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The Impact of Cellular Microenvironments and Biomaterials on Induced Pluripotent Stem Cell Generation

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Abstract – The implications of the microenvironment in reprogramming and cellular differentiation are very important and should not be overlooked. All components from the extracellular matrix (ECM) to physical properties of biomaterials play a significant role. Mechanotransduction, which happens through integrins and adapter proteins refers to cells' ability to sense mechanical stimuli. Studies have demonstrated that ECM stiffness affects fate and specification of cells. Similarly, composition of biomaterials such as toughness, diameter of fibers, and substrate topography may influence reprogramming process and cell growth. Therefore, when signaling molecules like growth factors are introduced, ECM composition may be critical in determining cell fate. Matrigels made from fibrous protein called fibrinogen or collagen display an apt medium for growth and differentiation during stem cell research over time. The paper discusses how microenvironmental factors control motility and cellular determination in detail. The article explores the distinct impacts of mechanical stimulation and nanotopography on the regulation of stem cells inside the extracellular matrix. Finally, it highlights that altering biomaterials may predestine the cell fate after they have been reprogrammed into a pluripotent state, since they induce pluripotency inside embryonic bodies. This comprehensive article provides information on the principles that impact regenerative medicine concerns, specifically in terms of stem cell biology.

Keywords – Induced Pluripotent Stem Cells, Embryonic Stem Cells, Extracellular Matrix, Endothelial Progenitor Cells, Mesenchymal Stem Cells.

I. INTRODUCTION

Stem cells possess the capacity to undergo differentiation and self-renewal. This is the process of cellular differentiation, when a pluripotent cell undergoes specialization into a certain lineage or cell type. During the majority of the first half of the 20th century, a widely held belief was that mature cells were permanently limited at a certain level and incapable of returning to their young, pluripotent condition. In 1962, John Gurdon made a groundbreaking discovery by demonstrating the reversibility of cell differentiation. He demonstrated this by showing that nuclei from fully developed Xenopus intestinal epithelial cells could generate fully functioning tadpoles when transplanted into oocytes with no nucleus [1]. The process of regenerating is now referred to as reprogramming.

The reprogramming procedures discussed by Lewandowski and Kurpisz [2] have enabled the somatic cells transformation into pluripotent stem cells that nearly resemble ESCs (Embryonic Stem Cells). ESCs are highly regarded as the ultimate solution for unlimited regeneration because of their capacity to transform into various types of cell in the body and repair injured tissue. Critics of this method often highlight the possibility of unethical use or allocation of ESCs, which possess the capacity for generating life and forming embryos. The discovery of a technique to generate iPSCs (induced Pluripotent Stem Cells) allows us to use their regenerative capabilities. iPSCs are closely similar to ESCs but have minimal ethical issues associated with ESC resources. iPSCs may be easily cultivated in the lab from various types of somatic cells for research reasons. The overexpression of four key pluripotent factors, namely Sox2, Oct3/4, Klf4, and c-Myc has been shown to enable different lineages` somatic cells undergo changes in both their morphology and pluripotent potential. It has been shown that Nanog and Lin28 can substitute c-Myc and Klf4 when combined with Oct3/4 and Sox2 in the process of

converting cells into iPSCs. Oct4, Sox2, and Nanog overexpression can transform human fetal gut mesentery-derived cells into iPSCs.

While Nanog is not necessary for reprogramming, it is crucial for the capacity of the creation and self-renewal of stable iPSCs [3]. For many instances, like adult mouse neural stem cells, the mere presence of a single factor (Oct4) is enough to create iPSCs, even with the use of episomal reprogramming. One possible explanation is that these neural stem cells generate Klf4, Sox2 and c-Myc intrinsically. Additionally, there might be a substantial increase in the efficiency of iPSC formation when p53 is downregulated using knockdown and knockout techniques. It has been found out that lack of p53 can be compensated for by the presence of Oct4 and Sox2 on their own leading to iPSC production. Somatic cell reprogramming, which is a new technique to cell identity and control over fate, has changed our traditional understanding. This process involves transforming one type of cell into another by specific proteins.

A direct conversion technology is now more preferable in creating therapeutic cells because it produces many types of cells today. Many different required cellular kinds have already been created using this technology during various investigations. Furthermore, in many cases it is usually shorter and simpler compared to an iPSC redifferentiation followed by comparison with them. Furthermore, it avoids the usual risk of teratoma formation that is often linked to the intermediate iPSC`s pluripotent stage. Currently, it is considered as one of the most auspicious methods for regenerative and personalized medicine, as well as for creating disease models tailored to individual patients for drug screening purposes. Furthermore, it enables the tracking of human disease development, like Alzheimer's, which was previously only possible at the last stage. Various non-integrating delivery methods are being investigated and utilized to achieve iPSCs and direct reprogramming. These methods include episomal plasmids, messenger RNA (mRNA), micro-RNA (micro-RNA), proteins, and small molecules, although lentiviral and retroviral vectors were the most often used in the early studies. Recently, biomaterials have been discovered to have a significant impact in controlling the reprogramming process.

This article delves into the crucial role of the microenvironment in determining guiding migration and cell fate and guiding migration. Through a focused lens on mechanical stimuli and nanotopography, we aim to uncover how these factors precisely shape cells. In addition, we will analyze the intricate control of stem cell development and self-renewal via the extracellular matrix (ECM) and other elements present in the niche. In addition, the paper examines the process of converting somatic cells into iPSCs, while also addressing the challenges and prospective applications of iPSCs in the fields of therapies and disease research. This study furthermore seeks to establish the part played by biomaterials in programming cells, and their physical or biological attributes that may affect cell reprogramming outcomes. On the other hand, it also places emphasis on growth factors and signaling molecules, which regulate differentiation and proliferation of stem cells.

This paper is organized as follows: Micro-environments on cell fate migration and determination are critically described in Section II. This section analyses mechanotransduction, cell differentiation microenvironmental regulation, and microenvironmental regulation of migration and adhesion. Section III reviews biomaterials in IPSC reprogramming. The concepts and aspects (physical aspects, biochemical aspects, and gene delivery biomaterials) of biomaterials in direct reprogramming have been described in Section IV. Lastly, Section V draws a summary of the research as well as future research.

II. MICROENVIRONMENTS ON CELL FATE DETERMINATION AND MIGRATION

Mechanotransduction

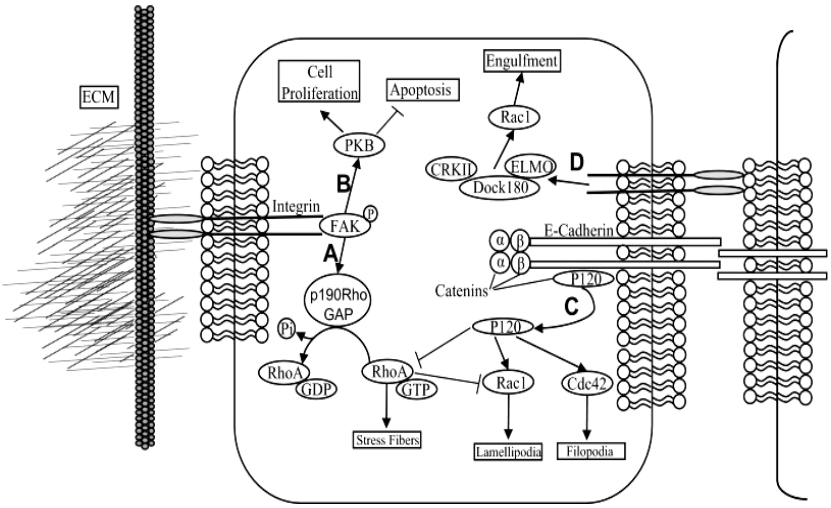

Fig 1. The Relationship Between Cells

Fig. 1 shows the relationship between cells and the ECM, as well as the communication between cells facilitated by E-cadherin and integrins. Integrin-mediated phosphorylation of FAK triggers the activation of p190 RhoGAP, which leads to the reduction of stress fibers (A). Additionally, it stimulates protein kinase B (PKB), which enhances cell survival (B). The p120 catenin dissociation from the E-cadherin-catenin complex (C) leads to the activation of Cdc42 and Rac1, while

simultaneously inhibiting the actions of RhoA. The activation of Rac1, facilitated by integrin, induces the engulfment of neighboring apoptotic cells (D).

