A REVIEW ON BIOMETERIAL FOR DIRECT RE-PROGRAMMING OF CELL

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ABSTRACT: Direct reprogramming is a very useful and interesting method for changing what happens to mature cells. recently This method can avoid the problems that come with using stem cells directly and allow stem cell therapy to be customized to each patient in regenerative medicine. Overexpression of different factors, like general reprogramming factors or lineage-specific transcription factors, can change what will happen to cells that have already specialized. On the other hand, biomaterials can give cells physical, topographical, and biochemical cues that can tell the cells what to do or be told what to do by the cells themselves affect the development of stem cells in a big way. Biomaterials haven't played much of a role in direct reprogramming so far, but they could be important for making direct reprogramming more precise or efficient. This review talks about how to use general direct reprogramming and biomaterials to guide stem cell differentiation.

KEY WORDS: Direct reprogramming, Biomaterials, Gene Delivery, Modulating**,** Transcription Factor, RNAi, Protein translation,

I. INTRODUCTION:

Biomaterials are another technology that has grown in popularity over the past few years and is used in regenerative medicine. Material science has come a long way, and now we know a lot about the bulk and surface properties of several different materials, as well as whether or not they are biocompatible [1]. So, research has started to look at how the benefits of biomaterial methods and stem cell developments can be used together to make human cell and tissue engineering more effective and possible. Scientists have figured out how to use these biomaterials as scaffolds for tissue engineering [3, 4]. This progress has been made possible by finding more materials that work well with stem cells. Biomaterials can affect how well cells can be reprogrammed and changed by acting as a niche or a way to send genetic or epigenetic information to cells. In this study, we will briefly discuss the present status of cell reprogramming and differentiation methods, their constraints, and how different biomaterial techniques could make this technology more beneficial. This involves utilizing the impacts of biochemical and biophysical cues that are crucial for cell growth, using approaches that do not rely on viral vectors while boosting cell phenotypic specificity, and building culture substrates to facilitate cell reprogramming and differentiation.

II. NEURAL CELL DIRECT REPROGRAMMING

Neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's are very dangerous and often deadly, but there is no clear cause and no good medical treatment. Necrosis and apoptosis are two ways that neural cells die, which is a common sign of neurodegenerative disorders. To treat these diseases, it is necessary to regenerate neural cells. So, neurodegenerative diseases can be treated with powerful regenerative therapies that use direct reprogramming [5]. because it is not a stem cell. A progenitor cell hasn't changed into a mature cell yet. It can change into some types of mature cells, but not all types, because it is not a stem cell. Directly reprogrammed

neural progenitors are different from directly reprogrammed neurons because they can grow in vitro and can change into different types of neurons. Using reprogramming, fibroblast cells derived from embryonic mouse brains may be converted into a variety of neural progenitor cells. First, induced neural progenitor cells (iNPCs) are created by introducing Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc) [6]. The same components used to generate iPSCs were used, but modifications were made to the signals that direct cell fate. This study also paved the way for novel signal-altering applications by using generic reprogramming factors rather than lineage-specific transcription factors. transforming mouse embryonic fibroblasts or adult tail-tip fibroblasts into dopaminergic-induced neural progenitors (iDPs) in the midbrain using the Yamanaka factors Shh and FGF8 [7]. Inhibiting the JAK-STAT pathway using a morphogen for midbrain development aided in the reprogramming process. Through the application of the transcription factors Sox10, Olig2, and Zfp536 fibroblasts from mice and rats were converted into induced oligodendrocyte precursor cells (iOPCs) [8].

III. CARDIOMYOCYTES DIRECT REPROGRAMMING

Heart disease is mostly associated with older age groups since it is primarily brought on by daily diet and lifestyle choices rather than only atherosclerosis and hypertension [9]. However, due to the incorrect growth of cardiomyocytes during embryogenesis, there is also a congenital cardiac abnormality. Heart illness may be difficult to treat because postnatal cardiomyocytes lack the ability to regenerate. Therefore, there are a lot of papers discussing stem cell-based treatments for cardiac disease. However, the functional heterogeneity, maturity, possible tumorigenicity, limited survival, retention of supplied cells, and inadequate stem cell supply of stem cell-derived cardiomyocytes are only a few of the issues [10]. A better answer could come from direct reprogramming. Cardiomyocytes, vascular cells, and cardiac fibroblasts make up the heart. Over 50% of them are cardiac fibroblasts [11]. When the heart is damaged, cardiac fibroblasts help to produce scar tissue, release signals, and sustain the cardiac structure. There have been attempts at direct reprogramming from cardiac fibroblasts to cardiomyocytes. Cardiomyocyte-like cells (iCMs) were produced by *M. Ieda et al.* [12] using mouse newborn cardiac and tail-tip dermal fibroblasts. Gata4, Mef2c, and Tbx5 were the three cardiac transcription factors they employed during development. Within seven days, this combination effectively and quickly produced iCMs. even though complete maturation needed more time after transduction. iCMs showed activation during spontaneous contraction. However, they found that iCMs produced from tail-tip fibroblasts oscillated at a lower frequency than iCMs obtained from cardiac fibroblasts. The second group used the identical Gata4, Mef2c, and Tbx5 factors, but started with mouse adult cardiac fibroblast [13]. They verified that the reprogrammed hearts had high potential for contractility and sarcomere formation. iCMs may also electrically link with functional endogenous cardiomyocytes. In vivo tests revealed that mice with iCMs injections had improved functionality across the board. The addition of Thymosin 4 improved in vivo cardiac function and scar size. Using Gata4, Mef2c, Tbx5, and Hand2, K Song et al. transformed mouse adult cardiac and tail-tip fibroblast into iCMs [14].

IV. DIRECT REPROGRAMMING IN HEPATOCYTES

The hepatocyte is the principal cell type in the liver. The liver controls many of the body's functions. Acute liver failure and other metabolic disorders of the liver are among the leading causes of mortality worldwide [15]. Liver transplantation is the gold standard for treating liver disorders. However, given the scarcity of donated livers, hepatocyte transplantation might be a viable option. Hnf4a, Foxa1, Foxa2, or Foxa3 were identified as reprogramming factors for mouse fibroblasts to become hepatocytes in a study by Sekiya et al. [16]. Both Foxa2 and Foxa3 were utilized to label fibroblasts, with Foxa2 being employed for embryonic fibroblasts and Foxa3 for adult dermal skin fibroblasts. iHeps are identical to mature hepatocytes in terms of gene expression, appearance, and hepatic function. iHeps were able to correct hepatic abnormalities when implanted into the liver of a rat. Ectopic production of Gata4, Hnf1a, Foxa3, and inactivation of p19Arf is another way to induce at the tips of mouse tails. The chromatin modification activities of Gata4 and Foxa3 were followed by Hnf1a's stabilization of inducible hepatic gene expression. The protein p19Arf plays a critical role in the cellular senescence cascade and also blocks iPSC reprogramming. Therefore, p19Arf inhibition may remove the restriction on cell proliferation. Similar studies using human cells have been conducted recently. *Huang et al*. created human-induced hepatocytes (hiHeps) using Foxa3, Hnf1a, and Hnf4a-expressing fibroblasts isolated from human fetal limbs and adult dermal tissue [18]. The hepatic capabilities of this HIV were fully developed, including biliary drug molecule excretion

and CYP enzyme activities. Adult dermal HiEps converted to hepatocytes at a much slower rate than fetal limb HiEps. HiHeps were created using human neonatal fibroblasts (Oct4, Sox2, and Klf) and a medium comprising known growth factors and the small chemical CHIR [19]. Some Yamanaka factors were employed, but the process did not reach the pluripotent phase

V. BIOMATERIALS TECHNIQUES FOR CELL FATE MODIFICATION

While performing their intended function, biomaterials must not pose a risk to the patient in any way, regardless of whether they are made of natural or synthetic materials. Biomaterials may interact with and integrate themselves into biological systems [20]. In regenerative cell therapy, the most important thing is to control the fate of cells, such as stem cells or the reprogramming of mature cells. If cell reprogramming is not well managed, the therapy may result in teratoma rather than treatment. There are several biomaterials that may be used to determine cell fate or to aid in the reprogramming process. Biomaterials may create microenvironments that replicate a particular cell niche, enabling cells to develop into the cell types they desire [21]. Homing signals from the artificial stem cell niche may attract and localize stem cells [22]. Direct reprogramming may function similarly [23]. The classification of these biomaterials is difficult since one biomaterial might have numerous functions. Biomaterials are classified based on their physical qualities, which include surface, mechanical, electrical, and morphological features. They may be classified according to their source, such as natural, polymer, ceramic, or metal, or according to their form, such as 2D or 3D materials.

