside the cell or on the cell membrane, and translated into a range of cell responses, such as matrix creation, migration, apoptosis, proliferation, and differentiation. The oxygen levels, mechanical stimulation, growth factors, extracellular matrix (ECM) chemicals, and other tiny molecules are the most significant signals that cells sense.

As predicted, it has been demonstrated that various tissues require various signal combinations, and that even the same tissue may need several signals at various depths or stages of maturation. For instance, the synthesis of type II collagen, the primary extracellular matrix component of articular cartilage, requires relatively low oxygen levels (below 5%) in the cells used to engineer the cartilage, a relatively simple tissue that is known to be avascular. Type II collagen is naturally synthesised in high quantities in the deeper cartilage layers. However, in order to modify the tissue's superficial layer, cells need a high oxygen concentration, which promotes the production of the superficial zone protein—a lubricating protein mostly produced by the superficial zone's chondrocytes. [74] Over the last ten years, the usage of bioreactors for growing tissues has increased dramatically. This is the outcome of the realisation that in order for tissues to achieve mechanical integrity, physiologic matrix composition, and an adopted natural phenotype, they must be subjected to specific stresses. Furthermore, mass transport is enhanced by bioreactors, which is necessary for the engineering of three-dimensional complex tissues and organs. The ability of bioreactors to automate, regulate, and standardise culture conditions is crucial for producing results that can be repeated. In tissue engineering, reproducibility is crucial, particularly when results could be used in clinical settings.

Small chemicals such as growth factors, cytokines, ECM compounds, and others have a significant impact on cell behaviour. Bone morphogenic protein (BMP) is required for bone formation, nerve growth factor (NGF) is essential for neural differentiation, and certain growth factors, such as basic fibroblast growth

factor (FGF-2), preserve the "stemness" of stem cells. Transforming growth factor beta (TGF-β) induces chondrogenesis. [75-79]

Depending on the stage of development, different ECM molecules including collagen and fibronectin are produced at different times during tissue morphogenesis. Variations in the ECM molecules' expression patterns are linked to a variety of events, including stem cell condensation, cell migration, and cell differentiation. [Singh, P. and Schwarzbauer, J.E. (2012) Fibronectin and stem cell differentia-tion – Lessons from chondrogenesis. J. Cell Sci., 125 (16), 3703–3712] The roles and functions of genes and proteins have been extensively studied in the past and present, but polysaccharides have received less attention. Polysaccharides, such as chondroitin sulphate, heparin, hyaluronic acid, and heparan sulphate (HS), are essential for numerous physiological and biological functions. They can also be used to encode the function of biological entities that are similar to DNA, RNA, and proteins. [80,81]

Repair of the central nervous system (CNS) has been linked to HS sulfation. During the gliotic scar formation process, Schwann cells show higher sulfation levels of HS than olfactory ensheathing cells.It is thought that the highly sulfated HS that Schwann cells produce causes a reactive astrocyte phenotype that prevents axon development after CNS damage. [82]

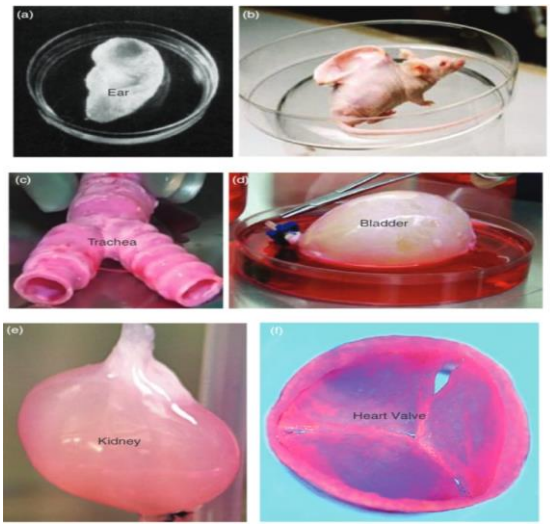

Figure: -3 Different tissue-engineered organs. (a) Scaffold prepared from synthetic biodegradable polyglycolic acid (PLA) in the shape of a 3-year-old auricle. (b) Scaffolds implanted subcutaneously on the back of an immunodeficient mouse. (Reproduced with permission from . (c) First trachea organ transplant using human's bone marrow stem cells. (d) Constructed artificial bladder seeded with human bladder cells and dipped in a growth solution. (e) Bioengineered kidney that mimics the function of a normal kidney concerning the control of the urinary system and blood filtration. (f) Tissue-engineered heartvalve using human marrow stromal cells. [11]