Cells possess the ability to translate mechanical stimuli into biochemical signals via mechanotransduction mechanisms. The capacity to perform this function is a result of the connection between several adaptor proteins and integrins, including as vinculin, FAK, paxillin, p130Cas, and talin. Cardiosphere-derived cells (CDCs) detect the rigidity of the nearby ECM by means of p190RhoGAP, a protein that activates guanosine triphosphatase (GTPase) for RhoA. This activation process facilitates the differentiation of CDCs into endothelial cells, as shown in Figure 1A. A worldwide decline in the occurrence of p190RhoGAP, together with a specific drop in the lamellipodia, causes a transformation in cell structure to a more circular cell shape. This, along with reduced integrin levels, may diminish cell interaction with the extracellular matrix, leading to the formation of spheroid-like clusters. These compact cell clusters need the presence of p120-catenin and E-cadherin, as well as Yes-Associated Protein (YAP) augmented nuclear localization.

The YAP and TAZ proteins, which are similar to the yorkie homologs, function as transcription regulators without relying on the NF2/Hippo/LATS pathway [4]. P190RhoGAP retains TFII-I in the cytoplasm of microvascular epithelial cells. TFII-I induces the transcription and overexpression of VEGFR2 (Vascular Endothelial Growth Factor Receptor 2) upon its release, leading to the occurrence of angiogenesis. Factors outside the stiffness and content of the ECM might potentially affect the mechanotransduction signals. The nanotopography of the ECM may influence the process of cellular differentiation, proliferation, and migration. The arrangement of cell skeleton and focal adhesions in human MSCs was changed by the ECM's nanotopography. This process is facilitated by FAK pathway, which controls the transcription of YAP/TAZ, hence defining the cell lineage.

Cell Differentiation Microenvironmental Regulation

The ECM, cytokines, growth factors, small molecules and morphogenic factors that are released by stem cells and neighboring cells in their microenvironment aid in the control of stem cell differentiation as well as self-renewal. Several stem cell lineages are determined directly by the stiffness of the ECM. Adult neural stem cells cultured on soft matrigel (<1000 Pa) underwent differentiation into glial cells, but those cultured on stiff matrigel (10-100 kPa) differentiated into neurons. When it becomes less than 10 Pa, adult neural stem cells lose their ability to differentiate or undergo self-renewal [5]. The undifferentiated MSCs also demonstrate how this happens during differentiation due to ECM stiffness. Osteoblasts can be obtained from a single colony simply by implanting isolated colonies onto these different matrices at 1 kPa osteogenic matrix stiffness after getting rid of any MSC contamination from other tissues. The three levels of stiffness mentioned correspond to the typical stiffness seen in bone tissue, muscle, and brain.

Through independent tests, it was shown that Mesenchymal Stem Cells (MSCs) grown in two-dimensional environments exhibited a direct relationship between the commitment of the cell type and the physical structure of the stem cell. Investigators have shown that, in 3D culture settings, the cell type commitment is not influenced by the shape of the cells, but rather by the stiffness of the environment. Adipogenic commitment occurs in situations with lower pressure levels ranging from 2.5 to 5 kPa, while osteogenic commitment occurs in conditions with higher pressure levels ranging from 11 to 30 kPa.

Cellular development of skeletal muscle necessitates interactions between cells and the ECM. In vivo, muscle stem cells present in adult tissues undergo fast proliferation. However, when same cells are cultivated on hard plastic plates with a stiffness of 106 kPa, their proliferation rate is not as rapid. Nevertheless, when the cells are cultivated in matrigel, which has 12 kPa`s stiffness, resembling the muscle tissue stiffness, the MSCs will persist in self-renewal within a laboratory setting. Moreover, these cells may be reimplanted into mice and substantially contribute to the restoration of muscle. The stiffness of the ECM controls the process of chondrocyte development by activating ROCK signaling and increasing the expression of TGF-β. Chondrocyte differentiation necessitates the phosphorylation of Smad3, which is most effective when the ECM stiffness is at a level that also triggers the production of chondrocyte genes.

Microenvironmental Regulation of Migration And Adhesion

The study of microenvironmental modulation of cellular function and shape has focused extensively on cell adhesion and migration. Extensive research has been conducted by Kong and Mooney [6] to comprehend the molecular and macromolecular regulators as well as the signaling cascades that are influenced by microenvironmental features. Specifically, there has been substantial research on integrins and their operations with the ECM in relation to their function in maintaining homeostasis and facilitating the development of various illnesses. Integrins are receptors of cell surface composed of subunits of α and β that form a heterodimer. They have the ability to bind to certain elements of the ECM and play a vital role in activating several pathways related to cell motility and cell survival.

After integrin ligation, FAK undergoes autophosphorylation at Y397. This phosphorylation creates phosphatidylinositide 3-kinase (PI3K) binding site for the p85 subunit, leading to protein kinase B (PKB) activation. As a result, apoptosis is inhibited and cell proliferation is promoted (see Figure 1B). When this route remains undamaged, the detachment from the extracellular matrix leads to a reduction in PI3K activity, ultimately causing anoikis. Integrins have a role not only in communication between cells and the ECM, but also in signaling between cells themselves. Integrins in other cells recognize apoptotic cells that have phosphatidylserine on their cell membranes. This initiates a signaling cascade that activates the

Dock180, CRKII, ELMO complex, leading to the activation of Rac1. This, in turn, induces the engulfment of the neighboring apoptotic cell.

III. BIOMATERIALS IN IPSC REPROGRAMMING

The ability to reprogram somatic cells into iPSC is no longer restricted to the researchers who have created different techniques in their own laboratories. Numerous advances in restructuring gene delivery have been made since "Yamanaka Factors" discovery in 2006, when four least genes—Oct3/4, c-Myc, Sox2, and Klf4—were discovered as important iPSC factors of reprogramming [7]. These developments include the delivery of restructuring genes as messenger RNA, or lentiviral transgenes. Each of these platforms is now economically accessible in kits of reprogramming, enabling most laboratories to reprogram iPSC from various tissues. The Sendai virus reprogramming vector has been the most effective for our group. The self-limiting and non-integrating murine parainfluenza virus effectively transmits the four necessary genes of iPSC in a single polycistronic message, as shown in Figure 2B. iPSCs have a remarkably pluripotent nature and may be used to differentiate into many lineages, regardless of the approach employed.