Table:1 Characteristics of biomaterials:

Physical characteristics	**Biochemical aspects**	**Gene delivery**
Surface rigidity	Extra cellular matrix	3D sphere growth of nano
Scaffold opening	Factor of expansion	micro particles in a nanochannel
The topographical pattern	The first signaling molecule	Protein

(1).

BIOMATERIALS FOR PHYSICAL ASPECTS:

For cell reprogramming, the mechanical strength (modulus) and surface topographies of biomaterials are important. First, the flexibility of the matrix of the microenvironment can alter the way a cell turns out. In general, stiffer substrates cause cells to be stiffer, and softer substrates cause cells to be softer [24]. In a similar way, the presence of a soft matrix can facilitate the transformation of mesenchymal stem cells into cells that exhibit behaviors similar to those of nerve cells. Myogenic differentiation is helped by a matrix with moderate flexibility, whereas osteogenic differentiation is helped by a matrix with a hard structure [25]. Engler A. et al. were the first to show that human mesenchymal stem cells can change into different types of cells by changing the elasticity of the matrix [26]. This includes neurogenic differentiation, which happens in the brain and has an elastic modulus between 0.1 and 1 kPa, myogenic differentiation, which happens in muscles and has an elastic modulus between 8 and 17 kPa, and osteogenic differentiation, which happens in bones and has an elastic modulus between 25 and 40 kPa (osteoid). The morphology of the cells also changed throughout the process. Hence, the characteristics of the matrix can serve as a powerful signal for mesenchymal stem cells to begin the process of transformation [27, 28]. In the same way, physical cues like the stiffness of the cell-substrate interface can influence the cell's fate. The use of soft hydrogel was essential in the production of iPSCs because it facilitated the acceleration of the transition from the mesenchymal to the epithelial state [29].]. Changes in cell fate could also be caused by the

size of the fibers in the substrates [30,31]. The diameter of the fibers in the laminin-coated electro spun polyether sulfone mesh could control how the neural stem cells changed and grew. In general, the rate of neural stem cell growth increased as the diameter of the fibers got smaller. Due to size limits, the shape of the cells in small fibers was stretched and went in many different directions, while the cells in large fibers were stretched along a single axis. Even though major signals such as retinoic acid, fetal bovine serum, growth factor, and others were required, fiber diameter had an effect on differentiation and proliferation. The scaffold's pores are also important. Levinger et al. [32] designed 3D porous biodegradable polymer scaffolds that altered cell growth, movement, and organization.

(2). BIOMATERIALS FOR BIOLOGICAL ASPECTS:

Growth factors, extracellular matrix (ECM) molecules, and other biochemical signals control cellular mechanisms. Integrin, in particular, regulates cell fate via structures and components of the extracellular matrix (ECM). Mescorted activation and signaling events that happen afterward [33]. The main ECM components are collagen, laminin, fibronectin, and Matrigel. Stem cells in the CNS were turned into functional neuronal circuits with the help of 3D collagen gel [34]. Neuronal stem cells need to anchor and connect to a solid surface in order to grow new neural tissue. This is why these solid polymer scaffolds are so important. ECM proteins like collagen must be added to synthetic hydrogel to help brain cells recognize and bind to it. On the other hand, stem cells trapped in a biologically made collagen hydrogel grew quickly in a serum-free medium with and turned into neurons, astrocytes, and oligodendrocytes. S. Battista et al. [35] looked at how the type of matrix in 3D constructs affected the development of embryonic stem cells. They used different amounts of collagen, fibronectin, and laminin to make semi-interpenetrating polymer networks. Depending on how much collagen was in each composition, the development and formation of the embryonic body were different. A high concentration of collagen stopped cells from dying off, which messed up the development of the embryonic body, and fibronectin sped up the differentiation of endothelial cells and the growth of blood vessels. Laminin caused embryonic stem cells to turn into cardiomyocytes. Direct reprogramming can also be done with a collagen hydrogel. Smooth muscle cells in a dense 3D collagen gel developed a blood vessel structure from endothelial progenitor cells [36]. Large fibrin is a non-globular protein. Fibrin is made when thrombin interacts with fibrinogen [37]. A good scaffold should have places for cells to stick and signals that help cells grow into different types. Researchers looked at fibrin scaffolds with different amounts of fibrinogen, thrombin, and aprotinin to create the right environment for neurogenesis [38]. They determined that fibrinogen concentration is more essential than thrombin concentration and that higher thrombin concentrations prevent cells from migrating inside the fibrin scaffold. Cell growth and differentiation increased with more cells planted. Aprotinin's optimal amount depended on the other ingredients because it controlled gel breakdown. Another group used a gel composed of fibrin to culture embryonic stem cells [39]. PEG was used as a secondary crosslinker to decrease fibrin gel degradation.

(3). BIOMATERIALS FOR GENE DELIVERY

Gene delivery is a method that is often used in research on cells. It is an important part of changing the fate of a cell in a lab or controlling how stem cells change. Viral vectors were used before new gene delivery methods were discovered. Nonetheless, this viral vector could arbitrarily alter the host sequence. The use of microscopic particles known as nanoparticles was the first novel method of delivering genes. As an example, as a non-viral gene vector, stimuli-responsive Hyaluronic acid (HA)-ss-Polyethyleneimine (PEI) nanoparticles were developed [40]. A nanocomplex made of positively charged PEI and negatively charged DNA can release DNA in the endosome. PEI's cytotoxicity places restrictions on this technique. There are HA receptors present in a wide variety of tissues, and HA possesses a polyanionic characteristic that prevents serum and PEI from interacting in a manner that is not very selective. In other words, HA has the potential to increase the particle's stability and specificity. DNA exits the endosome faster with disulfide links. *Zhao et al*. also made nanoparticles out of chitosan (CS)-ss-PEI to help osteogenic differentiation [41]. In the other case, a safe and effective gene delivery system based on magnetic nanoparticles was used [42]. Magnetic nanoparticles with a biocompatible surface coating were used to deliver therapeutic biomolecules like DNA, siRNA, and shRNA. In addition to that, the use of a micro- or nanochannel array in an electroporation system has been suggested [43–45]. This is a technology for transfecting a single cell that uses electrophoresis to inject charged agents directly into the cytosol, allowing for

high precision over the amount that is introduced. All of these methods for temporary gene expression using nonviral vectors have the potential to get around the problems of genes being overexpressed and viral vectors causing problems. Instead of using viruses as vectors, proteins are another risk-free way to send genes from one place to another. But because its half-life is so short, it must be consumed in large quantities, which drives up the associated expenses. Kim and his colleagues used reprogramming proteins that were attached to a peptide that can get into cells (CPP). This method gets around the problems of genetic integration and immunogenicity, but it didn't work very well [46]. Most of the time, getting protein into a cell is not very effective because protein is a large molecule that is hard to get through cell membranes. So, there is a strong need for biomaterials that make it easier for proteins to get into cells. *Qutachi et al*. employed PLGA microparticles to transfer proteins into the embryonic body so that they could control how the embryonic stem cells changed [47]. In addition to this, Bian et al. developed nanoparticles that are capable of holding growth factor proteins and combining with hydrogel scaffolds. Protein-loaded microsphere scaffolds turned mesenchymal stem cells into chondrogenic cells [48].

VI. BIO METERIAL FOR DIRECT RE PROGRAMMING:

Biomaterials are non-living substances used in medicine to restore damaged tissues and organs. As a consequence, biomaterial science and engineering have seen exponential development over the last 50 years. massive investment in the creation of new items Biomaterials consist of both natural and synthetic components. As was previously said, stem cell survival requires a certain environment. Biomaterials simulate in vivo cell-matrix interactions to influence stem cell fate. Biomaterial scaffolds may promote cell adhesion and stem cell characteristics. By incorporating chemical and physical cues into the ECM, 3D biomaterial scaffolds offer stem cells a better microenvironment than 2D cultivation. Scaffolds can directly modify cell signaling and induce stem cell lineage-specific differentiation through chemical cues or cell-matrix interactions [49]. As interest in biomaterial-based techniques has grown, it has been discovered that biomaterial properties influence stem cell lineage determination. When constructing a novel scaffold, surface, mechanical, electrical, electrostriction, morphological, and chemical qualities must be carefully examined. The organization may be modified to influence stem cell differentiation, according to *Y. Xu et al.*

