CELL FUSION: UNITING CELLS FOR DISCOVERY AND THERAPEUTIC APPLICATIONS

Abstract

Cell fusion, a fundamental biological process, encompasses diverse mechanisms such as membrane fusion leading to syncytium formation. Syncytia play pivotal roles across various biological processes, aiding in cell-cell communication and nutrient exchange. Notably, syncytium formation contributes significantly to physiological development, particularly in placental development by facilitating nutrient transfer and hormone production. In skeletal muscle formation, cell fusion is instrumental in embryonic development and postnatal differentiation, shaping the structure and function of muscles. Moreover, it serves as a mechanism for cellular reprogramming, evident in somatic cell nuclear transfer (SCNT) and induced pluripotent stem cells (iPSCs), holding promise for regenerative medicine and disease treatments. The applications of cell fusion in therapeutic avenues are extensive. Its relevance spans cell-based therapies, cancer research, tissue engineering, and organ transplants. Leveraging cell fusion holds potential for innovative treatments, offering avenues for disease management and regenerative interventions. This chapter provides a concise glimpse into the multifaceted roles of cell fusion, underscoring its significance in physiological processes, developmental biology, and its promising applications in therapeutic interventions across various fields.

Keywords: Therapeutic, Cell Fusion.

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I. INTRODUCTION

Cell fusion, a fascinating biological process, involves combining two or more cells and producing a hybrid cell with a combination of characteristics and functions. Theodore Schwann initially observed this phenomenon in pig embryos, noting that cell membranes "coalesce," while no such coalescence occurred between the nuclei of these cells. In subsequent studies and observations, nuclei have also been found to be capable of participating in the fusion process in a variety of cell types and organisms. This fusion can happen between similar and distinct cell types within the same species and even between cells of different species. Remarkably, cell fusion is not restricted to specific groups but occurs in invertebrates, vertebrates, eukaryotes, and prokaryotes [1].

Cell fusion occurs naturally in various biological processes, serving essential roles. It occurs during the fertilization of germ cells, contributing to the generation of progeny with significant genetic variations. Additionally, it facilitates placental development by trophoblast fusion [1, 2]. It is also important to note that myoblasts fuse to form skeletal muscles, while certain cells fuse to form giant cells (MGCs), which are involved in immunity [3]. These observations emphasize the widespread occurrence and significance of cell fusion in various biological mechanisms.

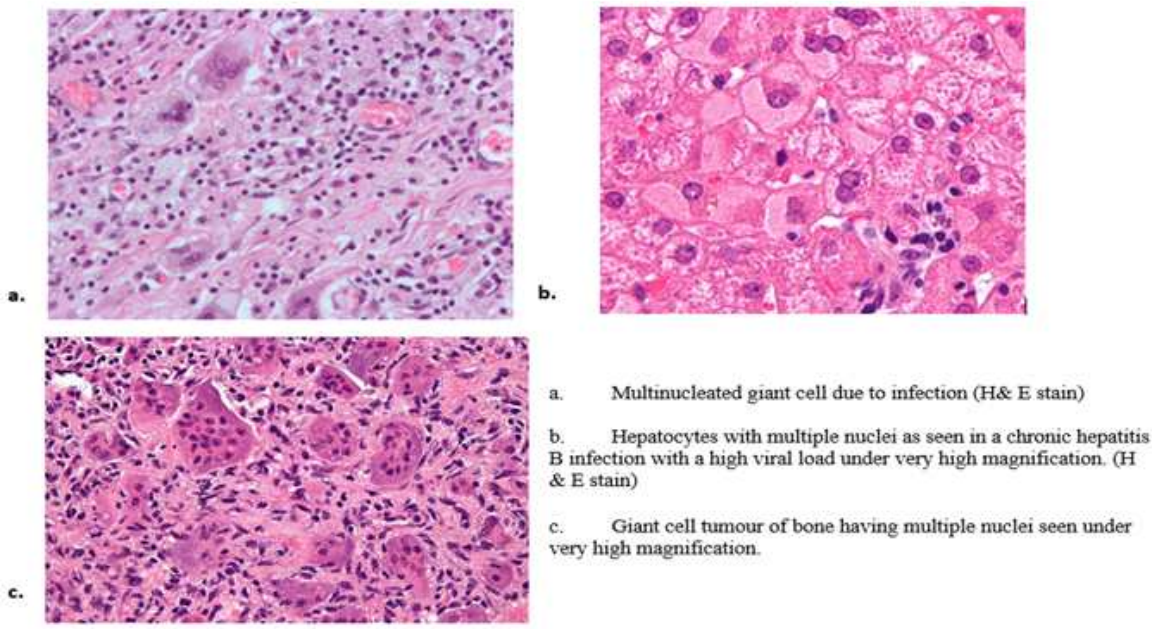

Figure 1: Multinucleate Cells Formed by Cell Fusion Observed under Very High Magnification.
(Source: Image A, B And, C: Online *Copyright © 2011 Michael Bonert*) [47, 48, 40].

However, certain aberrations caused by mutations, viral infections, and various physiological factors can give rise to abnormal cell fusion, leading to pathological conditions such as spontaneous fusion in tumours and cancer progression, including metastasis and the development of stem cells involved in cancer. This phenomenon renders cells drug-resistant, posing significant challenges in treating patients with malignant tumours. Additionally, in viral infections, the virus can effectively communicate and spread between infected and non-

infected cells through cell fusion, induced by the expression of viral proteins. Improper cell-cell fusion between osteocytes may contribute to osteoporosis, while defects in the sperm and egg fusion during eukaryotic fertilization can lead to infertility and preeclampsia during pregnancy [2, 5, 6]. Popular model organisms such as *Neurospora crassa* (fungi) and *C. elegans* are used for studying cell fusion mechanisms, highlighting the pivotal role of fusion mechanism in various growth factors of an organism's lifetime [2, 7]. A thorough understanding of cell fusion is essential to identify precursor defects during the developmental process.

Despite its significance, exploring the cell fusion mechanism poses several challenges. The process is highly spontaneous, occurring within minutes or seconds, making it difficult to differentiate between proteins involved in actual fusion versus those preparing for pre-fusion and post-fusion stages. Moreover, while successful in vitro induction of cell fusion is achievable, applying the same techniques and methods often encounters obstacles in in vivo processes. Addressing these complexities will be vital for comprehensive insights into the cell fusion process and its broader implications in biology and disease [2].