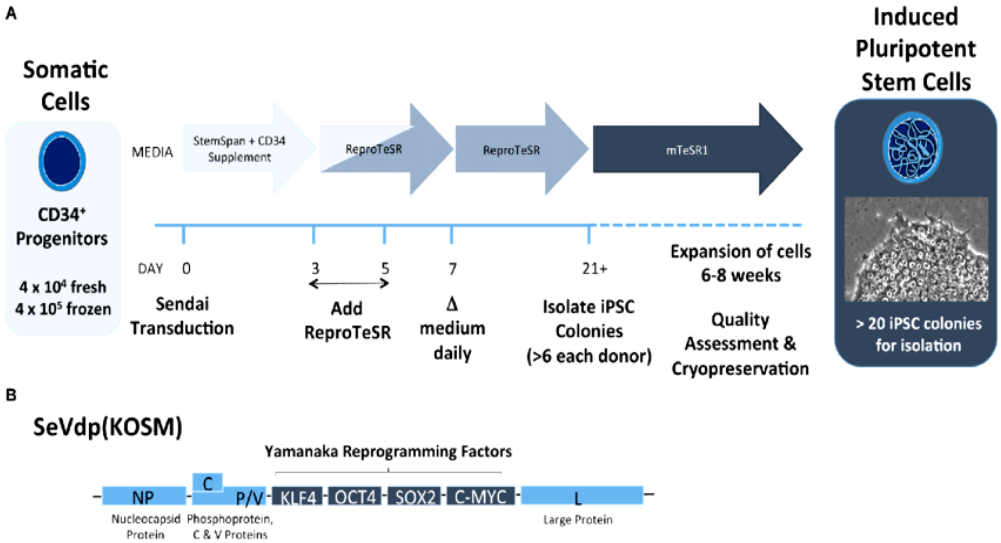

Fig 2. Schematic Representation of the Process of Converting CD34⁺ Cells into Ipscs Using Sendai Virus

Sendai virus affects CD34⁺ progenitor cells on day 0 and are then cultivated in circumstances without feeder cells on plates covered with Matrigel. The cells are cultured in a medium that supports the growth of CD34⁺ cells, such as StemSpan SFEMII supplemented with StemSpan CD34⁺ growth Supplement, for the first three days. On days 3 and 5, a medium that facilitates reprogramming, such as ReproTeSR, is introduced. From day 7 onwards, the medium is replenished every day until the colonies reach a sufficient size for clonal isolation, which usually occurs on day 21. Following isolation, cells are maintained in a PSC medium of maintenance, such as mTeSR1, for a period of 4 to 6 weeks to allow for growth. Following growth, iPSCs are evaluated for their capability to contrast into various cell types and their genetic stability, and may be stored by freezing for future use. Sendai genes NP (nucleocapsid), V protein, P (phosphoprotein), C protein, L (large protein), is comprised by the replication-defective Sendai virus (SeVdp KOSM) as well as Yamanaka reprogramming genes c-MYC, KLF4, SOX2, and OCT4.

A complete reordering of somatic cell epigenomes is necessary to convert a fully developed somatic cell into an induced pluripotent stem cell. This stage is crucial in going from specialization to pluripotency. In order to do so, dormant genes related with the pluripotency must be turned on, while those responsible for functions in tissues and organs other than reproductive ones have to be switched off. The first step of these changes begins through transcriptional conditions that result from the ectopic overexpression of Sox2, Klf4, c-Myc and Oct4. These proteins act as transcription factors that bind to DNA to control gene expression. Therefore, there are several mechanisms by which chemicals may enter cells, regardless of the use of viral vectors. Moreover, transgenes may sometimes integrate into the cellular genome during this process, while there are instances when they do not.

However, it is not easy delivering Yamanaka factors due to complex epigenomic alteration. Meticulously observing each step will help achieve success in reprogramming. Firstly, this involves scrutinizing the somatic cells then selecting specific ingredients and routes for administration. Besides that, it is important to know that inclusion of small molecules or growth factors in the media may disrupt signaling pathways too much like themselves when programmed as if it was very specific directions given by an instructor. Subsequently, reprogramming requires optimization of culture conditions after reprogramming that increase iPSCs proliferation without compromising their pluripotency. Consequently, the structure of this complex process may consist of different types of cells at various stages of reprogramming. Therefore, the categorization of these cells as iPSCs should undergo careful examination and meet some criteria for their correct identification. Thus, a number of issues might affect what kinds of iPSCs can be derived from them as well as their possible future applications in research on cellular programming methods. Naturally, one major objective is finding out useful therapeutic applications for

these cells. Nevertheless, these structures also pose additional challenges due to their complex organization and vulnerability to genetic changes during reprogramming.

The study of tumor tissues and related problems like immune response and tumorigenicity are currently being investigated by researchers. The former method involves the culture of human primary hepatocytes in co-culture with MSCs that are derived from bone marrow aspirates. Additionally, another one focuses on thwarting cytoplasmic tyrosine kinases linked to platelet-derived growth factor receptor VEGF receptors, along with other signaling pathways implicated in tumor growth, so as to hinder angiogenesis. Also, conditional knockout models using tissue-specific expression or deletion approaches are under development by scientists. All these activities involve converting iPSCs into specific cell lineages or generating a collection of haploid cells.

Using iPSCs in disease modeling is considered one of the most significant applications that have emerged due to the therapeutic advances made so far. Moreover, patient-specific iPSC generation procedures allow for formation of specialized cells with unique genetic imprints or modifications. These methods would be useful because they enable obtaining these cells which cannot be easily obtained directly from tissues. This is an important approach because it allows for retrieval of such cells whose direct collection from tissues could be problematic. In vitro disease modeling may provide more accurate animal data sets during preclinical stages of drug development thereby reducing the need for in vivo models. IPSCs can extensively contribute to basic research aimed at elucidating causes of diseases and physiology as well as toxicological testing or drug screening. Even though a new area requiring extensive examination, remarkable strides have been realized in terms of human aspect concerning iPSC technology.

Nevertheless, the area of veterinary iPSCs is much less advanced and has a scarcity of publications. The first documentation of canine iPSCs was published in 2010, followed by horse iPSCs in 2011, and felids iPSCs in 2012 [8]. Most research in veterinary species have mostly concentrated on the production of iPSCs and their use in laboratory settings, particularly for the development of cell types that are significant for clinical or disease modeling purposes [9]. However, only a small number of studies have explored the application of iPSCs in live animals. The understanding of human iPSCs might significantly enhance progress in the field of veterinary medicine, since many of the concerns and difficulties are common to both human and veterinary healthcare. Nevertheless, it is important to enhance our comprehension of the distinctions between various species. The analysis of epigenomic landscape, pluripotency networks, and iPSCs identity and their derivatives should be approached by comparing different perspectives rather than simply applying findings from human studies.

The use of conventional lenti- and retroviral-based vectors for delivering reprogramming genes may lead to the viral DNA random integration into the host genome, which might possibly activate oncogenes. The viral vectors usage is obviated in all in vivo implementation. Yamoah, Thai, and Zhang [10] achieved great efficiency in generating mouse iPSCs by using disposable cationic polymer PEI-coated superparamagnetic nanoparticles as biomaterials for temporary gene expression of iPS. This method outperformed existing non-viral methods. Furthermore, more than 60% of the iPSCs generated using this nanofection approach were devoid of incorporated foreign DNA. The molecular constituents of biomaterials, like other ligands or ECM, unquestionably have an impact on iPSC reprogramming. An instance of this is when chemically-defined self-assembled monolayers exhibit heparin-binding peptides that are extracted from vitronectin. These peptides enhance cell adhesion by interacting with glycosaminoglycans on the cell surface. It has been observed that these monolayers are particularly effective in the prolonged cultivation and preservation of pluripotency in human iPSCs. Moreover, Mao et al. [11] have shown that collagen acts as an obstacle to reprogramming. By inhibiting gene expression or using collagenase therapy on mouse fibroblasts, the efficacy of reprogramming may be enhanced.

The importance of the biophysical properties of substrates in guiding the reprogramming of adherent cells has been shown to be crucial. For instance, Zhang et al. [12] illustrated how the physical properties of biomaterials like parallel microgrooves and nanofibers can change cell shape and increase reprogramming efficiency. This leads to more colonies of cells that express Nanog, which is necessary for successful reprogramming. Mechanical stimulation modifies the epigenetic state of mouse fibroblasts resulting in this improvement. HDAC2 inhibition led to increased histone methylation whereas WDR5 overexpression induced a raised acetylation level. Additionally, using topographic cues resulted in enhancing mesenchymal-to-epithelial transition (MET), hence improving process of reprogramming iPSCs. Barui et al. [13] observed that different nanofiber polymer compositions had specific effects on MET during human fibroblast reprogramming.