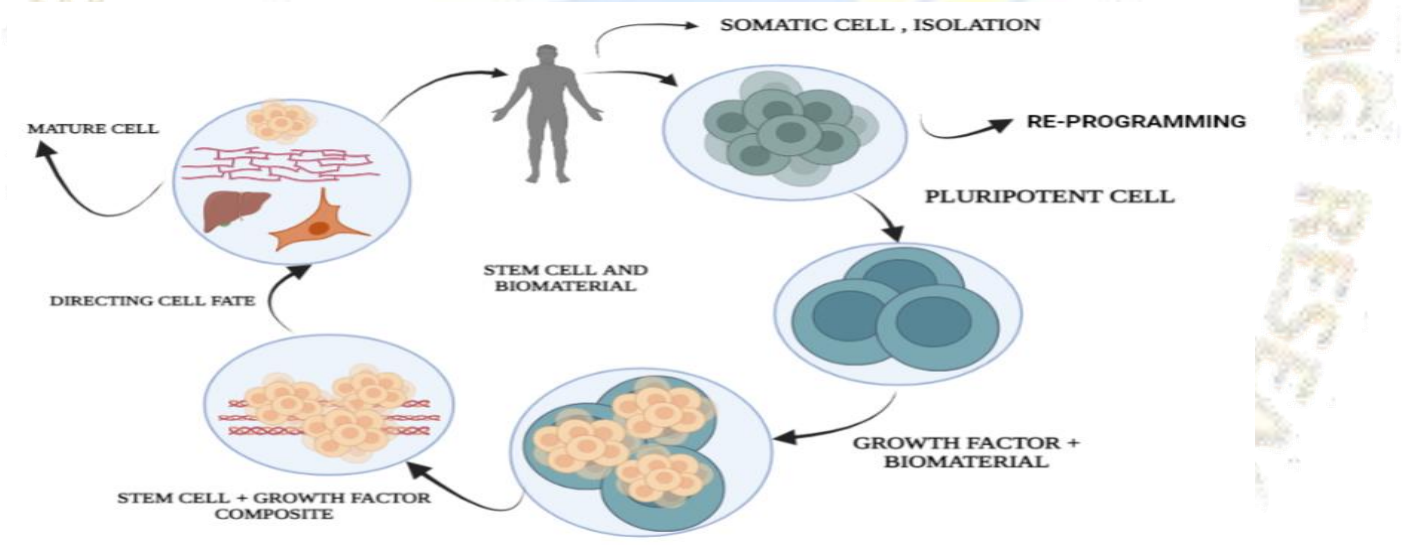


Figure 1: Extensive screening, the adhesion, transport, differentiation, and matrix proteins the use of stem cells and biomaterial.

Table 2: Classification of Biomaterials

Types	Examples	Properties	Application
NATURAL BIOMATERIAL	Collagen, hyaluronic acid, gelatin, laminin, fibrin	Good biocompatibility Self-existing bio signal Poor mechanical strength	Cornea repair Cartilage/bone repair
SYNTHETIC BIOMATERIAL	**POLYMRER-** PLA, PLGA, PCL, PEG, PVA, PHEMA, PM	Easy modification.	All kinds of stem cell culture and tissue
	CERAMIC- HA, TCP, bioactive glass	Good mechanical strength Poor degradability	Additives in bone tissue engineering
	METALS- Titanium, titanium alloy, stainless steel, cobalt alloy	compressive strength good fatigue resistance non-degradable non-bio adhesive	Orthopedic and dental treatment

VII. SYNTHETIC BIOMATERIAL

Even though natural biomaterials are biocompatible and have their own bio signals, they can't be used for as many things because they aren't very strong and are hard to change. To solve these problems, the main types of stem cells as a designed component, a synthetic biomaterial's structure and relative mass can be changed at will. Synthetic biomaterials, on the other hand, are not the best choice for this application because they don't have the properties that are needed for cells to attach or for biological signals to be sent. This indicates that they are unable to determine the fate of cells on their own. In stem cell production, biocompatibility and bioresorbable of the synthetic composite are frequently the most essential difficulties, and a lot of research is being done to discover solutions to these issues.

(1). SYNTHETIC POLYMERS

Some common polymers used in stem cell cultivation are poly (lactic-co-glycolic acid) (PLGA), poly (lactic-co-glycolic acid) (PCL), polyethylene glycol (PEG), polyhydroxy ethyl methacrylate (PHEMA), and polyvinyl alcohol (PVA). Since their invention in the 1700s, lactic acid polymers have been utilized in various fields [50]. PLA and PLGA are biocompatible, biodegradable, bioresorbable, immunogenic, and poisonous, making them superior to other synthetic polymers. They make ideal 3D scaffolds for dentistry, plastic surgery, and other applications [51]. PLA has been shown to promote and control the adhesion, growth, and differentiation of human fat-derived stem cells [52]. PCL was mixed with PLA to make engineered tissues more resistant to heat and improve their strength [53]. PEG is widely used for human MSC osteogenic differentiation because PEG gels leave a lot of space between stem cells and the extracellular matrix for nutrients and waste to move through [54, 55]. Also, glucosamine-modified PEG hydrogel for repairing cartilage has been shown to be more compatible with the body and stop fibrosis and hypertrophic cartilage markers [56].

(2). SYNTHETIC-CERAMIC

As ceramic additives, calcium phosphate, bioactive glasses, and calcium phosphate cements are frequently employed in orthopedics, dentistry, and bone tissue engineering to direct stem cell development. Even though ceramics are hard to break down and don't stretch well, they are often used in bone tissue engineering because they are better at being osteo-inductive, osteo-conductive, and mechanical. The initial mechanical strength and stiffness of the bone regeneration device can be improved by including hydroxyapatite-based calcium phosphate and bioactive glasses to build a composite for optimum osteofate direction [57, 58]. Bio ceramics can also be used to make a polymer implant more porous so that nutrients and waste can move more easily through the scaffolds. This is accomplished by incorporating ceramic-specific micro- and nanoscale voids [59]. But it can be tricky to use a ceramic scaffold. For example, calcium phosphate cements could be injected and were very biocompatible in vivo. However, when phosphate was released from an unmodified scaffold, the pH of the medium dropped and cell growth was slowed [60]. By sintering the scaffolds at extremely high temperatures, Link et al. converted the calcium phosphate component to a more stable form. In a laboratory setting, this improved the scaffolds' ability to detect cells similar to osteoblasts [60]. Our findings demonstrated the importance of bio ceramic physiochemical state in cytotoxicity and the need to consider both in vitro and in vivo performance when evaluating bio ceramics for clinical applications.