1.8.2.4 Bioprinting and three-dimensional (3D) printing

Three-dimensional (3D) printing systematically layers 2D medical pictures (such as CT, MRI, etc.) into 3D models that are saved as digital files (such as STL, AMF) that may be printed into actual 3D structures using computer-aided design (CAD) and segmentation software. [83,84,85]

Numerous medical specialities are using 3D printing technology for implantable medical device manufacture, surgical planning, and instructional modelling.[86]

To construct the 3D printed product, conventional 3D printing uses nonbiological, acellular materials like powders or gels. [87,88]

Cell-free scaffolds for surgical implantation have been printed using conventional 3D printing methods and additive manufacturing; now, 3D bioprinting is being investigated as a technology to combine living cells, biomaterials, and biochemicals in structures that resemble functioning tissues. [89,90,91]
The traditional method of 3D printing scaffolds and then seeding them with cells has been replaced with a simultaneous procedure that produces a 3D-bioprinted matrix and cells at the same time.[92]
Furthermore, 3D bioprinting offers accuracy and customisability since cells are carefully stacked using CAD and printed using 3D printers to closely mimic the patient's extracellular matrix. [93,94]

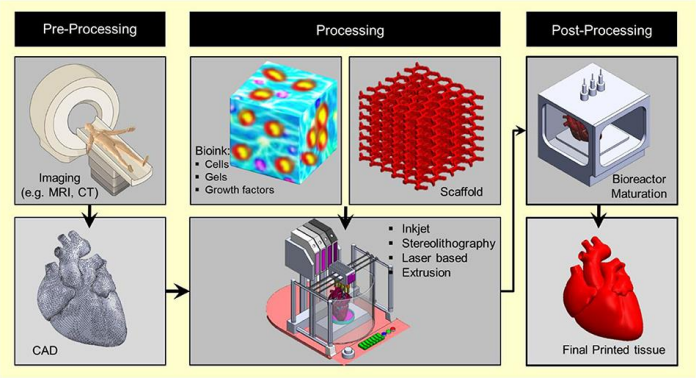

Figure: - 4 Overview schematic of the bioprinting processes.[120]

1.8.2.4.1 Benefits of 3D Printing

Although traditional tissue engineering techniques have proven effective in the past, it's vital to take into account the constraints of using these techniques to recreate patients' native tissues. The creation of scaffolds that are inaccurate in comparison to the anatomy of natural tissues, limitations on the biomaterials that can be delivered by classical engineering methods, erratic cell delivery, and improper interactions between various cell lines upon implantation in vivo are some of the drawbacks of classical tissue engineering techniques.[95]
Comparing 3D bioprinting to traditional tissue engineering techniques reveals numerous benefits. These more antiquated techniques are improved by three-dimensional bioprinting, which allows for great precision and customisation for each application and helps to automate the process.[96]
When compared to traditional tissue engineering techniques, the use of 3D bioprinting in scaffold building has resulted in more sophisticated and accurate anatomical features in the scaffolds' microstructures, enabling more exact co-deposition of cells and biomaterials.[97]

1.8.3 Tissue Engineering Applications

Tissue engineering is a relatively new field that engineer’s tissues using a combination of biomaterials, growth factors, cytokines, mechanical stimulation, biochemical signals, and physical signals. The creation of tissues that can be utilised to replace or repair bodily tissues that have lost all or part of their function is the most prevalent use of tissue engineering. Tissue engineering is beginning to discover new uses, though, including in vitro disease models, extracorporeal life support units (such bioartificial liver and kidney), drug screening tissues, smart diagnosis, and personalised medicine. The parts that follow will go into greater detail about these uses.