In the 1960s, scientists achieved successful artificial cell fusion between different eukaryotic cell types using a mouse virus called Sendai virus (SeV), known for its syncytium-forming ability. This breakthrough enabled the large-scale production of monoclonal antibodies [8]. Subsequently, a range of techniques are being developed and could help in inducing the fusion of cells, leading to the formation of hybrids with various applications in transplantation, immunity, regeneration, therapeutics, and cancer treatment [1, 2]. Biotechnologists now employ diverse technologies, such as Electric field-induced Cell-to-Cell Fusion, including cell-to-cell fusion induced by electric fields, fusion induced by polyethylene glycol, and various laser-induced techniques, to successfully recreate the fusion process artificially [4, 9, 10, 11].

The purpose of this chapter is to provide an overview of how cell fusion works, its role in developmental processes, cellular reprogramming, and its potential applications in regenerative medicine.

II. MECHANISMS OF CELL FUSION

A crucial and intricate process of fusion between cells is involved in a variety of biological processes, including reproduction, growth of organs and tissues, metastasis of cancer, viral mechanisms, and immune response. Viral envelopes are fused with cellular membranes by specific viral proteins. It is interesting to note that these proteins can also facilitate fusions between adjacent cell membranes, resulting in the merging of the cellular components and the formation of cells called syncytia which contain multiple nuclei in their cells. The membranes of adjacent cells are fused in the process of cell-cell fusion, forming a bilayer having continuity with the membranes of adjoining cells [16].

1. **Membrane Fusion:** The fusion of cells depends upon the process wherein the cellular membranes merge, a phenomenon that can be facilitated through diverse mechanisms, including direct contact, fusogenic proteins, or the involvement of viral envelopes. During this fusion event, lipid bilayers undergo rearrangement, and the contents of the cytoplasm blend together. Cells are dynamically organized by remodelling the membrane structure,

orchestrated by proteins, which enable the division and fusion of membranes. A number of essential cellular functions depend on intracellular membrane fusion, including secretion, cellular and protein trafficking, and the maintenance of networks in mitochondria and the endoplasmic reticulum. Any impairments during the normal fusion mechanism are proven to be associated with a range of disorders, encompassing mitochondrial dysfunction, lysosomal storage issues, and degenerative conditions. Moreover, many viruses with envelopes, including a number of pathogenic organisms affecting humans, use this mechanism to infect host cells. Beyond its role in viral infections, cell fusion serves as a critical process for fertilization, the growth of tissues, and the formation of various organs, including the formation of skeletal muscles and the placenta [2]. Nonetheless, if membranes could spontaneously fuse, it would lead to a state of chaos, as it could lead to the merging of various cell organelles and vesicles, causing the fusion of cells to occur indiscriminately, ultimately eradicating the partition between cells and undermining cellular integrity itself. Consequently, despite the proximity and duration of contact between biological membranes, spontaneous fusion is averted in many cases. At the interfaces between biological membranes, densely packed proteins generate impediments to membrane fusion, as well as high energy barriers associated with processes such as membrane deformation and mixing of lipids and the expansion of fusion pores. In order to achieve a fusion with favourable energy utilization, membranes should act against the repulsive forces exerted by hydrated phospholipids having charges, while also ensuring that their hydrophobic cores are minimally exposed during the mixing process. In this complex orchestration, only proteins exhibit the intricate coordination, execution, and control required for such a pivotal event in cellular biology [12].

Fusion processes exhibit considerable variations in the fusing membrane components, the context of biology in which they occur, and the regulatory mechanisms involved. Some fusions only require the presence of fusion proteins (also known as "fusion proteins" or "fusogens") on a single fusing membrane, which is referred to as a "unilateral mechanism." [2]. These fusogens act on the membranes, overcoming forces that hinder spontaneous fusion, and ensure a controlled and regulated fusion process. Initially, viral fusogens were identified as the first type of fusogens. They are evident in enveloped viruses like influenza, HIV, hepatitis, dengue, and Zika, where transmembrane glycoproteins on the virus surface facilitate attachment and fusion with host membranes. Based on the structural characteristics, the fusogens from virus can be divided into 3 classes with varied properties: class 1, mainly alpha-helical; class 2, predominantly composed of beta sheets; and fusogens of class 3, containing a combination of the former two classes. In spite of having structural differences, they have similar mechanisms of action. In the fusion of vesicles and organelles, intracellular fusogens, which function on the endoplasmic side of the membrane, are highly significant. The SNARE proteins, which mediate the fusion of vesicles with their target organelles, are among them and are more thoroughly researched and comprehended [12]. In other cases, fusion necessitates the presence of either identical or distinct fusogens in both fusing membranes; such cases are referred to as "bilateral homotypic vs. bilateral heterotypic processes." Bringing lipid bilayers into direct contact, catalysing the formation of energy-intensive fusion intermediates, and creating a fusion pore are the three primary functions of the fusion protein mechanism, which remain constant regardless of the type of fusion. Both lipid bilayers are locally ruptured during the fusion process and then rejoined [2].

Prior to the cell fusion process, distinct cell-to-cell adhesion proteins regulate the typical distances between opposing plasma membranes, ranging from 10 to a few tens of nanometres. Membrane proteins must be shifted towards the edges of the site of fusion in order to bring the membrane bilayers closer for fusion. Intense repulsive interactions brought on by hydration forces or thermal fluctuations must be overcome at extremely short distances, roughly equal to the size of the monolayer of lipids (around 2 nm) [2].

In a few nanometres, a considerable curvature in either or both of the membrane bilayers will bring them together, causing an internal disruption and reorganization within the lipid monolayers. Hemifusion, in which the monolayers of the fusing lipid bilayers merge, is a prominent initial step in the fusion process. Hemifusion enables lipid mixture among the membranes. The distal monolayers then merge with one another, forming a nascent pore during fusion that permits content mixing (refer to Fig. 1). Biological fusion frequently incorporates proteins as a vital part of the initial fusion intermediates, despite the fact that the hemifusion pathway mediated fusion was first discovered in protein-free lipid bilayers exhibiting monolayer curvatures typical of hemifusion intermediates or lipidic pores.

Example, research suggests that exocytosis triggered by Ca^{2+} may involve the development of a proteinaceous fusion pore, the rim of which is lined entirely or in part by amino acid residues from SNARE proteins' transmembrane domains. It is likely that proteins catalyse a membrane bilayer-specific fusion-through-hemifusion fusion pathway which is fuelled by the lipid bilayer stresses of the membrane (see Fig. 1 B) [2].