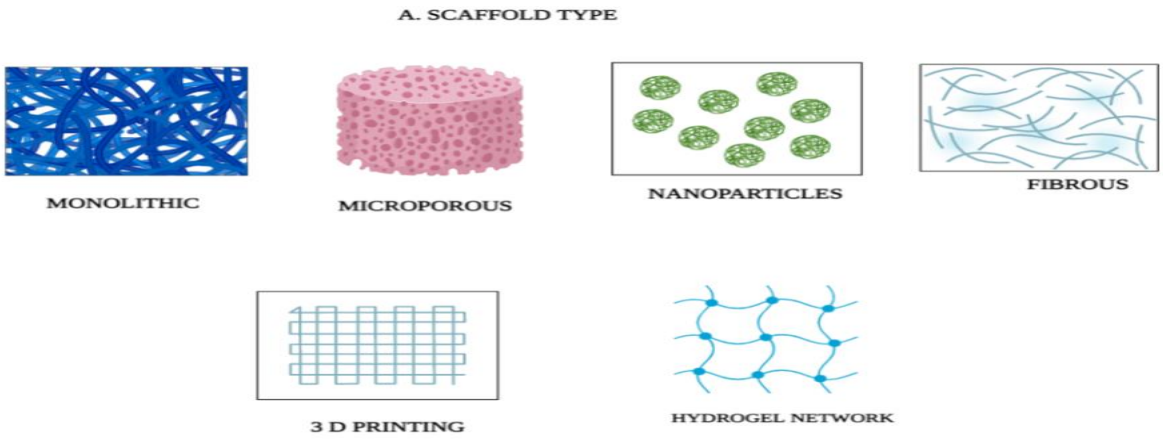
(3). SYNTHETIC-METALS

In orthopedic and dental care, titanium, titanium alloys, stainless steels, and cobalt alloys are frequently used to promote bone regeneration. Stainless steel implants are the most common type, likely because they are readily available. Non-specific protein adsorption and cell adhesion make it difficult to manage cell-metal interactions on the materials' surfaces, resulting in poor host tissue integration. A recent study tried to solve these problems by putting covalent bonds between adhesive peptides and stainless steel to control how stem cells stick to it [61]. Another study that looked at how to make stainless steels more biocompatible by coating them with ZrO2 and SiO2/ZrO2 showed that the way stem cells grew depended on the surface properties of the stainless-steel scaffold [62]. Titanium and tantalum showed good biocompatibility, resistance to corrosion, and mechanical properties. These properties could help MSC multilineage differentiation in vitro by giving cells enough places to stick to in 3D porous scaffolds [63]. Titanium and tantalum could keep the immunophenotypic features of MSCs in addition to making cells live longer [64]. Also, filtered cathodic vacuum arc deposition (FCVAD) can be used to deposit a tantalum film on titanium alloy (Ti6Al4V) to improve its mechanical and anticorrosion properties, as well as its compatibility with mammalian bone MSCs [65]. A tantalum layer and a polymer-titanium hybrid layer were employed in another fascinating work to make medical devices more compatible with MSCs [66]. These results show that biomaterial scaffolds made of titanium, tantalum, and their alloys are a good choice. Metallic biomaterials are strong and don't wear out easily, but there are risks with their current uses, such as the release of toxic ions and their inability to break down. However, magnesium (Mg) and its alloys present an opportunity to tackle these problems from a new perspective. Magnesium can be utilized to mimic the mechanical properties of genuine bone in cell culture since its elastic modulus and compressive yield strength are comparable to those of real bone. In addition, magnesium cation is a naturally occurring trace element in the human body, and its breakdown is relatively harmless [67-69].

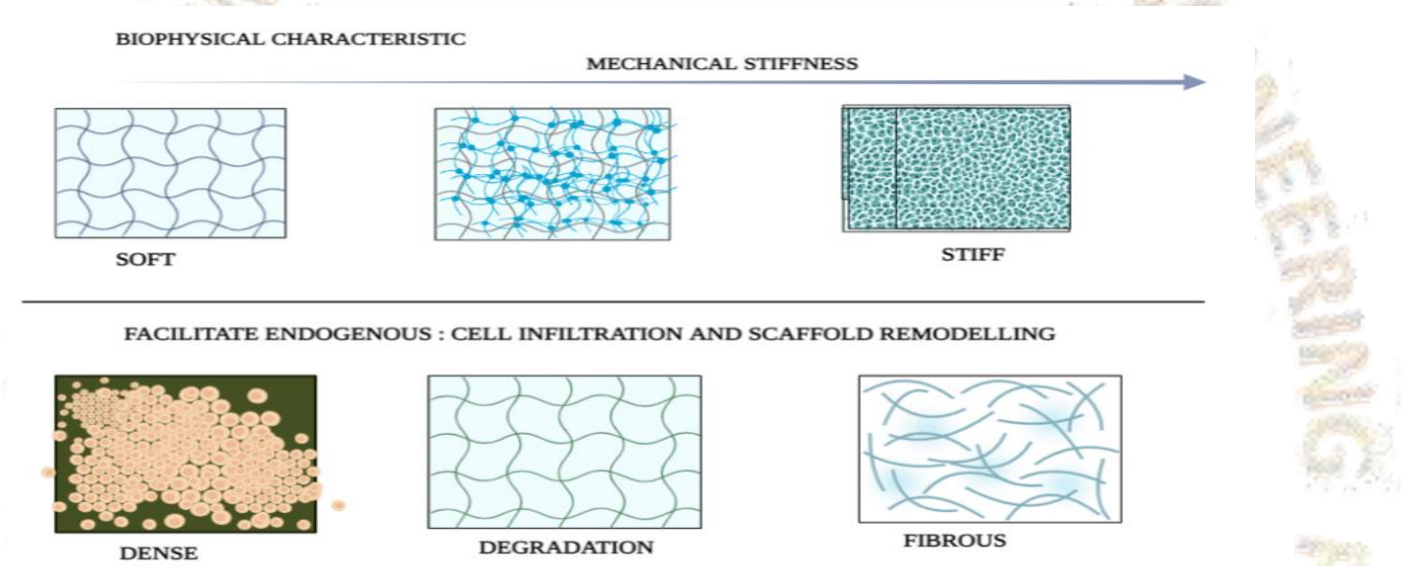
VIII. CHARACTERISTICS OF BIOMATERIALS

Scaffolds are made of biomaterials like polymers, ceramics, metals, and composites. Synthetic, natural, or a mix of the two, these materials must respond to biological signals and interact with the immune system and the body's own cells in order to help the body heal itself. Polymers, ceramics, metals, and composites are all examples of biomaterials that can be used to construct scaffolds. These materials, whether they are synthetic, natural, or a mix of the two, need to interact with the immune system and the body's own cells and respond to biological signals in order to stimulate regeneration. These responsive biomaterials can interact with the body and alter the microenvironment of local tissues by manipulating the immune system and regulating the rate and extent of healing by the body's own cells, thanks to their biophysical and biochemical features (from scarring to total regeneration). Tissues in close proximity to biomaterials can experience changes to their microenvironments as a

result of intracellular and intercellular signaling, biomaterial stiffness, structure, topography, and degradation [Fig.3].



[Fig.2]. Scaffolds like monolithic, microporous, nanoparticles, fibrous, hydrogel, and 3D-printed



[Fig.3]. intracellular and intercellular signaling, biomaterial stiffness, structure, topography, and degradation

The concentrations of enzymes, cells, ions, or radical species can be adjusted, as can the pH level or temperature. Cell adhesion, migration, and differentiation are all affected by the matrix's rigidity (positions 63–64). In the case of bone marrow, stiffer surfaces promote cell adhesion and proliferation, ultimately leading to the differentiation of stem cells into osteogenic lineages. In contrast, stem cells are guided towards the chondrogenic lineage by a softer matrix, which aids in their rounding and encourages differentiation. 12. As a result of its permeability, the scaffold allows cells to enter. Movement of nutrients, oxygen, and waste materials can be facilitated via networks of interconnected pores. As a result of its facilitation of angiogenesis, porosity also aids scaffolds in obtaining blood vessels. Cell adherence and cell fate can be aided or hindered by topological features such as patterned surfaces (high surface roughness). Biomaterials should degrade at the site of tissue injury or damage so that new tissue can grow in its place. The rate of tissue breakdown and tissue growth should be equal for optimal tissue development. Certain biomaterials, such as collagen or gelatin, can be degraded by cell enzymes to provide room for new tissue growth on top of scaffolds during a remodeling process. As biomaterials degrade and lose their stiffness, the newly produced tissue should be able to support load transmission. As a last point, healing won't be optimal if the biophysical properties of biomaterials don't coincide with those of the tissue. Due to this, the new tissue may not function well, tissue may be lost, and the implant may become dislodged. 65 In general, biomaterials' biophysical properties can be altered to influence cellular behavior and the surrounding environment

of living things. Signaling biomolecules, such as proteins and small molecules in the form of medicines, can be released from biomaterials, and the scaffold itself can degrade and dissolve, all of which are biochemical features of biomaterials.

IX. MODULATING EXTRACELLULAR SIGNALING:

There are two main types of in-situ tissue regeneration: activating native cells with signals from outside the cell and reprogramming cells directly through interactions between biomolecules inside the cell. Tissue regeneration can be started by priming cells outside of the cell by changing the biophysical and metabolic properties of the biomaterial [Fig.4] biomaterial scaffold that has adsorbed serum proteins modifies its surface characteristics after implantation [Fig. 5]. Attachment of endogenous immune cells to the adsorbed protein leads to the generation of cytokines and chemokines which can trigger pro-inflammatory or anti-inflammatory responses, respectively [66, 67]. These chemicals attract endogenous progenitor and stem cells into the scaffold during in situ tissue regeneration. Important step. After the cells have attached themselves to the biomaterial for the first time, they will begin the process of synthesizing and depositing nascent proteins on the surface of the biomaterial. Matrix metalloproteinases secreted by this mechanism modify the local extracellular matrix (ECM) and determine cell fate [66, 69]. This freshly produced extracellular matrix mediates bidirectional communication between indigenous cells and biomaterials.