Gerardo et al. [14] review the impact of stiffness by fibroblast cells on the reprogramming of iPSCs along with surface characteristics of biomaterials. The initiation of reprogramming of adherent cells using 0.1 kPa polyacrylamide gels resulted in higher expressions of Oct4 and Sox2 compared to the use of 20 kPa gels. Previous research has shown that altering the stiffness of the substrate may cause a reprogramming effect in HEK-293 cells. This effect occurs only via the modulation of actin force, without the involvement of any transcription factors. In a recent work, Caiazzo et al. [15] investigated the feasibility of developing a synthetic “reprogramming niche” that can precisely adjust and imitate the degradability, hardness, and biochemical composition of the natural microenvironment found in live organisms.

Romanazzo et al [16] sought to improve the efficiency of reprogramming in iPSC generation through optimization of PEG-based hydrogels. They found that when laminin and Epcam were present, an optimal range of hydrogel stiffness from 300-600 Pa resulted in the best performance during this process. The interplay between ECM-hydrogel interactions and substrate stiffness is important for cell differentiation programming and reprogramming.

In a study conducted by [17], it was observed that restraining cell shape using 3D systems increased reprogramming efficiency more than twofold in mouse and human iPS cells. This was due to mesenchymal-epithelial transition (MET) and epigenetic changes characterized by increased levels of Histone 3 acetylation and methylation. Also, colonies formed better because this method favors growth of only these colonies while restricting non-colony forming cells. In addition to improving the efficiency of reprogramming, this proof-of-concept work also established a model system that may be used in future studies to systematically analyze the relationship between biophysical parameters and genetic alteration, independent of transcription factors.

Remarkably, Kim, Hong, and Rhee [18] identified a similar confinement notion when they found that a microfluidic culture method may significantly enhance the efficacy of artificial mRNA-driven human iPSC postponement by up to 50-fold in feeder-free and xeno-settings. According to Totaro et al. [19], this impressive outcome is partly attributed to the reduction in the soluble signaling particles concentration in the culture medium resulting from the downscaling process. Physical confinement within the microfluidic ecosystem was proposed as an additional factor that contributes significantly to the improved efficiency. This is achieved through the secreted factors extracellular accumulation produced by cells that are already going through reprogramming. These factors likely serve as signals for neighboring cells to initiate the same reprogramming process.

The aforementioned confinement impact is not anticipated to be seen in a typical two-dimensional culture. Contrarily, Sia et al. [20] found an improvement of reprogramming mouse iPSCs efficiency by using dynamic culture throughout the reprogramming mid to late stages. Zhang et al. [21] ascribe this phenomenon to convective mixing, which hinders the cessation of the cell cycle upon reaching confluency. Remarkably, studies have shown that using suspension culture with stirring is a scalable approach to convert fibroblasts into iPSCs with enhanced efficiency. In this scenario, the act of stirring is crucial for preserving the size of the aggregate and might potentially hinder the process of differentiation by reducing the fluid shear stress impact.

Electromagnetic fields have recently been identified as a biophysical element that may affect cellular behavior and iPS reprogramming. Rana et al. [22] showed that subjection to extremely EL-EMF (low-frequency electromagnetic fields) not only hypered the regulation of changing mouse fibroblasts into iPSCs, but also eliminated the need for transcription factors in the reprogramming process. They were able to generate functional iPSCs by only expressing Oct4 with exposure of EL-EMF. EMF has the ability to actively control epigenetic modifications, namely the buildup of H3k4me3, by activating the histone lysine methyltransferase MII2.

In addition to their impact on the reprogramming onset, biomaterials have also been investigated for their function in the differentiation and maintenance of iPSCs. Graphene demonstrated a preference for maintaining mouse iPSC in the state of pluripotent. Additionally, it seemed to inhibit endodermal development. On the other hand, graphene oxide promoted endodermal differentiation and accelerated iPSC proliferation, particularly when contrasted to mesodermal or ectodermal lineages. Additionally, a thermos-responsive three-dimensional PNIPAAm-PEG hydrogel framework has been created to facilitate the long-term and efficient proliferation of human iPSCs in a scalable manner. The hydrogels, which are artificially created and have a precise chemical composition, decrease the clumping of cells and protect iPSCs from the harmful effects of mechanical forces that might cause cell death in a liquid environment.

IV. BIOMATERIALS IN DIRECT REPROGRAMMING

Biomaterials are substances, either artificial or derived from nature, that have the ability to come into touch with and merge with biological systems. However, they must not cause any damage to the patient while carrying out their intended activities. The precise cell fate control, including regulation of stem cell and the conversion of adult cells, is a fundamental challenge in the sector of regenerative cell treatment. If the process of cell reprogramming is not well regulated, the therapy may result in the formation of teratomas, rather than providing a beneficial treatment. Biomaterials exists in a wide array that may be used to ascertain the cells fate or facilitate the process of reprogramming.

Biomaterials have the ability to replicate a certain biological environment and prompt cells to undergo transformation into the target cell type. The artificial stem cell niche may attract and contain stem cells by chemotactic signals. This may bear resemblance to direct reprogramming. The categorization of these materials is challenging due to their multifunctionality. Biomaterials may be categorized based on their physical aspects, such as shape, electrical properties, mechanical traits, and surface characteristics. Materials may be categorized based on their origin, like metals, natural, ceramic, and polymer, or based on their physical structure, like 3D or 2D materials. This study will elucidate the roles of biomaterials in relation to their physical characteristics, biological attributes, and gene transfer capabilities (see Figure 3). Biomaterials that govern the fate of stem cells may also function in direct reprogramming, following the same underlying concept. Here, we will provide examples of biomaterials used for stem cell differentiation, as well as contemporary research on biomaterials for direct reprogramming.

Physical Aspects

Biomaterials used for cell reprogramming possess physical qualities such as mechanical strength (modulus) and surface topographies. The cell fate may be regulated by the matrix elasticity of the microenvironment. In general, substrates with higher stiffness lead to cells with higher stiffness, whereas substrates with lower stiffness lead to cells with lower stiffness. Similarly, a pliable framework has the capacity to induce the transformation of MSCs into cells that resemble neurons.

Myogenic variation is more likely to happen in a moderately elastic environment, whereas osteogenic differentiation is more likely to happen in a hard matrix.

In their study, Seib et al. [23] showcased the complete spectrum of differentiation in human MSCs by manipulating the elasticity of the matrix. This included the ability to induce neurogenic differentiation at 0.1 kPa and 1 kPa (brain) elastic modulus, myogenic variation at osteogenic differentiation at an elastic modulus of 25 kPa and 40 kPa (osteoids) and 8-17 kPa (muscle) elastic modulus. The cellular structure likewise underwent corresponding alterations. The matrix features may serve as a powerful distinguishing factor for MSCs. Likewise, mechanical cues like as rigidity at the boundary between a cell and its supporting surface may strongly influence the transformation of the cell's fate. The use of soft hydrogel was crucial in facilitating the iPSCs generation via the mesenchymal to epithelial transition stimulation.