- **Working Mechanism of Membrane Fusion Process:** As per the stalk hypothesis, the fusion process follows a systematic sequence of events, starting with the merging of the nearby monolayers, followed by the stalk formation, the creation of hemifusion intermediates, then eventually opening the fusion [13]. The pathway of fusion in cell-cell interactions involves a well-defined sequence of events:

  Initially, the cells that are ready to fuse recognize each other and come into close contact, leading to a process known as hemifusion (Fig. 2, Step 3). During hemifusion, the outer monolayers of the two membrane bilayers merge, allowing for the redistribution of lipid markers between the cells. The distant leaflets of the membranes and their contents stay different until a fusion pore forms during hemifusion, during which the external monolayers of the two membrane bilayers combine, allowing for the transfer of lipid markers between the cells. Exocytosis, protein transport, and viral entry are only a few biological processes that share the crucial event known as hemifusion [14]. An aqueous link among the fusing bilayers is established by the formation of fusion pores (see Figure 2). During the process of viral fusion and exocytosis, fusion pores with a diameter of around 2 nm occasionally open abruptly in a matter of microseconds. During the subsequent 10 to 20 milliseconds, fusion pores have the potential to experience alterations in conductance, leading to the formation of relatively stable intermediates displaying diverse conductance states. During this phase, rapid and irregular openings and closures of the fusion pore, known as flickering, are often observed, lasting from milliseconds to seconds. Sometimes the reactions may stop, causing the pore to seal once more. The fusion pore expands gradually and irreversibly after this stage in most cases. The

initial conductivity of fusion pores and the presence of fusion pore flickering during protein-free liposome fusion indicate that, even during protein-mediated fusion, fusion pores are largely composed of lipidic components. [13].

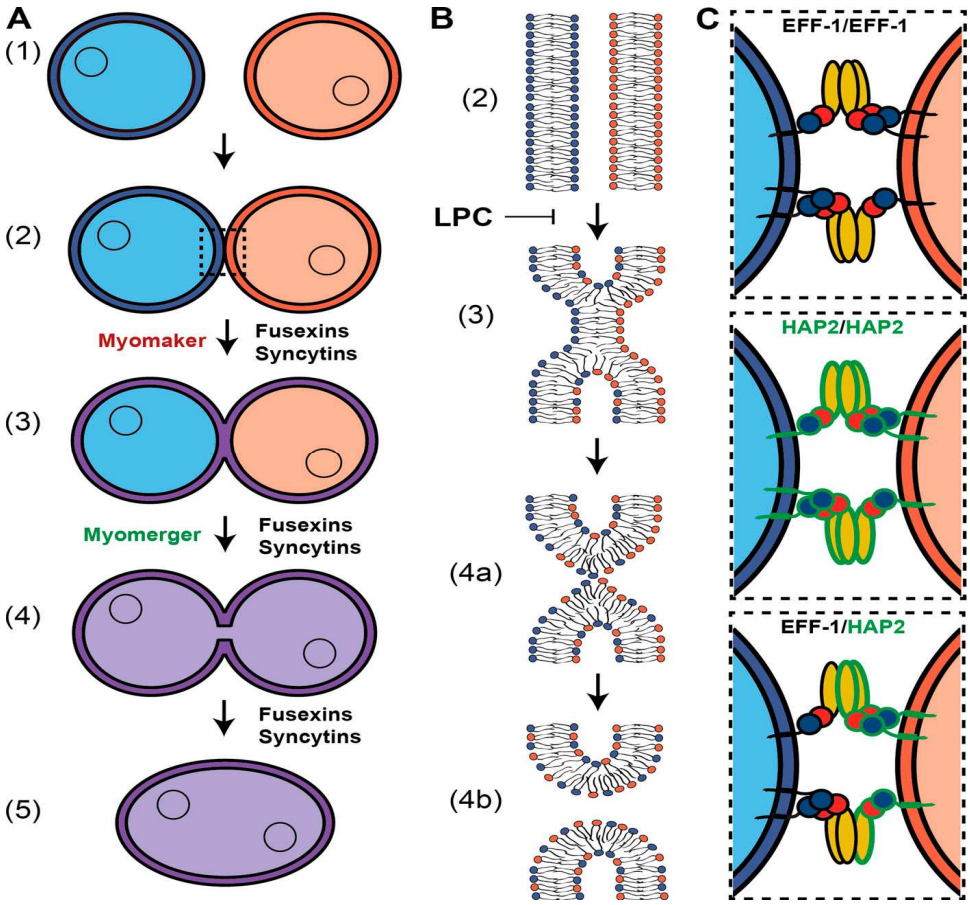

Figure 2: Membrane Fusion Mechanism in Cell-Cell Fusion with Schematic Representation of Rearrangement of Lipid
(Source: Brukman Et Al., 2019: Online) [50]

Once the fusion pore opens within the hemifusion structure, the cytoplasmic contents mix (Fig. 2, Step 4), and the process of fusion is completed by the expansion of fusion pores, which fuses cells into a single entity (Fig 2, Step 5). While current research identifies Myomaker/Myomerger, syncytins, and fusexins as critical proteins for specific fusion processes, it is highly likely that they collaborate with other proteins, particularly those involved in myoblasts, which have already been identified. Every phase of the process of fusion depends on fusexins and syncytins, although the early stage, when Myomaker is directly involved in the initial stages, helps the transition to hemifusion, and the latter stage before the fusion pore opens after the hemifusion process, where Myomerger is necessary [2].

LPC (lysolecithin) prevents the bends of the interacting monolayers from taking place, which inhibits hemifusion. (Fig. 2 B). Protein fusogens are vital components in overcoming the energetic barriers associated with hemifusion, as well as the subsequent processes of fusion pore opening and expansion. Bilateral and

heterotypic fusions between the cells of C. elegans EFF-1 and the cells of Arabidopsis HAP2, as well as the bilateral and homotypic fusions in between them, are examples of fusion processes (Fig. 2 C) [2, 12,].

In eukaryotic cells, the fusing of intercellular membranes is mediated by a number of protein families, including SNARE proteins, Rab proteins, as well as Sec1/Munc-18 associated proteins (SM proteins). The SNARE group of tiny, primarily proteins anchored to the membrane, is made up of SNAREs, which are distinguished by a common structure made up of approximately 60 amino acids known as the SNARE motif. These SNARE proteins can form core complexes, which have tightly wound helical bundles that are essential for drawing the fusing membranes closer and promoting fusion. SM proteins, on the other hand, are proteins that are soluble and belong to a unique family and bind to particular classes of SNARE proteins in order to prevent the core complexes from being formed. Additionally, Ras-associated binding (Rab) proteins carry out tightly controlled GTP-GDP cycles as GTPases. They engage in interactions with particular effector proteins when they are GTP-bound. Despite not being directly engaged in the process of fusion reaction itself, recent data suggests that the Rab proteins contribute to the primary contact between membranes that joins the concerned fusing membranes [15].

Formation of multinucleated cells in animals is closely regulated by cell fusion, which accounts for up to one-third of the nucleus of all cells in species ranging from *C. elegans* to human beings. In *C. elegans*, cell fusion primarily occurs in epithelia, while in humans, it is predominantly observed in skeletal muscle. Nonetheless, the majority of cells remain mononucleated, underscoring the precise control and regulation of the mechanism of fusion of cells [2].