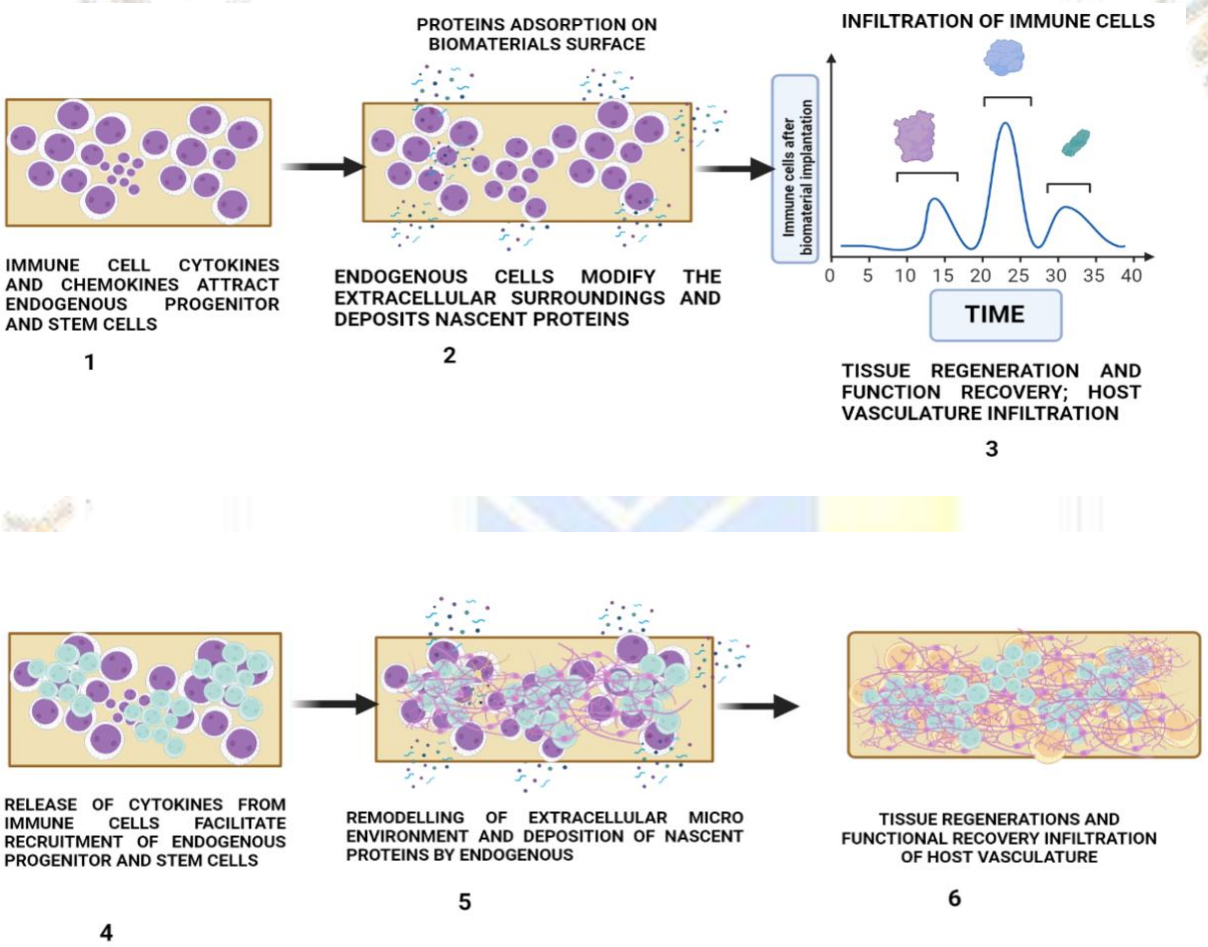


Fig. 4 Influences endogenous cells to regenerate tissue by manipulating the extracellular microenvironment. In particular, biomaterials with suitable biophysical and biochemical cues influence the immune response, enhance endogenous cell homing, tissue ingrowth, and functional tissue formation.

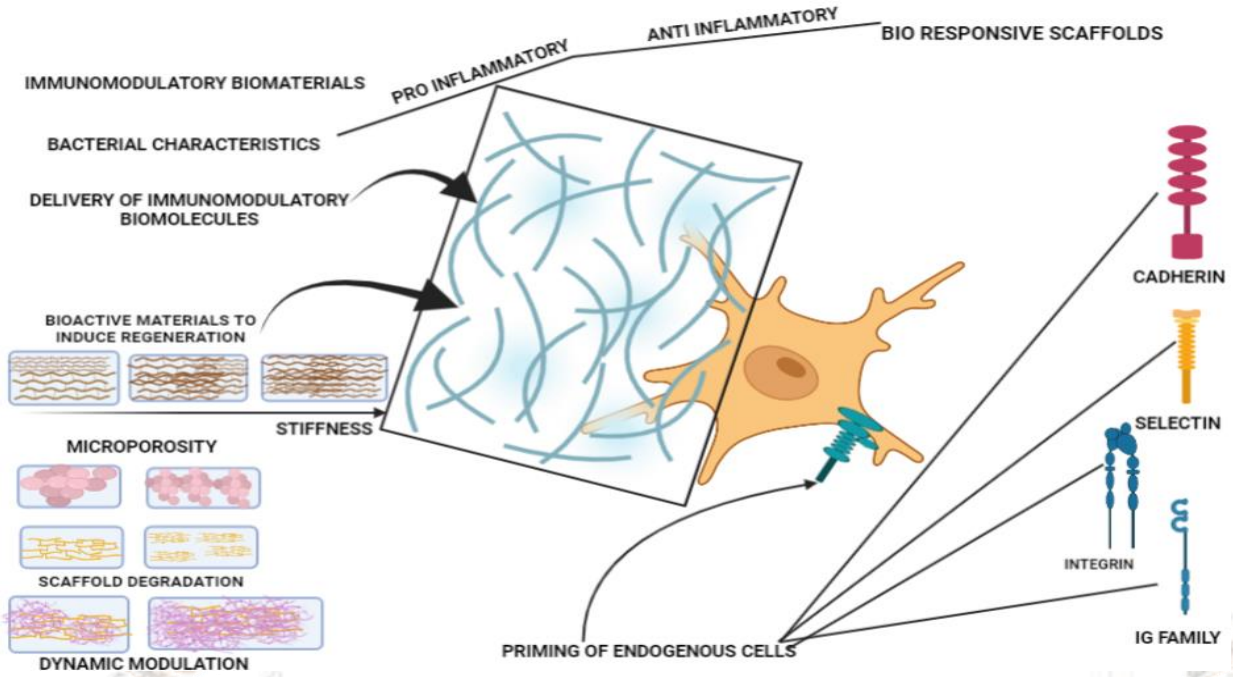


Fig.5: The adsorbate triggers an immunological response from the body's own cells, which may be pro-inflammatory or anti-inflammatory depending on the type of cytokine or chemokine they produce.

X. CELL SENSE THE MECHANICAL PROPERTIES OF BIOMATERIAL

Mechanical properties of biomaterials may have an effect on biological activities such as adhesion, migration, proliferation, and differentiation. The role that stiffness plays in regulating adhesion, spreading, and differentiation of stem cells in 2D culture settings was established in the earliest days of in vitro research. Under the same conditions, stem cells sown on matrices of varying stiffnesses (0.1-1 kPa, 8-17 kPa, and 25-40 kPa) enhanced neurogenic differentiation, myogenic differentiation, and osteogenic differentiation, respectively. Matrix stiffness influences cell fate 70, and this was found in 3D culture. When the results of 3D culture were compared to those of 2D culture, it was found that the cells' morphology and their capacity to protrude from the matrix were not significantly different due to the stiffness of the matrix. In contrast, it was shown that cellular activities are determined by the stiffness of the matrix, which regulates the molecular interface between cells and the matrix via integrin binding. Nanoparticles were used as crosslinking agents to examine the effect of matrix stiffness and structure in a distinct experimental setup (71). Without changing the polymer concentration or microstructure, this resulted in a tenfold increase in matrix stiffness48. The stiffness of the matrix determined the form and protrusion of the cells within the 3D microenvironment. To demonstrate the impact of matrix stiffness on in vivo bone regeneration, a collagen-hydroxyapatite composite was applied to decellularized bone scaffolds of varied stiffnesses [72]. The ability of these scaffolds to attract endogenous stem cells was established by subcutaneous implantation of the scaffolds. They deposit their own extracellular matrix (ECM), which remodels the microporous scaffolds, but the origin of these cells is unknown. It was shown that increasing the scaffold's rigidity led to an increase in both the synthesis of osteo-related proteins, including osteocalcin and osteopenia, as well as the amount of vascularization, which is indicative of a strong linkage between the growth of blood vessels and bone formation. This study showed that endogenous stem cells can figure out how stiff the matrix is and deposit extracellular matrix that is specific to the tissue. Using a similar method, matrix rigidity affected angiogenesis [73]. Matrixes of intermediate stiffness (800 psi) had more cellular infiltration, angiogenesis, and vascular endothelial growth factor receptor 2 expression than more elastic (700 psi) or stiff (900 kPa) gels