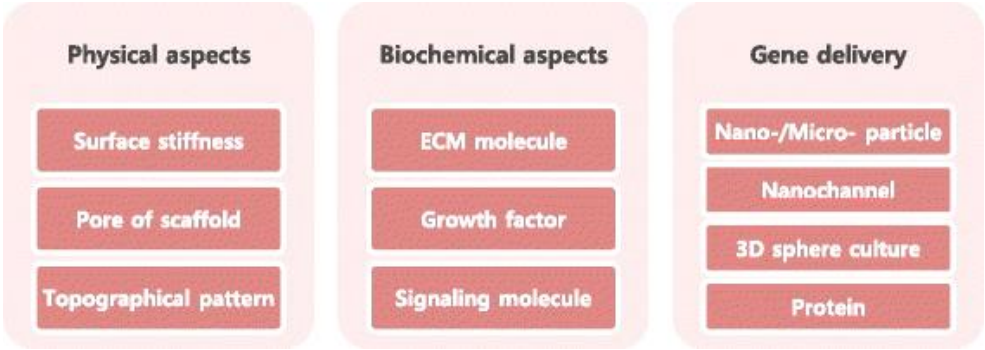

Fig 3. Categorization Of Biomaterials Approaches for Determining the Cell Fate

The cell fate shift may also be influenced by the substrates fiber diameter. The neural stem cell proliferation and differentiation may be regulated by a polyethersulfone mesh fiber diameter that is coated with laminin. In general, a reduction in fiber width resulted to an increase in the proliferation rate of neural stem cells. The minor fiber exhibited multi-directional and elongated cell morphology, whereas the giant fiber had an elongated structure along a single axis, likely owing to its size limitation. While several key signals, including as retinoic acid, growth factors, and fetal bovine serum, are essential for the processes of proliferation and differentiation, the fiber diameter has a significant impact on the efficiency, leading to either an increase or reduction.

Nikolova and Chavali [24] manufactured biodegradable polymer scaffolds with three-dimensional porous architectures that effectively altered tissue architectures, cellular spreading and cell orientation. The advent of 3D scaffolding can help restore the complex features of stem cell niches. In addition, they preferred to use pore disordering in the scaffold to enhance uniformity. The strength of PLGA was improved using Poly (lactic-co-glycolic acid), while PLLA that has a pore diameter ranging between 250-500 μm was chosen for its role in controlling mechanical stiffness. Differentiation into specific lineages from embryonic stem cells (ESCs) was achieved by various growth factors such as retinoic acid (RA) for neuroectodermal-like structure development, Activin-A for liver-like cell generation, and TGF-β for cartilage formation. Alternatively, a different team used RGD peptide as well as alginate scaffolds riddled with large pores by way of heparin binding peptide. These peptides may result in several activating pathways at ECM-cell interfaces resulting in an environment that supports normal functioning of cardiac stem cells.

In determining sources of stem cells, it is secure and encouraging to use carrier with specially designed surface structure without harmful substances included on it. Li et al. [25] reviewed the impact of micropatterned nanoridges on the surface topography on MSCs, leading to the acquisition of neuronal properties. When comparing the cell shape on a smooth surface to the cell shape on a ridge patterned hydrogenated amorphous carbon sheet, the stem cells exhibited an extended morphology. Alternatively, the fibronectin arrangement on a coverslip created using printing of microcontact altered the inclination for distinction based on its location. In the absence of a pattern, human MSCs have a tendency to develop into osteogenic cells near the periphery, while the cells in the middle prefer to contrast into adipocytes. Nevertheless, the cells that were cultivated on the coverslip with a specific pattern altered the positioning of their differentiation into either osteogenic or adipogenic lineages.

The presence of nanotopography caused alterations in the behaviors of stem cells. The expression levels of integrin and FAK were lower in human MSCs cultured on a nanopatterned substrate compared to an unpatterned substrate, regardless of whether the substrate was soft stiff Tissue Culture Polystyrene (TCPS) or polydimethylsiloxane (PDMS). This suggests that the nanotopographical cue has a greater impact on stem cell behaviors than the stiffness of the substrate. Research findings indicate that human MSCs cultivated on PDMS with nanograting have the ability to undergo differentiation into neuronal-like cells [26]. This research provides proof that nanotopography may direct stem cells down certain pathways, rather than allowing them to follow the default choice. Chen et al. [27] discovered that hydrogen-terminated ultra-nanocrystalline diamond sheets had the ability to boost the growth and specialization of brain stem cells. Hydrogen-terminated ultra-nanocrystalline diamond sheets enhanced the capacity for brain stem cells to expand and develop. Reports have shown the use of substrate topography in the process of direct reprogramming. Significant alterations in the epigenome occur during direct reprogramming, specifically involving modifications to histones.

In their investigation, Kulangara et al. [28] conducted an experiment where they transformed fibroblasts into neurons by manipulating the topology of the substrates. They showed that the interactions between cells and their physical surroundings may have a vital effect on gene expression, the advancement and branching of nerve fibers, and ultimately the function and structure of cells. During the neuronal induction process, the cytoskeleton reorganization is an essential and pivotal stage. The cytoskeleton underwent reorganization in response to both the topography and stiffness of the substrate. Alternatively, when microtopography is present on a substrate that cells may adhere to, it causes a change in cell shape, which in turn affects the epigenetic state of the cells. In connection with this finding, the presence of micro- or nano-scale surface features on PDMS material caused noticeable alterations in the patterns of histone acetylation and methylation. Furthermore, it greatly facilitated the transformation of adult fibroblasts from a mesenchymal to an epithelial state. More precisely, the flat, microgrooved, and nanogrooved substrates exhibited distinct variations in cell alignment. A group conducted a comparison by employing UV-based capillary force lithography to manufacture the patterns. They also studied the process of directly transforming somatic fibroblasts into neurons. Passive topographical cues have the potential to significantly improve the cell reprogramming effectiveness.

A microparticle is an additional illustration of functioning of biomaterials as a physical mechanism. Microparticles have been extensively utilized in biomaterial research for the encapsulation and controlled release of various compounds. Various types of microparticles were synthesized by Samba et al. [29] utilizing different materials including gelatin, agarose, and PLGA. The microparticles were effectively incorporated into clusters of ESCs in a way that depended on the dosage. Stem cells may develop particular differentiated characteristics based on the produced biomaterials due to the inclusion of microparticles inside stem cell aggregates. However, the efficacy of just combining microparticles and stem cells was significantly poor, therefore necessitating forceful aggregation. Maximal formation of desirable differentiated cells was consistently seen when both chemical stimuli and physical cues were administered simultaneously in almost all instances. Furthermore, the precise interactions between ligands and receptors of matrix molecules or growth factors were shown to be more significant than physical cues in the regulation of cells. Nevertheless, the physical characteristics of cell culture settings may be vital in defining cellular fate and function.

Biochemical Aspects

Cell fate is regulated by crucial biochemical signals, including growth factors, ECM molecules, and other signaling molecules. Specifically, ECM elements and structural control the cell fate by means of integrin-mediated activation and subsequent signaling processes. Matrigel, collagen, laminin, and fibronectin are the primary constituents of the ECM. The CNS stem cells differentiation into functioning neural circuits was achieved using a 3D collagen gel. Neuronal stem cells depend on being firmly attached to a solid surface, rendering solid polymer scaffolds essential for the formation of neural tissue. Neural cells do not effectively adhere to synthetic hydrogel; thus, it is crucial to alter the hydrogel with ECM proteins like collagen to promote proper identification and attachment of biological cells. On the other hand, stem cells that were confined inside a collagen hydrogel obtained from biological sources were able to quickly multiply in a medium without serum, while also demonstrating the ability to grow into astrocytes, oligodendrocytes, and neurons.

In their study, Battista et al. [30] examined how the composition of the matrix in 3D structures affects the development of embryonic stem cells. The researchers created semi-interpenetrating polymer frameworks consisting of varying concentrations of collagen, laminin, and fibronectin. The growth and creation of embryonic body varied based on the collagen concentrations in various compositions. An elevated level of collagen hindered cellular death, hence disrupting the creation of the embryonic body. Conversely, fibronectin expedited the endothelial cells differention and the process of vascularization. ESCs were induced to contrast into cardiomyocytes by the stimulation of laminin. Collagen-based hydrogel may also be used for direct rescheduling. Endothelial Progenitor Cells (EPCs) underwent reprogramming into smooth muscle cells, resulting in the formation of blood vessel structures composed of smooth muscle cells inside a densely packed collagen gel.