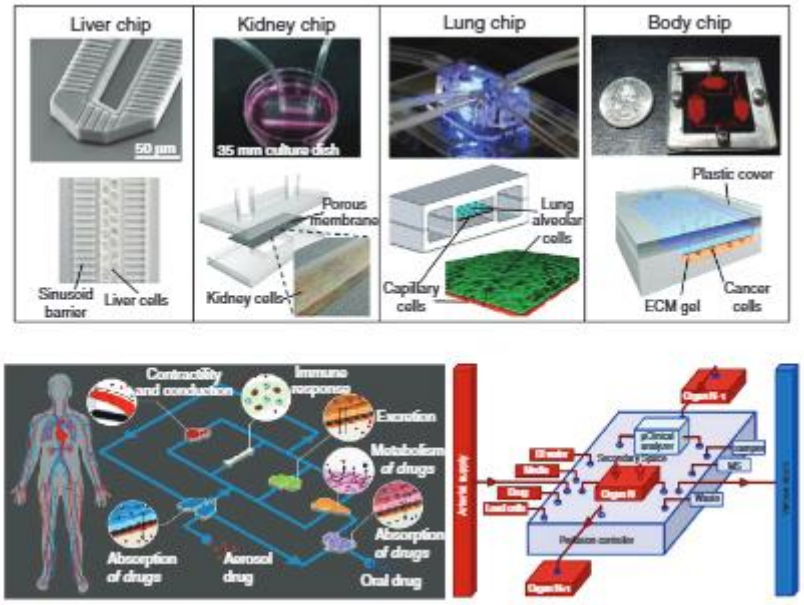

Figure: - 5 The latest advances in the use of microfluidics to create "organs-on-chips" models for in vitro studies of organs and tissues that have been engineered.[11]

1.9 Gene Editing

The process of altering the genome at specific sites using designed nucleases is known as "genome editing" [**98**]. Zinc finger nuclease (ZFN), transcription activator-like effector-based nuclease (TALEN), and clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) are examples of genome editing systems that are intended to cause DNA double-strand breaks (DSBs) at specific genomic loci. These breaks can be repaired in one of two ways.

In the absence of homologous template DNA, nonhomologous end-joining (NHEJ), an error-prone method that binds broken chromosomal ends directly and produces insertions and deletions (indel) . It is possible to accomplish significant DNA deletions if the nuclease is engineered to cause two cuts at separate genomic locations. By using a template DNA that shares sequence homology with the targeted locus, the homology-directed repair pathway can effectively repair the double-strand break. The HDR route makes it possible to accurately integrate, remove, or alter DNA sequences at pre-determined places based on the homologous sequences in the template donor DNA. While the nuclease-elicited DSB greatly increases the HR efficiency by several orders of magnitude, the technique is comparable to standard homologous recombination (HR).

Naturally occurring in bacteria, CRISPR/Cas9 is an immune system that has been modified for RNA-guided genome engineering. The cells in the model type II system express the spacer sequence-encoding CRISPR RNA (crRNA) and transacting crRNA (tracrRNA). Under the supervision of the spacer sequence on crRNA, tracrRNA and crRNA associate with each other and work with Cas9 to identify a protospacer-adjacent motif (PAM) and surrounding protospacer sequence. After then, the chromosomal DNA is cut into a blunt-ended double-strand break (DSB) by the catalytic domains of Cas9. A chimeric single guide RNA (sgRNA) containing the 5' spacer sequence and motifs imitating the structure of crRNA:tracrRNA can be used to replace the necessity for the crRNA:tracrRNA. [**99**]. In contrast to earlier genome editing techniques (TALEN and ZFN), CRISPR/Cas9 is simpler to use and can be programmed by simply altering the sgRNA's spacer sequence. As a result, CRISPR/Cas9 has taken the position of ZFN and TALEN as the most widely used genome editing technique. [100]