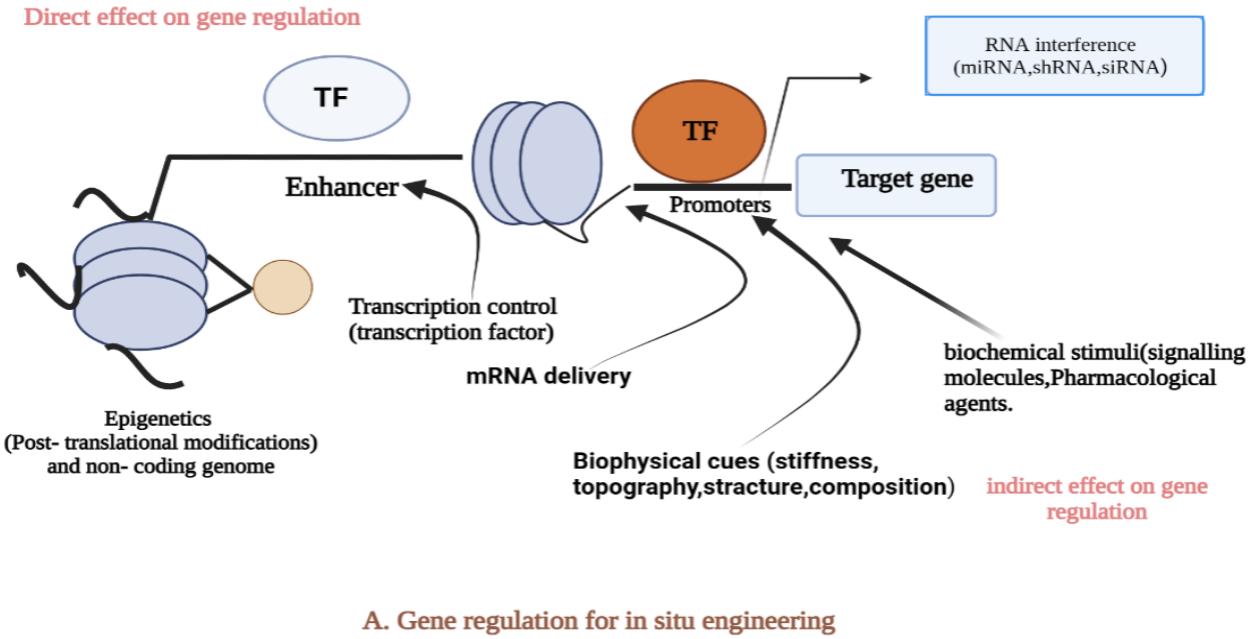
(VEGFR2). It is intriguing that the optimal stiffness necessary to stimulate angiogenesis in in vivo settings was substantially lower than in 2D in vitro environments (4 kPa). In a segmental defect model, cyclic mechanical stimulation stimulated in vivo angiogenesis and bone regeneration [74]. Bone stimulation showed this.

XI. MICROPOROSITY AIDS CELL MIGRATION AND TISSUE IN GROWTH

Cell migration and tissue ingrowth are both helped by microporosity. The microstructure and surface topology of the scaffold have an influence on the adhesion, infiltration, and lineage-specific differentiation of the cells. For instance, the adherence of human mesenchymal stem cells is facilitated by the use of microporous scaffolds, which also enhance osteogenic differentiation. In a similar manner, the osteogenic development of human MSCs is affected by the surface topography of biomaterials [75]. In addition, the microstructure and surface topography contribute to the organization of the cytoskeleton by modulating the clustering of integrins and the assembly of focal adhesions. This results in integrin-mediated mechanic transduction, which determines the destiny of the cell. The provision of an interconnected porosity network for cellular movement and tissue integration is made possible by the use of microporous scaffolds, which allow for vascularization and tissue ingrowth. In one study, for example, microporous scaffolds were created by an enzyme-mediated annealing process of homogeneous hydrogel microparticles (also known as microgels). This method was employed in the construction of the scaffolds. In the subsequent steps, these microgels might be loaded with cells or bioactive signals in order to induce migration and modify the extracellular matrix. This would be done in order to accomplish these goals. When the scaffold was evaluated under in vitro conditions, it was successful in stimulating cell migration, proliferation, and the creation of a three-dimensional cellular network within the scaffold. The development of vascular networks made it possible for the scaffold to be rapidly incorporated into the host tissue when it was subjected to testing in an in vivo environment. The presence of endothelial cells as well as pericytes within the microporous scaffolds is proof that a functional vascular network has been established. When compared to the non-porous scaffolds, it is interesting to notice that the microporous scaffold promoted the infiltration of inflammatory cells yet demonstrated a lower apparent inflammatory response. When compared to the non-porous scaffolds, the microporous scaffolds demonstrated significantly more wound re-epithelialization as well as the growth of subcutaneous tissue. This research shows that in-situ tissue regeneration can take place even when there are no exogenous growth triggers present from the surrounding environment.

XII. INTRACELLULAR REPROGRAMMING

All cells have the same DNA, but the expression of specific genes determines the cell's specific function and identity. Every gene has cis-regulatory regions (promoters and enhancers) that contain transcription factors that determine the cell's lineage and transcription factors that respond to signals. These areas control the expression of a gene. These transcription factors permit the recruitment of chromatin-remodeling complexes, which in turn trigger epigenetic alterations that unwind the DNA and render it accessible to other crucial cofactors and, ultimately, RNA polymerase II, which converts the DNA into RNA. As a result, much control occurs throughout the transcription process. In addition, the way promoters and enhancers communicate with one another across long distances determines the extent to which transcription occurs. Both internal and external influences regulate the degree to which a cell expresses a certain set of genes [Fig. 6]. Transcriptional control (through regulatory transcription factors and pause-release regulation of RNA polymerase II), RNA processing (capping, splicing, alternative cleavage, and polyadenylation), translational control (factors determining how effectively RNAs are translated), and the microenvironment all play roles in determining gene expression. There are currently five basic approaches to altering cellular function: Genetic reprogramming via clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9); overexpression of lineage-determining transcription factors; silencing (downregulation) of particular gene expression by the administration of tiny biomolecules like microRNA or pharmaceutical medicines; mRNA delivery; epigenetic alterations that occur when physicochemical characteristics of materials change or when biochemical signals are changed. Here, we provide a comprehensive breakdown of different approaches to in situ cellular reprogramming, discussing the challenges and potential solutions that lie ahead in their translation to the clinical setting.



[Fig. 6]. Internal and external influences regulate the degree to which a cell expresses a certain set of genes

(1) DELIVERY OF TRANCRIPTION FACTOR

Lineage-specific differentiation may be induced by external lineage-determining transcription factors [Fig.7]. Preserving protein integrity and function is difficult with this technique. Retroviral, lentiviral, adenovirus, and plasmid transgene incorporation methods have been developed to address this obstacle. Retroviral transduction of four master transcription factors—OCT4, SOX2, KLF4, and MYC—can reprogram somatic cells to pluripotency (trans differentiation). The modified Waddington "epigenetic landscape" illustrates trans differentiation [Fig.8]. Trans differentiation is promising for osteoarthritis, neurological disorders, and myocardial infarctions when endogenous stem cells fail to migrate and regenerate tissue. These methods can induce lineage-determining transcription factors through viral DNA transcription, although foreign sequences may cause genetic changes in target cells [76]. This method might reprogram endogenous cells if an alternative transcription factor carrier can overcome these problems. Intracellular delivery uses synthetic nanoparticles, injectable hydrogels, electro spun scaffolds, and microspheres. Synthetic nanomaterials are ideal for cellular reprogramming due to their stability, biocompatibility, shelf life, and loading efficiency [77, 78]. PEG81 bioinert polymeric nano capsules may passively carry transcription factors. In situ polymerization encapsulated MYOD1 in PEG nanoparticles. The nanoparticles delivered MYOD1 intracellularly to myoblast cells, which translocated to the nucleus and started myogenic differentiation into skeletal, cardiac, or smooth muscle cells. The lipofectamine-transfected MYOD1 plasmid findings were identical. Thus, transcription factors may control cell differentiation ex vivo without incorporating external genetic information into the host genome. Passive nanoparticle intracellular delivery yields less than active targeting.