- **Significance of Cell-Cell Fusion Mechanism:** Fusogens have the power to modify behaviour, affect the future of cell and serve as a protective barrier against invasive cells. There are interesting therapeutic applications that take advantage of their capabilities, such as the targeted delivery of medications or the absence of genes to particular cells in any species. On the other hand, blocking fusogen activity may help in the creation of contraceptives or provide vaccinations that stop the transmission of inheritable material. However, uncontrolled and unintended fusion can have disastrous effects. Fusogens must be tightly regulated at the level of subcellular structures to maintain the identity of organelles and membrane differentiation, which enables eukaryotic cells to carry out distinct metabolic and biochemical processes in a variety of spatial chemical conditions. Defects in SNARE proteins and dynamin have been related to a number of clinical disorders, including centronuclear myopathy and the CEDNIK syndrome. Defects in respiration and neurological illnesses including Charcot-Marie-Tooth syndrome and cases such as predominant optic atrophy are also brought on by impaired mitochondrial fusion, which also results in the buildup of mitochondria without the mitochondrial DNA. Atlastin, a protein that works in sensory neurons, is essential for fusion of the Endoplasmic reticulum. Hereditary sensory neuropathy and spastic paraplegia have both been linked to specific type of atlastin mutations. In addition, a number of illnesses and disorders, such as infertility and muscular dystrophy, have been linked to the decrease of fusogen activity [12].

Some viruses are thought to use cell-cell fusion in addition to virus-cell membrane fusion, which is an important step in enveloped virus penetration, which helps to infect the neighbouring cells. FAST proteins are expressed by cells infected with nonenveloped viruses like baboon orthoreovirus. These FAST proteins promote the fusing of infected and uninfected cells, which aids in the transmission of the virus. A syncytia forms in the lymph nodes of patients with HIV, HIV-infected humanized mice, and cell culture systems when T cells that are infected with the virus also express viral fusogens. However, the precise role of these Env-mediated syncytia in HIV replication and pathogenesis in vivo, including the broader significance of the fusion of cells in various viral infections, still requires further investigation [2]. Fusion can take place between cells inside a tumour or among cancer cells and macrophages when cellular or fusogens of virus are not specifically produced (for example, after being infected by an enveloped virus). Consequently, heterokaryons are created, which may eventually develop metastatic properties as a result of the increasing genetic and epigenetic variety brought about by fusion. Interestingly, the human genome contains 18 known retroviral elements that encode entire viral fusogens, albeit it is yet unclear how these elements specifically contribute to cell fusion in health or sickness [12].

Several challenges are faced while trying to identify and analyse the fusion process between cells. Factors including influx of calcium (exocytosis), endosomal acidification during internalized virion entry (influenza virus), and the interaction of viruses with host cell receptors and the cofactors of the fusion mechanism (e.g., HIV and Dengue virus) are frequently responsible for the activation of fusion mechanisms involving viral proteins and proteins within a cell. These events typically occur rapidly, within milliseconds to hours. Contrarily, the intricate and multi-step cell differentiation mechanisms that prepare for cell fusion can require days, and the precise environmental factors specifically initiating the fusion events itself—which take place in a matter of seconds to minutes—have not yet been fully identified. Differentiating between proteins that only operate prior to and post the fusion stages and those that only participate particularly in the fusion event is one of the biggest hurdles. Different experimental methods, including assessing their fusogenic activity and structural characteristics, are used to identify the fusogens. Three factors serve as the ultimate standard for determining a fusogen's function (or fusogenic compound): (1) it must be required in the process where cells need to fuse; (2) it must be situated on the membranes involved in fusion at the proper time and position; and (3) it must be efficient in fusing the membranes that typically may not undergo fusion. Only proteins that satisfy each of these criteria are recognized as true fusogens [2].

While many proteins have a part to play in regulating various elements of cell fusion, primarily fusogens have the special ability to be both essential and sufficient to promote the merging of cells. It's now possible to identify novel potential fusogens because of strong similarities in structure among fusogens of eukaryotes and better-studied viral and intracellular fusion proteins. The regulation of the mechanisms involved in fusing cells at the levels of transcription, translation, and posttranslational modifications is an additional significant factor. Operations of fusogens, their localization, and expression are further influenced by aspects like membrane lipid arrangement, intracellular trafficking, and the cytoskeleton, ensuring that the

appropriate cells are brought together at the appropriate time and location. Further research will explore how cells are efficiently fused by fusogens, how cellular machinery controls its functions, and the identification of fusogens that could be discovered in the future [2].

2. **Syncytium Formation:** Syncytium refers to a multinucleated cell resulting from the fusion of multiple cells. It is commonly observed in developmental processes, such as muscle and placental formation. The formation of syncytia involves repeated rounds of cell fusion without subsequent cell division.

The fundamental principle of the "cell theory" asserts that all living organisms are comprised of cells, and they form the basic building blocks of all living systems. While this theory initially implied the distinct and inviolable nature of individual cells, it also acknowledged the phenomenon of cell fusion, wherein two or more cells can merge. Remarkably, stem cells or the offspring they produce are able to undergo fusion with several cell types under specific conditions, leading to the blending of cytoplasmic and even genetic materials from diverse origins (heterotypic fusion). Such heterotypic cell fusion may hold significant implications for development, tissue repair, and the onset of diseases [1].

Theodor Schwann made notable observations on the fusion of cells in pig embryos' superficial dorsal muscles, noting that the cell walls at the junction points merge, allowing communication between the cell cavities without nuclear coalescence. These fused cell masses, later known as syncytia, facilitate the mixing of homotypic cell cytoplasm while keeping the nuclei separate. Schwann's observations extensively discussed cell fusion in muscle, nerve, and bone tissues in his historical monograph on microscopic structures of plants and animals. As observed in the placenta, bone, and muscle, and also in mature persons, syncytia formation through fusion is thought to take place during growth and development, giving rise to multinucleated giant cells. Importantly, because the genesis of such multinucleated cells has been identified or determined, the creation of syncytia fits with the cell hypothesis [1].

Challenges may arise if different cell types were to fuse (heterotypic fusion), as it might not be straightforward to determine the origin of the resulting cell. Nonetheless, syncytia formation has been observed spontaneously in nature, even within normal tissues. Recent studies provide evidence that the fusion of diverse cell types indeed takes place, resulting in cells termed heterokaryons to underscore their heterologous origin. Harris and colleagues originally hypothesized the likely scenario of the fusion of cells from different origins in 1965 when they used the Sendai virus to cause the fusion of Ehrlich ascites tumour cells and HeLa cell lines. The nucleus of every fusion partner had maintained individuality and stability over time within the experimental arrangement, which is noteworthy [1].