Fibrin is a substantial protein that is created by the polymerization of thrombin and fibrinogen. Scaffold that is optimal should have an attachment and relevant cell indicators for the purpose of facilitating distinction among the cells [31]. The aim of this study was to investigate fibrin scaffolds with various concentrations of aprotinin, thrombin and fibrinogen to establish suitable neurogenesis conditions. Higher concentration of fibrinogen than thrombin has been demonstrated. Additionally, a larger concentration of thrombin hinders the movement of cells inside the scaffold of fibrin. Furthermore, a higher cell seeding density resulted in increased cell differentiation and proliferation. The inclusion of extra aprotinin might regulate the pace at which the gel breaks down, so the ideal concentration of aprotinin varied based on the other components present. A different cohort used fibrin gel as a medium for cultivating embryonic stem cells.

In order to slow down the deterioration of the fibrin gel, a secondary crosslinking approach using poly(ethylene glycol) (PEG) was used to modify the gel. The use of PEGylated fibrin culture resulted in enhanced cell growth efficiency and regulated the embryonic stem cells differentiation. The PEG-based hydrogel has the ability to effectively change cell fate. PEG inhibits the undesired adsorption of proteins, which may lead to the modification of cell signaling. The feature of PEG has the potential to provide a more favorable environment for cell reprogramming. Additionally, it resulted in an almost twofold increase in the effective of generating cardiomyocyte-based cells from fibroblasts. This indicates the prospective pathway for creating a completely artificial rescheduling microecosystem by the incorporation of adhesion peptides or molecules into a PEG-based hydrogel.

Similarly, the creation of vessel-like structures by using EPCs produced from human umbilical cord blood was achieved by a particular group. These cells may be readily obtained from umbilical cord blood or from the peripheral blood of adults. Akbarian et al. [32] created PEG molecules that were linked to bioactive peptides, which served as signals for cell adhesion and degradation by proteolytic enzymes. The integration of the comparison capabilities of EPCs with the properties of polyethylene glycol (PEG) hydrogel resulted in the formation of native-like matrices that mimic the natural environment. These matrices facilitated the development of three-dimensional microvessel networks without the need for additional angiogenic growth agents.

The density and affinity of ligand-receptor relations at the interface of biomaterial may be altered by a certain group in order to control cell fates. Osteogenesis was enhanced by a monolayer that was either coated with fibronectin or presented cycRGD. A low density linRGD surface facilitated neurogenesis whereas, myogenesis was enhanced by a high density linRGD. Furthermore, ligands with a strong attraction to a specific target increased the formation of bone tissue, while ligands with a weaker attraction predominantly promoted the formation of muscle tissue at a high concentration and the formation of nerve tissue at a low concentration. By controlling the density and affinity of the ligand, it is possible to guide the development of certain stem cells. In addition, matrigel is valuable as a cell culture scaffold due to its ability to provide suitable extracorporeal biochemical signals for stem cell development.

Growth factor is a significant biological stimulus as well. None of the growth factors have the ability to solely alter cell fate to a single cell type. However, each growth factor may stimulate the development of a certain cell fate lineage. For instance, TGFβ1 or Activin-A primarily stimulate the mesodermal cells advancement, whereas BMP-4, bFGF, retinoic acid, or EGF stimulate the differentiation of ectodermal and mesodermal cells. On the other hand, NGF and HGF facilitate the differentiation of all three embryonic germ layers. Willerth, Rader, and Sakiyama-Elbert [33] used a mixture of growth conditions on a 3D fibrin culture to investigate the effect of these factors on the differentiation of embryonic stem cells. By using five distinct growth factors, the researchers determined the ideal concentration of each element to effectively stimulate the development of neurons and oligodendrocytes.

The notch signaling framework is fundamental in regulating epithelial differentiation. The availability of Jagged-1, a Notch ligand on the surface of biomaterial, may control the development of stem cells. HEMA-bound or Polystyrene notch ligand may enhance both tight clustering and epithelial differentiation. However, the Jagged-1 in a soluble form existence does not possess the ability to function as an antagonist. Therefore, it is essential for it to exist in a fixed state on the surfaces of other biomaterials. Dishowitz et al. [34] have shown that notch signaling biomaterials have the ability to work in a particular and adjustable way, depending on the timing of activation. The activation of the notch route in embryonic stem cells amounted in the upregulation of genes associated with the ectodermal lineage. Additionally, the availability of Jagged-1 on the cell surface directed the differentiation of cardiovascular progenitor cells towards a cardiac fate.

Wazan, Urrutia-Cabrera, and Wong [35] have shown that, rather of relying on transcription factors for direct reprogramming, it is possible to alter the cell fate by utilizing a combination of small chemical compounds. Small molecules have the ability to pass across cell membranes, do not trigger an immune response, and are economically advantageous. After careful screening, certain small compounds were chosen for direct reprogramming. When these small molecules were integrated to the medium of the cell culture, they effectively produced a change in cell fate. Examples such as Forskolin, ISX9, CHIR99021, and SB431542 have been shown to stimulate the development of functional neurons from murine fibroblasts. Cao et al. [36] generated functioning cardiomyocytes from human fibroblasts using a chemical induction method including a mixture of nine chemicals.

The categories in this article were separated into biochemical and physical aspects; however, in stem cell studies, combining biochemical and physical cues, like controlling matrix rigidity and the amount of proteins on substrate jointly, is an efficient and vital schedule to alter the evolved cells fate more effectively.

Gene Delivery Biomaterials

Gene delivery is a commonly employed method in cell-based study. It is a crucial procedure for artificially altering the cell fate or controlling the development of stem cells. Prior to the advent of novel gene delivery techniques, viral vectors were often used. However, this viral vector presents safety concerns since it has the potential to disrupt the host sequence in a random manner. Hence, the need to design secure gene delivery techniques arose. The first approach was the use of nanoparticles for gene delivery. An instance of stimuli sensitive nanoparticles was created by combining Polyethylenimine (PEI) and Hyaluronic acid (HA), which were used as a non-viral gene vector. Polyethyleneimine (PEI) that has been positively charged has the ability to create nanocomplexes with negatively charged DNA and then release it inside the endosome. However, some limitations are imposed by its cytotoxicity. HA receptors are present in several organs, and HA has a polyanionic property that hinders the non-particular binding between PEI and serum. Consequently, HA contributes to the specificity and stability of the molecule. The presence of a disulfide bond may facilitate the more efficient release of DNA inside the endosome.

In a similar manner, Zhao et al. [37] developed nanoparticles using Chitosan (CS) and ss-PEI to facilitate osteogenic differentiation. Alternatively, a vector system using magnetic nanoparticles was used to provide secure and effective gene delivery. The magnetic nanoparticles were coated with a biocompatible surface and modified to transport therapeutic biomolecules like shRNA, siRNA, or DNA. Furthermore, a proposed technique for electroporation included the use of a micro/nanochannel array. This is a technique for transfecting single cells, allowing charged chemicals to be delivered directly

into the cytosol via electrophoresis. This enables exact control over the dose. Transient gene expression via non-viral vectors effectively mitigates issues associated with gene overexpression and viral vector interference.

An alternative strategy for safe gene delivery using viral vectors involves using the protein itself. However, the protein has a brief half-life, necessitating a substantial dosage and incurring significant expenses. Liu et al. [38] employed reprogramming proteins that were combined with a CPP (Cell Penetrating Peptide). This approach may circumvent issues related to genetic integration and immunogenicity, albeit its efficacy was suboptimal. Typically, the efficacy of protein distribution into the cell is significantly limited due to the macromolecular nature of proteins and their difficulty in penetrating cell membranes. Therefore, there is significant biomaterials need that may enhance the intracellular protein delivery efficacy. In their study, Rafi et al. [39] used PLGA microparticles to administer proteins into embryonic bodies, therefore exerting control over the advancement of embryonic stem cells. In addition, Sivashankari and Prabaharan [40] synthesized nanoparticles capable of integrating with hydrogel scaffolds and encapsulating growth factor proteins. The protein filled microsphere scaffolds were able to stimulate the MSCs differentiation into chondrogenic lineage.