Point mutations are the most prevalent genetic variations linked to human disease. Additionally, CRISPR was adapted for base editing such that DSB creation was not necessary. In this regard, cytidine deaminases like APOBEC1, which cause the conversion of cytosine (C) to thymine (T), were fused with Cas9 nickase to create cytosine base editors (CBEs). [101] Additionally, adenine base editors (ABEs) were created for effective A/T to C/G editing. [102], It might be used to modify just one amino acid in transgenic plants. [103]

1.10 Applications in regenerative medicine and stem cell engineering

CRISPR, CRISPRi, and CRISPRa have been utilised in different stem cells, such as human PSCs, for genome engineering and gene regulation**.** [104]

Although still in its early stages, the use of CRISPR technology in stem cell and regenerative medicine is becoming more and more popular. But before applying CRISPR technology to regenerative medicine in a clinical context, a few problems must be resolved. These obstacles include immunological reactions, off-target consequences, and ineffective gene transport and editing. [105]

1.10.1Cutting-edge technology

The most recent developments are represented by cutting edge technologies, which are the outcome of progressive innovation. Cutting edge therapies are ones that are administered to patients and have a track record of success. Haematopoietic progenitor cell transplantation (HPCT) is the only FDA-approved stem cell therapy available for the treatment of haematologic diseases at this time. Additionally, cell-based gene therapy treatments have FDA approval.

[106] Therefore, the number of cell-based therapies that satisfy state-of-the-art standards is still restricted.

1.10.2 Cellular immunotherapy

However, it is not stem cell therapy, CAR T cell therapy is a type of cell-based gene therapy that is used to treat some haematologic malignancies. As such, it falls within the category of cutting-edge therapy. This novel immunotherapy involves genetically modifying a patient's T cells, or immune cells, to produce receptors on their surface called chimeric antigen receptors (CARs). These receptors will identify a particular antigen on cancer cells and launch an attack to eradicate the cancer cells. Adoptive cell transfer (ACT) is a growing field of immunotherapy that includes CAR T cell treatments. A patient's blood is drawn, their T cells are isolated, and an inert virus is used to modify the T cell so that it expresses the CAR on its surface. After receiving lymphodepleting chemotherapy, the cells are then grown in a lab and injected into the patients. [107] The first report of lymphoma regression utilising CAR T cell treatment was made in 2010 by Kochenderfer and associates. [108]

There are now two CAR T cell treatments that have FDA approval. In August 2017, the FDA authorised Kymriah (tisagenlecleucel), the first cell-based gene therapy to be made available in the United States. Patients with B cell precursor acute lymphoblastic leukaemia up to the age of 25 who have refractory illness or have had two or more relapses may receive Kymriah treatment.[108]

In May of 2018, the FDA authorised the same medication for individuals with relapsed or refractory diffuse large B cell lymphoma.[109] The FDA authorised Yescarta (axicabtagene ciloleucel), the alternative CAR T cell therapy, in October 2017 for the treatment of adult patients with relapsed or refractory diffuse large B cell lymphoma. [110]

1.11 Future challenges and limitations

Regenerative medicine is a new topic, particularly in terms of stem cell use. While there's a lot of potential,

there are a lot of obstacles that must be overcome. These difficulties include obstacles in the form of science and technology as well as ethical and public perception problems. The future of this profession will be determined by how well these issues are overcome and how previous knowledge is incorporated into new initiatives for research and development.[1]

1.11.1 Regulation: It can be challenging to navigate the intricate regulatory structure, and it's not always clear which regulatory route is ideal for novel treatments and technological advancements.

1.11.2 Stem cell research: Selecting the appropriate stem cells raises ethical questions. There are also problems with genetic instability, processing, and manufacturing. Concerns about the economy and a lack of knowledge about how stem cells function at their target places are also present.[111]

1.11.3 Clinical application: Stem cell-based protocols are often applied to patients without a strong understanding of the pathophysiology. [112]

1.11.4 Manufacturing: To realize the full potential of regenerative medicine therapies, several manufacturing obstacles must be addressed. [113]

Ethics: Safety, effectiveness, patient consent, information, professional obligations, equity, and fairness are among the ethical concerns.