A syncytium is described as an epithelium or tissue characterized by the presence of continuous cytoplasm, forming a large mass without individual cell boundaries and containing multiple nuclei [17]. Typically, cells complete their division with cytokinesis

at the end of mitosis. But in particular tissues, partial cytokinesis takes place, creating a syncytium of cells that continue to be connected by intercellular links. The germline of numerous organisms, from fruit flies to human beings, exhibits this behaviour. Another method of syncytium formation involves the membrane fusion of specific cells that are ready to fuse and serve particular functions. All of these activities play crucial roles in diverse biological processes, as observed in a wide range of organisms [16].

Mammalian cell fusion is a strictly regulated mechanism that specifically takes place in certain tissues under normal physiological settings. Male gamete fusion with a female gamete, macrophage fusion to generate osteoclasts with multiple nuclei, myoblast differentiation into syncytial myofibers, and fusion of cells in the placenta to form the trophoblast layer are a few examples. When cells merge, their plasma membrane's lipid bilayer must undergo an active merging process, overcoming the energetic barrier that typically prevents such fusion [19]. This mechanism of fusion among cells is extensively discussed under the topic - "Membrane fusion."

Role of Syncytium Formation in Various Biological Processes: Role in physiological development: For example, spermatocyte and oocyte union is required for fertilization. The syncytiotrophoblast, the outermost layer in the human placenta, is also produced by the unio of trophoblast cells. Additionally, the fusing of myoblasts results in the formation of muscle fibres, whereas the fusion of bone macrophages produces osteoclasts having multiple nuclei, which are essential for regulating bone homeostasis [16].

Cell-cell communication: A well-coordinated regulatory mechanism involving intracellular calcium signalling and intercellular electrical transmission through the syncytium system is necessary for the coordinated pumping mechanism of the heart. Cardiomyocytes and cardiac-specific macrophages communicate inside this network, especially around the atrioventricular node. The macrophages and cardiomyocytes have integrated communication and this has the ability to affect how the heart contracts [20]. Individual cells (cardiomyocytes) of the heart muscle group together to produce syncytia at intercalated discs. These intercalated discs additionally give the muscle fibres structural support and also have gap junctions that permit the action potential to go smoothly along the entire stretch of the fibre [21].

Diseases: Cell-cell fusion has significant implications in cell transformation and the progression of cancer. Genetic integrity may be jeopardized, metastasis may be aided, and resistance to drugs can be developed. In addition, multinucleated cells can be found in a variety of granulomatous disorders, including leprosy, schistosomiasis, sarcoidosis, and tuberculosis, where they develop in response to the environment that is chronically inflamed by various bacteria and protozoans [16].

Enveloped viruses play a vital part in the fusion of cells as they express particular proteins that facilitate fusion between the viral envelope and the membrane of target cells. In addition to being present in the viral envelope, fusogenic proteins are also produced throughout the process of viral replication and transferred to cell membranes where they are then included in newly formed viral particles during the process of budding. When a sufficient amount of fusogens on the infected cell engage with receptors on neighbouring cells, syncytium formation occurs. This triggers cell-cell fusion. Cell-cell fusion is a

strategy used by several human-infecting enveloped viruses to increase viral persistence and dissemination. With the aid of this technique, they are able to fend off antiviral immune responses and survive without extracellular virions [16]. Notably, syncytia formation requires viruses capable of fusing directly at the cell surface without relying on endocytosis [22].

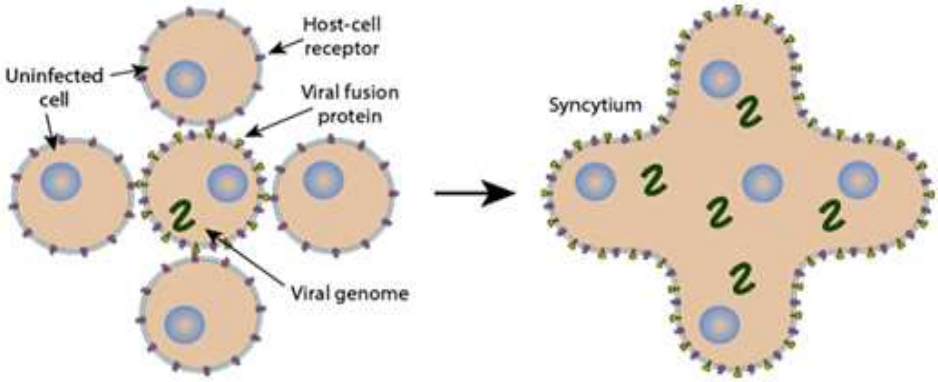

Figure 3: Syncytium Formation Caused by Viral Infection
(Source: Swiss Institute Of Bioinformatics. *Syncytium Formation Induced By Viral Infection*: Online) [51]

The epithelial lining of the airway is where many respiratory viruses, such as the human respiratory syncytial virus, influenza viruses, and SARS-coronaviruses, enter the human body. These viruses use cell-cell fusion as a tactic to escape the mucociliary blanket, which includes antibodies and effectively traps viral particles. These viruses readily propagate in the lung by using cell-cell fusion. Furthermore, human immunodeficiency virus infection is characterized by the development of syncytia in human beings and animal models such as mice. Syncytia have been seen in the brain and lymphoid organs of patients with HIV and primarily consist of merged monocytes, lymphocytes, and dendritic cells. In all of these situations, syncytium development is reliably linked to viral pathology, disruption of the function of cells, and increased disease severity [16].

Disease treatments: SeV (Sendai virus) possesses the unique capability to merge eukaryotic cells and create syncytium, making it a valuable tool for generating hybridoma cells. These hybridoma cells are capable of producing monoclonal antibodies on a large scale [8]. Monoclonal antibodies have found extensive clinical applications in diagnosing and treating various human disorders, such as cancer and infectious diseases. Additionally, they have been employed to modulate immune responses effectively [23].

In conclusion, syncytium formation plays a pivotal role in various biological processes, including developmental processes like muscle and placental formation. The process of cell fusion and the creation of multinucleated syncytium have been linked to viral pathogenesis, cancer progression, and immune modulation. Overall, understanding the mechanisms and implications of syncytium formation provides valuable insights into disease processes and potential therapeutic approaches.