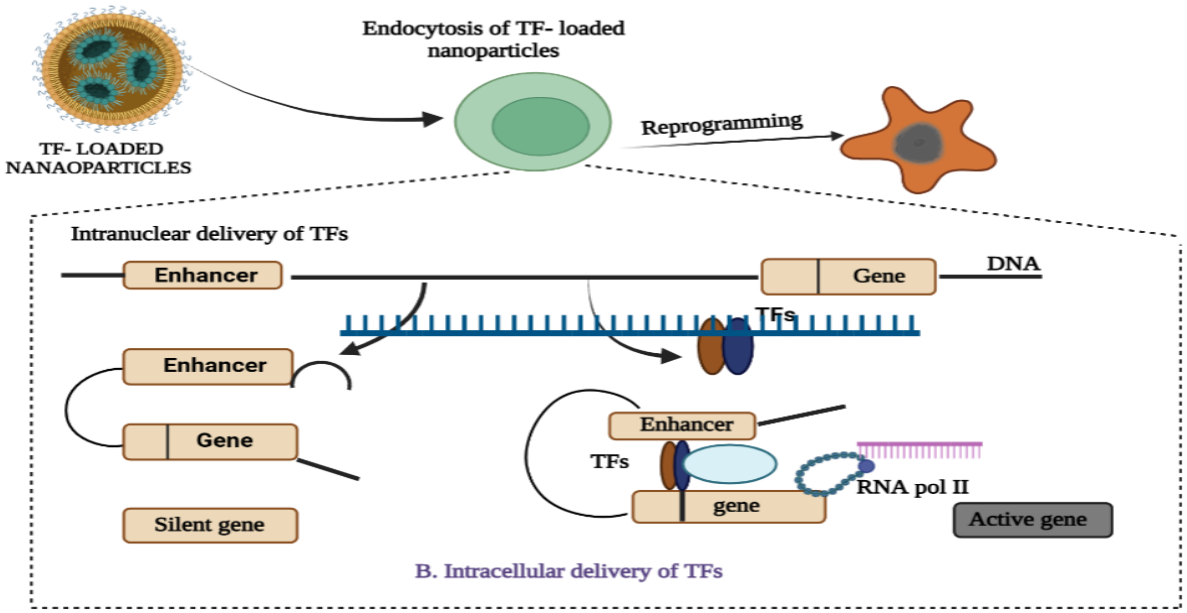


Fig.7 Intracellular Delivery of TFs Loaded Nanoparticles.

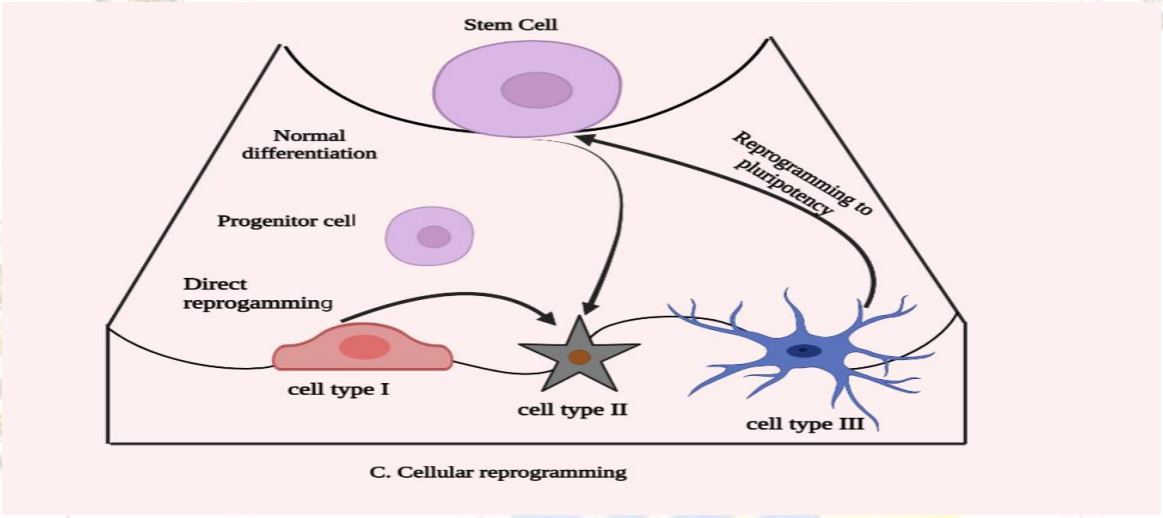


Fig.8 cellular reprogramming epigenetic.

(2). RNAi-BASED THERAPEUTICS

MicroRNAs (miRNAs) or small interfering RNAs (siRNAs) may be delivered intracellularly to inhibit gene expression through RNA interference (RNAi) (Fig. 6d). RNAi molecules are difficult to transport. Due to their phosphate backbones, nucleic-acid biomolecules cannot diffuse across negatively charged surfaces [Fig.9]. Reprogramming of cells in situ for the purpose of tissue regeneration Epigenetics, transcriptional regulation, RNA processing, the local biophysical and biochemical environment, and external stimuli are just a few of the variables that can affect how genes express themselves at various stages.

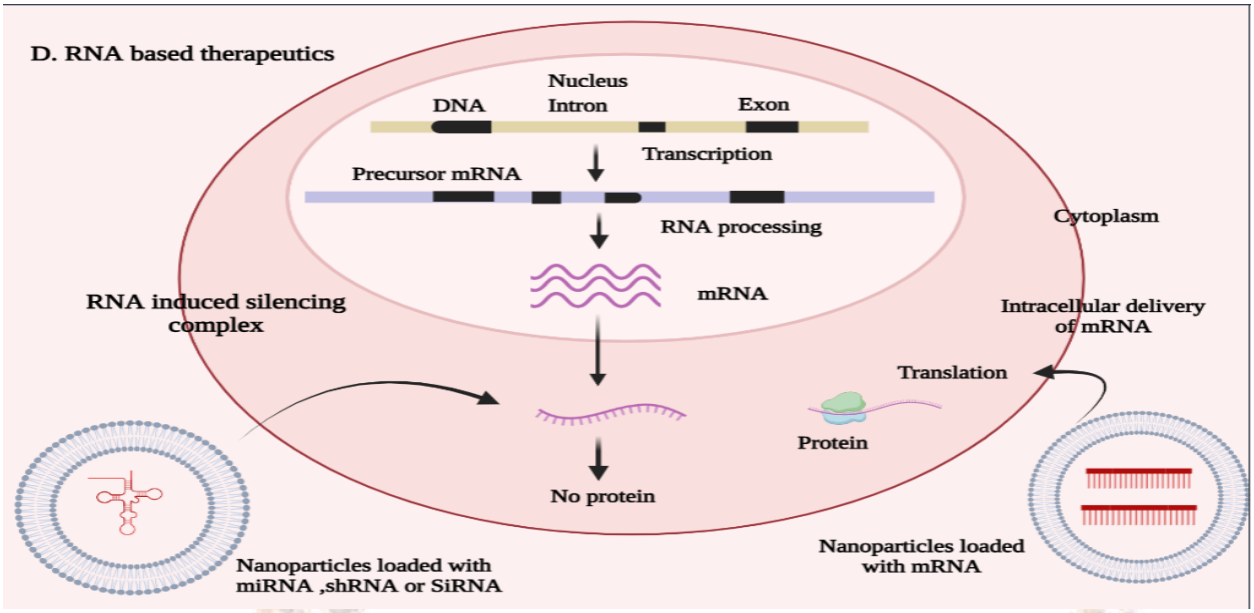


Fig.9: RNA based therapeutics

There is potential for the development of biomaterial-based gene regulatory technologies. It is possible for transcriptional factors, also known as TFs, to remodel chromatin when they are supplied intracellularly to cells that have been reprogrammed from one cell type to another. This may activate and repress certain gene-expression programmes. Pioneer transcription factors have the ability to reprogrammed cells. RNA-based proteins and gene silencing therapies RNA interference may be done through intracellular administration of miRNA, shRNA, or siRNA (siRNA). Biomaterials may cause epigenetic changes, including DNA methylation and histone deacetylation. RNAi molecules are often expressed via viral delivery vectors, which may cause mutagenesis and immunogenicity. Liposomes, polymeric nanoparticles, hydrogels, nanofibers, and microporous scaffolds are being explored as synthetic biomaterials. MicroRNAs are small RNA molecules that do not code for proteins and are produced in the nucleus by RNA polymerase II. They are then processed to attach to messenger RNA (mRNA) by base pairing complementary sequences. Because of its binding, translation is repressed and mRNA is degraded, both of which contribute to the silence of genes and the regulation of gene expression. MiRNA overexpression or inhibition regulates many growth factors. Angiogenesis, immunity, and tissue regeneration depend on miRNAs. Localized miRNA administration increased scaffold vascular volume by more than twofold. Electro spun scaffolds guided indigenous cells, whereas collagen hydrogels included miR-222-loaded micellar nanoparticles 81. In vivo axon regeneration and remyelination show that biophysical and pharmacological techniques may regenerate tissue in situ. siRNAs, exogenous double-stranded RNA, attach to and cleave their target mRNA to silence genes. SiRNAs have one mRNA target and need complete complementarity, whereas miRNAs have numerous targets and just need 2–7 nucleotide seed complementarity. Cancer therapies based on siRNA have received little attention in regenerative medicine. An early study used a synthetic hydrogel to deliver noggin siRNA (a BMP blocker) and rhBMP2 to mice's dorsal muscle pouch, resulting in ectopic bone growth 82. siRNA did not affect bone healing. Noggin siRNA and miRNA-20a were co-delivered in rat calva rial defects to suppress PPAR, a negative regulator of BMP2-mediated osteogenesis83. SiRNA and miRNA influence mRNA levels via being transported to the cytoplasm, making them easier than gene editing.