Finally, the use of 3D sphere culture shows great potential as a secure and effective technique for gene delivery. Neural crest-based cells were generated by cultivating fibroblasts with the FOXD3 sole factor, without the need of a viral vector, on a chitosan substratum rather than TCPS. Subsequently, the cells assumed a spherical morphology and maintained cohesion with the plasmid. The results indicated a relatively poor efficiency of transduction, but a high vitality of around 100% after transfection. Furthermore, there have been reports of the generation of brain progenitor-like cells from fibroblasts via 3D sphere induction. Gene disruption, instabilities, and tumor development are possible risks associated with viral vectors, yet recurrent transduction is still required because of the poor transduction efficiency.

V. CONCLUSION AND FUTURE SCOPE

The behavior and reprogramming of cells are significantly impacted by the microenvironment, particularly in relation to the ECM and other biomaterials. Cells possess the ability to detect and transform mechanical inputs into chemical signals via mechanotransduction pathways, which rely on the presence of integrins and adaptor proteins. Cellular processes, including proliferation migration and differentiation, among others are significantly influenced by changes in stiffness and surface properties of ECM. Induced pluripotent stem cells (iPSCs) or reprogrammed adult cells is one of the avenues that medicine and research have been focusing on. Despite this, there are barriers to the complete realization of the therapeutic uses and disease modeling potential of reprogramming that arise from modifications in cellular genetics. This conveys a better efficiency and safety in iPSC generation, as well as the new gene delivery strategies like Sendai virus reprogramming vectors.

The field of direct reprogramming within cell biology has evolved into an aspect that is applicable to both basic research and practical applications. Direct conversion of living organ cells through direct programming has some benefits compared to other similar methods thus making it an option for treatment of many human disorders. Advanced approaches like CRISPR-Cas9 screening and computational modeling could be used to create non- directly programmed somatic cells based on specialized genetic engineering techniques developed through extensive international cooperation. These strategies are useful in delineating the necessary reprogramming factors for specific lineage which further allows their experimental verification in vitro models. In addition, identification of small molecules, non-coding RNAs or synthetic proteins that can be injected into an organism but still be safe enough is opening up new avenues for medical applications. Single-cell omics technology has made significant progress, allowing us to study the processes of direct reprogramming with exceptional accuracy and detail. These advancements, together with cooperation amongst many disciplines, are creating many chances to gain a deeper knowledge of cell fate conversion and to create novel treatment approaches. Many scholars have emphasized previous advancements in the differentiation of iPSC into important immunological, endothelial, and endocrine cell types.

However, the future use of this method will further broaden as the scientific community generates more types of cells from iPSC progenitor populations. The capacity to alternate between protective and vulnerable variations and efficiently regulate the activation or suppression of genes would enable the implementation of reductionist mechanistic investigations that were previously limited to transgenic animal or gene knockout models. Future models using iPSC have the potential to include more intricate combinations of cells to recreate the micro-environments of tissues, including several microvasculature, acinar tissues, endocrine cell types, and maybe even nerve supply.

CRediT Author Statement

The author reviewed the results and approved the final version of the manuscript.

Data Availability

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interests

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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References

[1]. R. Ramprasad, B. Chaudhary, and R. Bandopadhyay, "Induced Pluripotent Stem Cells—A milestone in Regenerative Medicine: 2012 Nobel Prize for Physiology or Medicine," National Academy Science Letters, vol. 37, no. 2, pp. 203–206, Mar. 2014, doi: 10.1007/s40009-013-0214-3.

[2]. J. Lewandowski and M. Kurpisz, "Techniques of human embryonic stem cell and induced pluripotent stem cell derivation," Archivum Immunologiae Et Therapiae Experimentalis, vol. 64, no. 5, pp. 349–370, Mar. 2016, doi: 10.1007/s00005-016-0385-y.

[3]. C. V. Echegaray and A. Guberman, "Nanog in iPS cells and during reprogramming," in Elsevier eBooks, 2022, pp. 319–348. doi: 10.1016/b978-0-323-90059-1.00006-3.

[4]. S. W. Plouffe et al., "The Hippo pathway effector proteins YAP and TAZ have both distinct and overlapping functions in the cell," Journal of Biological Chemistry, vol. 293, no. 28, pp. 11230–11240, Jul. 2018, doi: 10.1074/jbc.ra118.002715.

[5]. J. Keung, P. Asuri, S. Kumar, and D. V. Schaffer, "Soft microenvironments promote the early neurogenic differentiation but not self-renewal of human pluripotent stem cells," Integrative Biology, vol. 4, no. 9, pp. 1049–1058, Aug. 2012, doi: 10.1039/c2ib20083j.

[6]. H. J. Kong and D. J. Mooney, "Microenvironmental regulation of biomacromolecular therapies," Nature Reviews Drug Discovery, vol. 6, no. 6, pp. 455–463, Jun. 2007, doi: 10.1038/nrd2309.

[7]. G. Liu, B. T. David, M. Trawczynski, and R. G. Fessler, "Advances in Pluripotent stem cells: history, mechanisms, technologies, and applications," Stem Cell Reviews and Reports, vol. 16, no. 1, pp. 3–32, Nov. 2019, doi: 10.1007/s12015-019-09935-x.

[8]. L. Galarza, T. E. Arshaghi, A. O'Brien, A. Ivanovska, and F. Barry, "Induced pluripotent stem cells in companion animals: how can we move the field forward?," Frontiers in Veterinary Science, vol. 10, Apr. 2023, doi: 10.3389/fvets.2023.1176772.

[9]. Z. Jiang, H. Yun, and X. Cao, "Induced pluripotent stem cell (iPSCs) and their application in immunotherapy," Cellular & Molecular Immunology, vol. 11, no. 1, pp. 17–24, Dec. 2013, doi: 10.1038/cmi.2013.62.

[10]. M. Yamoah, P. N. Thai, and X.-D. Zhang, "Transgene delivery to human induced pluripotent stem cells using nanoparticles," Pharmaceuticals, vol. 14, no. 4, p. 334, Apr. 2021, doi: 10.3390/ph14040334.

[11]. J. Mao et al., "Reprogramming stem cells in regenerative medicine," Smart Medicine, vol. 1, no. 1, Dec. 2022, doi: 10.1002/smmd.20220005.

[12]. Zhang et al., "Zero-dimensional, one-dimensional, two-dimensional and three-dimensional biomaterials for cell fate regulation," Advanced Drug Delivery Reviews, vol. 132, pp. 33–56, Jul. 2018, doi: 10.1016/j.addr.2018.06.020.

[13]. Barui, F. Chowdhury, A. Pandit, and P. Datta, "Rerouting mesenchymal stem cell trajectory towards epithelial lineage by engineering cellular niche," Biomaterials, vol. 156, pp. 28–44, Feb. 2018, doi: 10.1016/j.biomaterials.2017.11.036.

[14]. H. S. Gerardo et al., "Soft culture substrates favor stem-like cellular phenotype and facilitate reprogramming of human mesenchymal stem/stromal cells (hMSCs) through mechanotransduction," Scientific Reports, vol. 9, no. 1, Jun. 2019, doi: 10.1038/s41598-019-45352-3.

[15]. M. Caiazzo, Y. Okawa, A. Ranga, A. Piersigilli, Y. Tabata, and M. P. Lütolf, "Defined three-dimensional microenvironments boost induction of pluripotency," Nature Materials, vol. 15, no. 3, pp. 344–352, Jan. 2016, doi: 10.1038/nmat4536.