III. DEVELOPMENTAL SIGNIFICANCE OF CELL FUSION

1. **Placental Development:** Cellular fusion is a finely tuned and dynamic process that holds immense importance in both development and the maintenance of a harmonious internal environment. However, this remarkable fusion capability is restricted to a select few specialized human cell types with the ability to merge and transform into multinucleated cells. Within the placenta this phenomenon contributes to the emergence of myotubes, osteoclasts and a layer called syncytiotrophoblasts (STB).[24]

Beyond its role in development, cellular fusion also actively engages in tissue healing and viral infections, and intriguingly, it could potentially be relevant to the growth and advancement of cancer. The intricacies of fusion of cells continue in to unravel, revealing its far-reaching impact on diverse biological processes.[24]

Under normal circumstances, the capability for cell fusion is restricted to a select few cell types within the human body.Within the placenta, this fusion process assumes a pivotal role in the development of syncytiotrophoblast, which represents a specialized lineage of trophoblast cells. The syncytiotrophoblast forms an extensive interconnected cytoplasmic network containing multiple nuclei. This intricate layer acts as a crucial barrier, facilitating the efficient exchange of nutrients and gases of substances between maternal blood and fetal tissue, while concurrently blocking the transfer of detrimental substances and maternal immune cells via intercellular junctions.[25]

In order to ensure the integrity regarding the syncytiotrophoblast, precursor cells called cytotrophoblasts undergo significant transformations. They cease their self-renewal capabilities, activate specific genes associated with differentiation, and ultimately merge with the syncytium above them. Any disruptions in these processes can lead to various complications during pregnancy, underscoring the vital role of proper syncytiotrophoblast formation in maintaining maternal-fetal health.[25]

The placental syncytiotrophoblast (STB) layer is an uninterrupted and multinucleated barrier that arises from the fusion and differentiation of mononuclear cytotrophoblasts (CTB). This mechanism of differentiation is crucial for the continuous growth and sustenance of the placenta throughout pregnancy. The syncytial layer plays a vital role in regulating the interchange of gases, nutrients, and other substances between the circulatory systems of the mother and the fetus. Furthermore, it acts as a safeguard, shielding the fetus from the maternal immune system. Additionally, the syncytiotrophoblast layer holds the responsibility for the synthesis and secretion involving a variety of hormones, proteins, and growth factors, including human chorionic gonadotropin (hCG) and glycoproteins unique to pregnancy.[24]

- **Exchange of Nutrients:** The syncytiotrophoblast, which comprises a microvillous plasma membrane (MVM) facing the maternal side and a basal plasma membrane (BM) directed towards the fetal circulation, serves as the primary barrier for the transportation of nutrients through the human placenta. The transport properties of these two plasma membranes play a crucial role in determining the overall transfer of nutrients across the placenta and subsequently affect growth of the fetus. Additionally,

the plasma membranes of the syncytiotrophoblast exhibit a variety of nutrient carriers that can be influenced by signals from the foetus, mother, and placenta.[26]

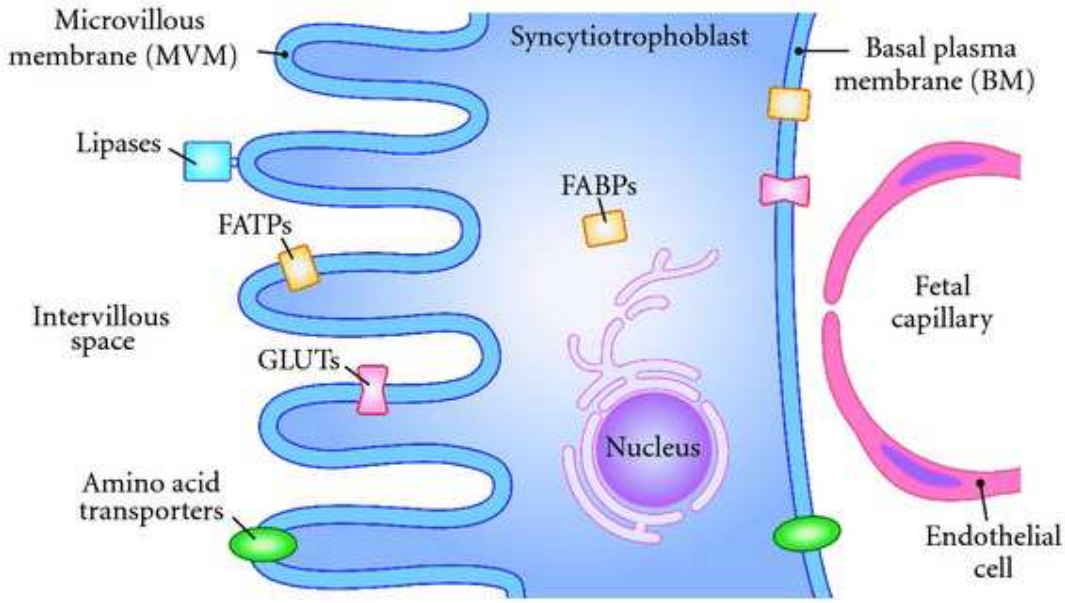

Figure 4: Syncytiotrophoblast Regulates Nutrient Passage, a Vital Maternal-Fetal Barrier. (Susaane Lager, Theresa L. Powell ,2012; Online)[52]

The syncytiotrophoblast assumes its crucial role as the primary barrier, regulating nutrient passage from mother to fetus. Encircling the microvillous membrane and aligned with the fetal circulation, maternal blood plays a vital part. The syncytiotrophoblast employs transporters to enable the movement of glucose, essential amino acids, and fatty acids. Furthermore, lipid transfer takes place through a meticulous process wherein lipases located outside the cell liberate fatty acids sourced from maternal lipoproteins, expertly guided by intracellular proteins that bind to fatty acids (FABPs) within the syncytiotrophoblast's cytosol.

The primary barrier to nutrient transfer from the mother to the foetus is the syncytiotrophoblast. Within the intervillous space, maternal blood collects and surrounds the microvillous membrane (MVM). Conversely, the basal plasma membrane (BM) of the syncytiotrophoblast faces the foetal circulation. The plasma membranes of the syncytiotrophoblast, both of them contain transporters that facilitate the movement of amino acids, glucose (through GLUTs), and fatty acids (via FATPs). When it comes to lipid transfer, extracellular lipases are responsible for releasing fatty acids derived from maternal lipoproteins, as intracellular binding proteins (FABPs) direct these fatty acids within the cytosol of the syncytiotrophoblast. [26]

- **Production of Hormones:** Within this intricate world of placental development, the cytotrophoblast (C), acting as a stem cell, forms an intimate bond with the multinucleated syncytiotrophoblast (S) through graceful desmosomes. The syncytiotrophoblast takes on a wondrous role as a producer of protein and steroid hormones, generously releasing them into the maternal blood (M).[27]

As the blastocyst finds its abode in the uterine wall, the syncytiotrophoblast orchestrates a grand performance, synthesizing human chorionic gonadotrophin (hCG), a glycoprotein hormone, in its rough endoplasmic reticulum (ER). Like a maestro, the syncytiotrophoblast dons a new role around the eighth week of gestation, transforming into the corpus luteum, and embarks on a symphony of steroid hormones—estrogen and progesterone—that fill the maternal realm with harmony. In this harmonious composition, the syncytiotrophoblast obtains the necessary cholesterol for progesterone synthesis from low-density lipoproteins (LDL) elegantly circulating in the maternal blood. The LDL receptors on the syncytiotrophoblast's deftly play their part in this intricate exchange.[27]