Ethics: Safety, effectiveness, patient consent, information, professional obligations, equity, and fairness are among the ethical concerns. [114]

1.11.5 Costs: The treatment of stem cells may be costly.

1.11.6 Difficulties: Long-term adverse effects and problems are possible.

1.12 Future Opportunities for the Field of Regenerative Medicine

1.12.1 Specific treatment plans and personalised medication Research and development (R&D) can now be conducted in ways that would not be feasible on Earth because to advancements in space travel and the availability of low Earth orbit (LEO) habitats like the International Space Station (ISS), run by the United States National Laboratory. The ability to conduct studies in a setting with constant microgravity is one of the LEO environment's many advantages for life science research. This unique setting has made it possible to conduct ground-breaking research in tissue engineering and regenerative medicine, particularly in the fields of biofabrication, disease modelling using microphysiological systems (MPS), stem cell proliferation, and differentiation. These kinds of studies have improved our understanding of biology as well as the rate at which healthcare and medical technology have developed. These benefits mean that biological research conducted in low Earth orbit (LEO) may yield results that are not attainable on Earth. [115]

1.12.2 The consequences of stem cell banking

A part of this environment are biobanks, which are crucial to the field of regenerative medicine. They manage the procurement, distribution, and preservation of superior cells, tissues, body fluids, and other biospecimens. The flow of information needed by increasingly sophisticated research methods, like systems biology, extensive gene-environment studies, and epidemiological research, is centralized and made easier by biobanks. Biobanks are a crucial channel for sending biospecimens and the health information they are associated with them to users downstream [116]

For use in drug discovery, developmental biology, regenerative medicine, cell therapy, and toxicity, stem cells hold great promise. In order to maintain their biological properties, avoid contamination and deterioration, and enable their efficient use in fundamental and translational research as well as present and future clinical application, stem cell banks have been established more and more throughout the world.

[117]

The continuous existence of the ISS in Low-Earth orbit provides a useful platform from which to promote the ISS's positive effects on Earth's economy. To bridge the gap between the early stages of space-based biomedical research and the establishment of a sustainable, investment-worthy biomanufacturing market in LEO supported by future commercial platforms, the ISS National Lab's provision of access to the space station is crucial.

1.12.3 Future prospects for the use of induced pluripotent stem cell (iPSC) technology

AI has the potential to significantly transform and speed up the development of iPSC technology in a number of areas, including disease modeling, cellular treatments, drug discovery, and cell culture. Artificial Intelligence (AI) has the potential to greatly progress iPSC research and development by gaining meaningful insights from enormous volumes of molecular and genomic data. These tasks would be impractical for humans. Even though AI has the potential to improve iPSC research, significant technological obstacles need to be removed before its uses can be extensively used. Therefore, in order to overcome these obstacles and completely realize the potential of AI in iPSC technology, a great deal of study is needed.

The potential to improve and tailor AI algorithms for use in regenerative medicine is growing as high-quality data becomes more widely available and AI technology mature. Advances in robotics, AI, and computer vision have the potential to yield novel insights that could result in revolutionary advancements in iPSC technology. Personalized regenerative therapy development may advance with the combination of AI with other nanotechnology, genome editing, and 3D bioprinting. Utilizing AI to its fullest potential can lead to the development of specialized and successful regenerative treatments through interdisciplinary collaboration, ethical development, and use of these technologies.