(3). STIMULATION OF PROTEIN TRANSLATION

The development of mRNA-based therapies has recently seen a resurgence due to breakthroughs in chemically improving the stability and immunogenicity of mRNA [84–86]. The transfer of mature mRNA from the nucleus to the cytoplasm triggers the initiation of protein synthesis via the ribosomal machinery. Hence, several nanoparticles, both polymeric and inorganic, may be used for mRNA intracellular delivery [Fig.9]. One major advantage of mRNA transport over plasmid DNA (pDNA) delivery is that intranuclear dispersion is not required. Direct mRNA injection further decreases the potential for unintended outcomes since it does not involve permanent integration of the genetic material into the host genome. In addition, the ability of non-dividing or

slowly dividing cells to transfer mRNA to the cytoplasm may enhance protein synthesis. In contrast, pDNA is more efficient at promoting cell growth, but it must be provided in the nucleus. A recent study compared pDNA and mRNA delivery for ocular applications using cationic polymer nanoparticles [87]. Enclosing mRNA in polymeric nanoparticles greatly improves its stability and reduces its immunogenicity. As compared to pDNA delivery, the amount of protein generated via mRNA injection was much higher in vitro. More than 1,800-fold more protein was synthesized when mRNA was chemically modified instead of pDNA. Nanoparticles were required for mRNA translation in vivo (bolus administration did not result in protein creation). Related studies transferred mRNA using lipid nanoparticles to generate therapeutic proteins for the treatment of genetic diseases (93). To improve the efficiency of drug delivery, a library of lipid nanoparticles with varying compositions was developed. Only endothelial Kupffer cells, not hepatocytes, were transported by oxidized cholesterol-containing nanoparticles [88]. Nanoparticle-specific protein coronas are considered to be responsible for the selective uptake of nanoparticles by some cells, although the exact mechanism for this absorption is uncertain. The researchers also demonstrated that therapeutic mRNA may be administered selectively in vivo to boost endogenous protein production.

(4) IN VIVO GENE EDITING

Mega nucleases, zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), and CRISPR-Cas9 are just a few examples of the engineered nucleases, or molecular scissors, that may be used to directly edit the genome and control endogenous genes (see references 89–91). Two processes, non-homologous end joining (which results in indel mutations and gene silencing) and homology-directed repair (HDR), are responsible for repairing the site-specific double-strand breaks in the genome caused by these nucleases [Fig.10]. As a nuclease, CRISPR-Cas9 has the potential to be used for in situ tissue regeneration given to its low cost, high efficiency, wide range of applications, flexibility in adapting to different cell types, and little off-target effect. CRISPR-Cas9 gene editing is transmitted by adeno-associated viruses [92,93]. Nevertheless, delivery by virus results in immunogenic issues, off-target repercussions, and weak efficacy. Non-viral CRISPR therapeutics may make use of gold nanoparticles loaded with guide RNA, donor DNA, and Cas9 protein [94]. Donor DNA and Cas9 protein were loaded into nanoparticles using this technique because to their DNA coating. After being taken up by cells, this nanoparticle mixture was designed to escape the endosomes. HDR was confirmed in human embryonic stem cells in vitro. After injecting the loaded nanoparticle into mouse skeletal muscle, gene editing happened in the area immediately around the injection site. Dystrophin protein synthesis was revived and the mutated gene was fixed in a mouse model of Duchenne muscular dystrophy. Muscle damage from cardiotoxins triggers natural HDR processes, although this toxin is never used therapeutically. Cardiotoxin increased the efficiency of editing from 5% to 9%. Even with 1% efficiency, editing only partially restored muscle function. Injections of these nanoparticles were given repeatedly with no ill effects, suggesting that in situ gene editing is safe. In vivo gene editing with synthetic nanomaterials for tissue regeneration: a possible application Gene editing in vivo employs a different class of synthetic nanoparticles. These range from lipid nanoparticles to exosome-liposomes to PEGylated helical polypeptide nanoparticles to lipid-coated gold nanoparticles to PEG-PLGA nanoparticles [95–98]. Just as before, these nanoparticles have low editing efficiencies and cause off-target gene editing. In order to get over these restrictions, biomaterial implants are used. We used bio adhesive coating 99 to apply Cas9 and a single-guide RNA complex to electro spun scaffolds. In-situ tissue regeneration by targeted gene editing may be feasible using biomaterial scaffolds.

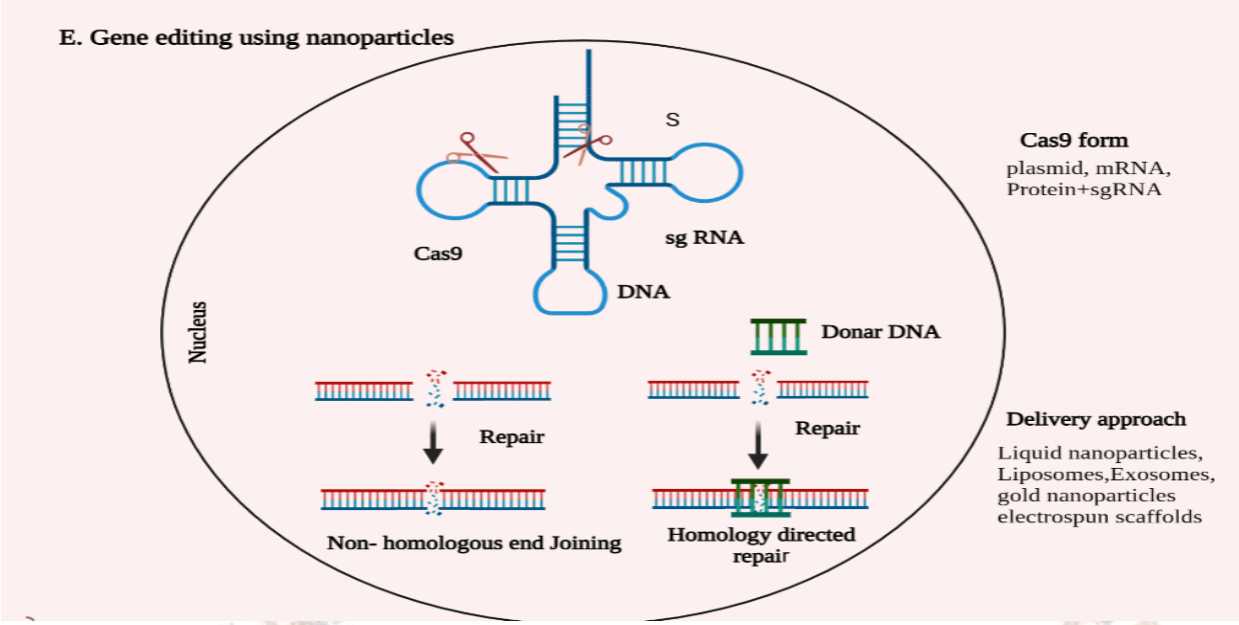


Fig.10. Gene editing using Nanoparticles

XII. CONCLUSION

In this summary, direct reprogramming is an encouraging medical approach for treating degenerative diseases, curing incurable diseases, simulating diseases, and creating new drugs. More so, biomaterials may enable this method to be more effective and stable. There are, nevertheless, a great number of obstacles that must be conquered. The method for interlineage conversion by direct reprogramming is not yet well-established. The efficiency of direct reprogramming is currently too low to create enough cells for therapeutic therapy, and the safety has not been shown in longer-term clinical studies. It is also important to reduce the heterogeneity and immaturity of converted cells. Biomaterials need a completely synthetic reprogramming microenvironment for quality assurance and handling of immunogenic issues. On the other hand, biomaterials have not been shown to significantly boost the efficiency of direct reprogramming in any published studies or reports. In general, it is necessary to form the biochemical cue and physical cue combination that is most efficient for any direct reprogramming procedure. In addition, for clinical use, it is important to think about how to balance treatment efficiency, convenience, and the amount of time it takes to complete.

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