As the performance reaches its crescendo, another hormone takes the stage within the rough ER of the syncytiotrophoblast. This hormone, human chorionic somatomammotropin (hCS), dances in tandem with growth hormone, harmonizing with the enchanting melody of prolactin to foster the development of the majestic mammary gland.[27]

2. **Skeletal Muscle Formation:** Several subcellular events contribute to the process of myoblast fusion, which is a crucial step during the formation of mature skeletal muscle. Myoblasts, the precursor cells of skeletal muscle, undergo fusion to generate myotubes with multiple nuclei during the process of myogenesis. Additionally, myoblasts are capable of merging with existing myotubes which facilitates muscle growth and repair. Inside myoblasts, numerous proteins play essential roles in coordinating various intricate mechanisms like elongation, movement, cellular attachment, restructuring of the cytoskeleton, merging of membranes, and, eventually, fusion [28].

To study myoblast fusion, researchers employed three model organisms: Danio rerio (zebrafish), Drosophila melanogaster (fruit fly) and Mus musculus (mouse). By examining myoblast fusion in these models, we have reached the conclusion that myofibers in various organism’s form through the fusion of mononucleated precursors.[28]

The process of myoblast fusion encompasses several steps which is explained, each involving specific subcellular events. These steps are as follows:

- Initially, cells migrate towards their partner for fusion while simultaneously undergoing actin polymerization and expressing transmembrane attractants to guide their migration.
- Subsequently, the cells make contact and adhere to each other, leading to the localization of cell type-specific transmembrane proteins.
- As a result, actin accumulates, and the Fusion-Related MyoAdhesive Structure (FuRMAS) forms at the fusion site. Along with vesicular trafficking, a group of proteins involved in fusion localize to the fusion site.
- Following this, disintegration of membrane occurs, and the membrane undergoing vesiculation and components of mechanisms of fusion are removed.
- Lastly, the cell needs to restore itself in regard to subsequent accomplishing fusion cycles by producing suitable levels of transmembrane attractant. This repeating procedure persists until the desired muscle or fiber size is attained. [30].

The regulation of muscle formation and development involves a group of specific regulatory factors called Myogenic Regulatory Factors (MRFs). MRFs are muscle-specific proteins that belong to the basic helix-loop-helix family of transcription factors. The four MRFs, namely Myf5, MyoD, myogenin, and MRF4, play a crucial role in controlling the determination and differentiation of skeletal muscles during embryogenesis and postnatal myogenesis [29].

MyoD, the pioneer member of the MRF family, was initially identified and cloned by screening a library comprising an equal mix of myoblasts. Subsequently, the three other MRFs were also uncovered using comparable functional screens, along with the recovery of cDNAs showing similarity to MyoD. [29].

During embryogenesis, the expression of MRFs is restricted solely to myogenic cells, and each MRF displays dissimilarities in the level and timing of its expression. These variations highlight the distinctive reactions of each MRF to the signals and interactions occurring at the protein level. However, this aspect remains relatively unexplored until now.[29]

- **Embryonic Development of Skeletal Muscles:** Myogenic Regulatory Factors (MRFs) have a substantial impact on the development of skeletal muscle. The sequence commences as the Paraxial mesoderm forms on both sides of the neural tube in the mouse embryo, subsequently accompanied by segmentation along the head-to-tail axis, leading to the creation of somites. Signaling factors like Wnt, Sonic Hedgehog, and BMP factors impact the transformation of somites into an underlying mesenchymal sclerotome and a partially overlapping dermomyotome. The presence of MRFs becomes evident for the first time approximately one day E8.0. The lateral edges of the dermomyotome move towards its lower surface, experiencing a change from an epithelial to a mesenchymal state. This process leads to the formation of a distinct myotome beneath the dermomyotome, which holds significant importance in the development of skeletal muscles in both the body and limbs. Development in the myotome is influenced by communication originating from the notochord and floor plate, particularly through Shh signaling, which triggers the expression of Myf5 mediated by the action of the transcription factor Dmrt2. The upper middle region of the myotome gives rise to the myotome on the dorsal side, contributing to the development of muscles in the posterior region of the back, while the lower side region, referred to as the hypaxial myotome, controls muscle development in the body wall. Around E10.5, Wnt signaling originating from the upper endoderm and BMP4 from the side mesoderm prompt the expression of MyoD in this specific area. The activation of MyoD additionally relies on the paired-like homeodomain factor Pitx2, which operates before Pax3 in the hierarchy. Transcription factors Six1/4, coupled with their coactivators Eya1/2 and Pax3, play a significant role in governing MyoD expression, particularly in the hypaxial somite and limb domains. Eventually, hypaxial myoblasts situated close to the emerging limb buds disengage and move towards the limbs due to stimulation, thus participating in the establishment of limb muscles.[29]

Myogenin and MRF4 transcripts emerge around E8.5 and E9.0, respectively, with an even distribution throughout the myotome. MRF4 transcripts demonstrate a

two-phase expression pattern, initially undergoing downregulation by E11.5, however, resurfacing within differentiated muscle fibers around E16.0. The molecular differences between the epaxial and hypaxial myoblast groups are evident because they rely differently on Myf5 and MyoD.Myogenesis encompasses several stages of differentiation, commencing with embryonic myoblasts that give rise to the initial muscle fibers. These fibers serve as a framework for secondary myofibers formed by the fusion of fetal myoblasts around E14.0. Following this, satellite cells, which constitute a third generation of myogenic progenitors located in proximity to existing fibers, come into existence toward the conclusion of postnatal development. They facilitate the expansion of skeletal muscle size post-birth and are accountable for continuous muscle rejuvenation throughout the organism's lifespan.Some satellite cells enter a quiescent state while remaining primed for activation. The presence of the paired-box transcription factors Pax3 and Pax7 marks the emergence of embryonic myogenic progenitors originating in the central region of the somitic dermomyotome. The sequential triggering of Myf5, MyoD, myogenin, and MRF4 controls the process of myogenesis in this phase. There is a hypothesis that a subgroup of myogenic precursor cells, which don't express MRF but still retain the expression of Pax3 and Pax7, may function as precursors for adult satellite cells. [29]