Establishing strong benchmarking standards and criteria is crucial for the creation and evaluation of AI systems. These ground truth datasets provide for an objective assessment of an algorithm's efficacy because of their precise labeling and widespread acceptance. They act as a pillar for contrasting different models and approaches and supporting the identification of the most effective methods for certain uses. Standardized reporting procedures are also essential for greatly raising the caliber of research in AI applications [118]

1.12.4 Technological developments in tissue engineering and regenerative scaffolding

Furthermore, commercially financed R&D with terrestrial applications is becoming more significant than government-funded fundamental research, which is fostering innovation and industry in low-Earth orbit. This modification highlights the significance of funding and public-private collaborations in advancing critical R&D that leverages the advantages of the LEO environment. Recent ISS research has emphasized the potential economic worth and benefits of space-based biomanufacturing to life on Earth. This entails blending biological and non-biological elements to create commercially viable biomolecules and biomaterials that might be applied to preclinical to therapeutic settings [1]

1.12.5 Artificial intelligence in optimizing stem cell therapy and research

During the Biomanufacturing in Space Symposium, numerous interesting areas for further research and development (R&D) in space-based biomanufacturing were identified. Viable options include illness modeling, stem cells and their byproducts, and biofabrication. Throughout the workshop, there was also emphasis on the need for additional study to assess and reduce the hazards connected to these opportunities. The first step is to generate and gather the required data, and experts concur that automation, artificial intelligence, and machine learning are essential. The seminar attendees acknowledged the

necessity of funding and public-private partnerships to explore these opportunities for a manufacturing sector in LEO. [1]

1.13 Conclusion

In conclusion, the fields of tissue engineering, gene editing, and stem cell therapy are exciting areas of regenerative medicine that have the potential to completely change the way diseases and injuries are treated. However, there are issues that must be resolved, including immunological rejection, tumorigenicity, and ethical issues. Technological developments such as CRISPR-Cas9 and 3D bioprinting portend positive things to come. Over the past few decades, tissue engineering has advanced significantly, bringing solutions that were previously thought to be science fiction to the clinic. Even though tissue engineering concepts are not yet widely used in clinics, the field is predicted to have a very bright future as additional tissues will be added to the list of "clinically applicable tissue engineered constructs.It will probably to be possible in the future to combine immune-transparent cells with a commercial scaffold cultured in a sophisticated bioreactor that provides customized signals for the target tissue. It still takes a lot of fundamental and applied scientific research to get off-the-shelf body parts to the point where they are therapeutically meaningful.Future research will concentrate on creating innovative biomaterials for various uses in tissue engineering and regenerative medicine. The biomaterials' mechanical characteristics and structure will be modified to better fit the target tissue. These biomaterials ought to be able to overcome the field's present main obstacles, particularly those related to mass transit. Additionally, it is anticipated that the biomaterials that are developed will be more specifically designed to preserve the phenotypic of cultivated cells and provide the ideal combination of growth factors and cytokines when needed. Moreover, materials with injectability or flexibility that enable minimally invasive surgical techniques should be the focus of research in order to reduce implant complexity. Lastly, it is important to create materials with improved stability or integration in the implant site. Covalent bonding based on naturally occurring tissue residues and engineered residues on the scaffold, as well as biomaterials with muscle-adhesive proteins and other glue interfaces, may be studied. In order to promote better repair or regeneration, future research will also concentrate on cell manipulation techniques including transfection and silencing. It will be necessary to get more fundamental scientific knowledge of cell activity in tissue engineering systems, including interactions between cells and scaffolds as well as in vitro and in vivo.For the various tissue engineering applications, the effects of various growth factors as well as the optimal dosages and time of supplementation should be established. In order to imitate the conditions of thick tissues in vivo, in vitro culture methods should also be updated, especially the transition from 2D to 3D systems and oxygen levels. The biological implications of the currently utilized culturing procedures may be clarified by conducting systematic investigations that contrast the in vivo scenario with the in vitro culture systems currently used in tissue engineering.With this information, current cell culture methods can be enhanced to promote tissue repair. Lastly, efforts should be undertaken to optimize existing ethical and regulatory issues in order to facilitate the safer and easier introduction of tissue engineering and regenerative medicine solutions into clinical settings.To overcome these obstacles and fully achieve the potential of stem cell treatment, tissue engineering, gene editing, and regenerative medicine, future research should concentrate on artificial intelligence, stem cell banking, personalized medicine, and tissue engineering.

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