[16]. S. Romanazzo, L. Kang, P. Srivastava, and K. A. Kilian, "Targeting cell plasticity for regeneration: From in vitro to in vivo reprogramming," Advanced Drug Delivery Reviews, vol. 161–162, pp. 124–144, Jan. 2020, doi: 10.1016/j.addr.2020.08.007.

[17]. K. Shukla, G. Gao, and B. S. Kim, "Applications of 3D bioprinting technology in induced Pluripotent Stem Cells-Based tissue engineering," Micromachines, vol. 13, no. 2, p. 155, Jan. 2022, doi: 10.3390/mi13020155.

[18]. J. A. Kim, S. Hong, and W. J. Rhee, "Microfluidic three-dimensional cell culture of stem cells for high-throughput analysis," World Journal of Stem Cells, vol. 11, no. 10, pp. 803–816, Oct. 2019, doi: 10.4252/wjsc.v11.i10.803.

[19]. Totaro et al., "Downscaling screening cultures in a multifunctional bioreactor array-on-a-chip for speeding up optimization of yeast-based lactic acid bioproduction," Biotechnology and Bioengineering, vol. 117, no. 7, pp. 2046–2057, Apr. 2020, doi: 10.1002/bit.27338.

[20]. J. Sia, R. W. Sun, J. Chu, and S. Li, "Dynamic culture improves cell reprogramming efficiency," Biomaterials, vol. 92, pp. 36–45, Jun. 2016, doi: 10.1016/j.biomaterials.2016.03.033.

[21]. F. Zhang, D. Lin, S. Rajasekar, A. Sotra, and B. Zhang, "Pump-Less platform enables Long-Term recirculating perfusion of 3D printed tubular tissues," Advanced Healthcare Materials, vol. 12, no. 27, Sep. 2023, doi: 10.1002/adhm.202300423.

[22]. Rana, S. Kumar, T. J. Webster, and M. Ramalingam, "Impact of induced pluripotent stem cells in bone repair and regeneration," Current Osteoporosis Reports, vol. 17, no. 4, pp. 226–234, Jun. 2019, doi: 10.1007/s11914-019-00519-9.

[23]. P. Seib, M. Prewitz, C. Werner, and M. Bornhäuser, "Matrix elasticity regulates the secretory profile of human bone marrow-derived multipotent mesenchymal stromal cells (MSCs)," Biochemical and Biophysical Research Communications, vol. 389, no. 4, pp. 663–667, Nov. 2009, doi: 10.1016/j.bbrc.2009.09.051.

[24]. M. P. Nikolova and M. Chavali, "Recent advances in biomaterials for 3D scaffolds: A review," Bioactive Materials, vol. 4, pp. 271–292, Dec. 2019, doi: 10.1016/j.bioactmat.2019.10.005.

[25]. X. Li, Y. Sun, L. Liao, and X. Su, "Response of mesenchymal stem cells to surface topography of scaffolds and the underlying mechanisms," Journal of Materials Chemistry B, vol. 11, no. 12, pp. 2550–2567, Jan. 2023, doi: 10.1039/d2tb01875f.

[26]. B. K. K. Teo, S. Ankam, L. Y. Chan, and E. K. F. Yim, "Nanotopography/Mechanical induction of Stem-Cell differentiation," in Methods in Cell Biology, 2010, pp. 241–294. doi: 10.1016/s0091-679x(10)98011-4.

[27]. Y.-C. Chen et al., "The effect of ultra-nanocrystalline diamond films on the proliferation and differentiation of neural stem cells," Biomaterials, vol. 30, no. 20, pp. 3428–3435, Jul. 2009, doi: 10.1016/j.biomaterials.2009.03.058.

[28]. K. Kulangara et al., "The effect of substrate topography on direct reprogramming of fibroblasts to induced neurons," Biomaterials, vol. 35, no. 20, pp. 5327–5336, Jul. 2014, doi: 10.1016/j.biomaterials.2014.03.034.

[29]. Samba, R. Hernández, N. Rescignano, C. Mijangos, and J. M. Kenny, "Nanocomposite hydrogels based on embedded PLGA nanoparticles in gelatin," Nanocomposites, vol. 1, no. 1, pp. 46–50, Jan. 2015, doi: 10.1179/2055033214y.0000000006.

[30]. S. Battista et al., "The effect of matrix composition of 3D constructs on embryonic stem cell differentiation," Biomaterials, vol. 26, no. 31, pp. 6194–6207, Nov. 2005, doi: 10.1016/j.biomaterials.2005.04.003.

[31]. M. Akhmanova, E. O. Osidak, S. P. Domogatsky, S. Rodin, and A. Domogatskaya, "Physical, spatial, and molecular aspects of extracellular matrix ofIN VivoNiches and artificial scaffolds relevant to stem cells research," Stem Cells International, vol. 2015, pp. 1–35, Jan. 2015, doi: 10.1155/2015/167025.

[32]. M. Akbarian, K. Ali, S. Eghbalpour, and V. N. Uversky, "Bioactive peptides: synthesis, sources, applications, and proposed mechanisms of action," International Journal of Molecular Sciences, vol. 23, no. 3, p. 1445, Jan. 2022, doi: 10.3390/ijms23031445.

[33]. S. M. Willerth, A. Rader, and S. E. Sakiyama-Elbert, "The effect of controlled growth factor delivery on embryonic stem cell differentiation inside fibrin scaffolds," Stem Cell Research, vol. 1, no. 3, pp. 205–218, Sep. 2008, doi: 10.1016/j.scr.2008.05.006.

[34]. M. I. Dishowitz, F. Zhu, H. G. Sundararaghavan, J. L. Ifkovits, J. A. Burdick, and K. D. Hankenson, "Jagged1 immobilization to an osteoconductive polymer activates the Notch signaling pathway and induces osteogenesis," Journal of Biomedical Materials Research Part A, vol. 102, no. 5, pp. 1558–1567, Jul. 2013, doi: 10.1002/jbm.a.34825.

[35]. L. E. Wazan, D. Urrutia-Cabrera, and R. C.-B. Wong, "Using transcription factors for direct reprogramming of neurons in vitro," World Journal of Stem Cells, vol. 11, no. 7, pp. 431–444, Jul. 2019, doi: 10.4252/wjsc.v11.i7.431.

[36]. N. Cao et al., "Conversion of human fibroblasts into functional cardiomyocytes by small molecules," Science, vol. 352, no. 6290, pp. 1216–1220, Jun. 2016, doi: 10.1126/science.aaf1502.

[37]. X. Zhao et al., "Enhanced gene delivery by chitosan-disulfide-conjugated LMW-PEI for facilitating osteogenic differentiation," Acta Biomaterialia, vol. 9, no. 5, pp. 6694–6703, May 2013, doi: 10.1016/j.actbio.2013.01.039.

[38]. H. Liu et al., "Emerging landscape of cell penetrating peptide in reprogramming and gene editing," Journal of Controlled Release, vol. 226, pp. 124–137, Mar. 2016, doi: 10.1016/j.jconrel.2016.02.002.

[39]. Md. K. J. Rafi, S. Singh, V. Kanchan, C. Anish, and A. K. Panda, "Controlled release of bioactive recombinant human growth hormone from PLGA microparticles," Journal of Microencapsulation, vol. 27, no. 6, pp. 552–560, Aug. 2010, doi: 10.3109/02652048.2010.489974.

[40]. P. Sivashankari and M. Prabaharan, "Prospects of chitosan-based scaffolds for growth factor release in tissue engineering," International Journal of Biological Macromolecules, vol. 93, pp. 1382–1389, Dec. 2016, doi: 10.1016/j.ijbiomac.2016.02.043.