- **Differentiation of Skeletal Muscle in Postnatal Development:** During the postnatal stage of satellite cell development, Pax3 and Pax7 serve as markers for muscle progenitors situated beneath the the foundational layer of mature myofibers. Pax7 is found within every satellite cell after birth, Pax3 is not expressed by every satellite cell. Research incorporating gene expression analysis and ChIP-seq studies of Pax7 and Pax3 in primary myoblasts has unveiled that both transcription factors identify identical DNA motifs, however, Pax7 displays a greater preference for homeodomain-binding motifs in contrast to Pax3. Pax3 interacts with a subset of Pax7 target genes primarily linked to embryonic functions and the preservation of an uncommitted state. In contrast, Pax7 selectively stimulates genes responsible for upholding the adult satellite cell characteristics, encompassing the control of proliferation and the inhibition of differentiation.[29]

Extensive research has been dedicated to comprehending the control of Myf5 and MyoD expression within satellite cells and their dedication to the myogenic lineage. Recent research suggests that even though adult satellite cells do not exhibit MyoD expression when at rest, insights from employing a MyoD-iCre mouse strain containing a lineage-tracing reporter allele indicate that all progenitors originating from satellite cells express MyoD during prenatal phases, irrespective of their anatomical position or embryonic source. Significantly, adult muscles house discrete groups of satellite cells that are either Myf5-positive or Myf5-negative, as evidenced in Myf5-nlacZ reporter mice and by directly observing Myf5 protein levels.[29]

To investigate whether Myf5-negative satellite cells constitute a distinct population that has never exhibited Myf5 expression during development, researchers employed the Myf5 Cre/R26R-YFP mouse model. This innovative model allowed for the labeling of cells expressing Myf5 and their subsequent generations with yellow fluorescent protein (YFP). The research findings unveiled that roughly 10% of the total satellite cell population had never displayed Myf5 expression throughout

development. Interestingly, the satellite cells lacking Myf5 transcription (YFP-) demonstrated a notable capability for prolonged self-renewal. In contrast, the satellite cells that did express Myf5 (YFP+) appeared to exhibit behavior more akin to committed progenitors.[29]

Within the intricate dance of gene regulation, Myf5 expression hinges on a fascinating process involving Pax7 and its arginine methylation by Carm1. This methyl "signal" prepares the context for the arrival of the histone methyltransferase complex Wdr5-Ash2l-Mll2 (Kmt2) at the Myf5 locus.Once the complex takes center stage, it orchestrates an enchanting performance of chromatin modifications, choreographing the transcriptional activation of Myf5. This activation is not just a solo act but rather an integral part of the graceful ballet of asymmetric divisions in muscle stem cells. Meanwhile, a secret has been unveiled: satellite cells do indeed whisper the Myf5 gene, but their words remain hushed and untranslated. This subtle quietness represents their method of preserving a dormant state, achieved by skillfully containing Myf5 transcripts within mRNP granules. A tiny conductor known as miR-31 seems to be directing this symphony of sequestration. But when the time is right, when the spotlight falls upon satellite cell activation, the mRNP granules part ways, releasing their sequestered treasures. The result is an astonishing display of rapid translation, transforming the once-muted Myf5 mRNAs into a harmonious melody of cellular activation.[29]

In the realm of proliferating myoblasts, MyoD's expression finds its inspiration from an ensemble of transcription factors: FoxO3, Six1/4, Pax3, and Pax7. Yet, an uncharted territory in this domain lies in exploring the interplay between the nuclear localization of gene-containing loci expressed during myogenesis and their transcriptional status. An example of this captivating phenomenon can be witnessed with the initiation of low-level transcription of MyoD, where the role of TFIID comes into play, harmonizing with the spatial localization of the MyoD locus near the nucleus's periphery. As the symphony of differentiation unfolds and myotube formation takes the stage, the MyoD locus gracefully relocates to the luminous core of the nucleus, guided by the presence of factors such as TAF3.[29]

IV. CELLULAR REPROGRAMMING THROUGH CELL FUSION

1. **Somatic Cell Nuclear Transfer (SCNT):** Somatic cell nuclear transfer (SCNT) encompasses the movement of nuclei from fully developed cells into either blastocysts or oocytes. These transplanted nuclei then undergo growth and differentiation, resulting in the generation of pluripotent cells. This mechanism has significant implications in both reproduction and therapy. Both aspects share a common initial procedure, which entails removing the nucleus from a mature cell, inserting it into an oocyte, and then stimulating its development using electric impulses or chemicals.As a result, an embryo with genetic identity identical to the donated cell nucleus is produced. When implanted in a uterus, this embryo develops into a clone. On the other hand, if the embryo is employed for tissue growth, it is regarded as therapeutic. The resultant cells are immunologically indistinguishable from the donor and hold the capacity to develop in a similar way to an embryo conceived naturally.[31] However, there is a broad consensus that the relatively low rate of successfully producing healthy and viable offspring through SCNT in animals

(1-5%) and the significant occurrence of abnormalities in these cloned animals can be attributed to the inadequate accomplishment of epigenetic reprogramming.[32]

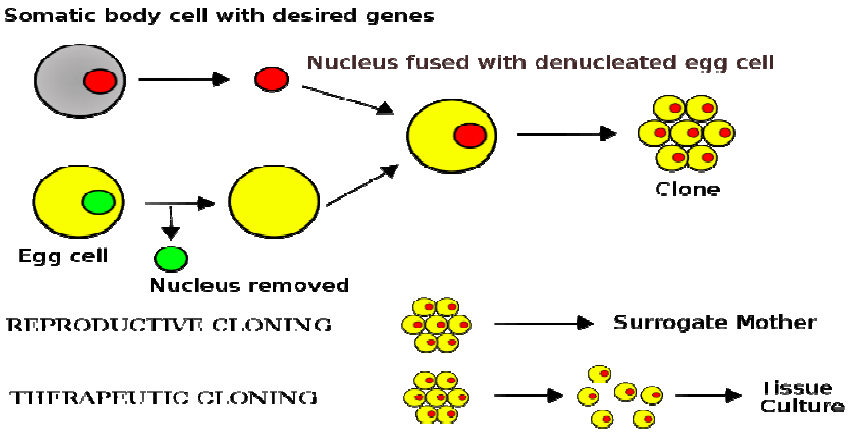

Figure 6: Schematic Representation Showing Pluripotent Stem Cell's Versatility for Cell-based Therapies Through Differentiation into Various Cells
(Derived from Image Drawn by/De: Quelle:Zeichner:Schorschski /Dr. Jürgen Groth; 2007; Online)[53]

The impressive capacity of pluripotent stem cells to transform into diverse cell types throughout the body offers significant promise for utilizing cell-based therapies to restore impaired tissues or organs resulting from injuries, degenerative conditions, aging, or cancer.
[33]

The acquisition of pluripotent stem cells from the inner cell mass of a blastocyst is limited because of ethical considerations. However, alternative methods exist for obtaining pluripotent cells from a variety of fully developed adult cells, along with adult stem cells through processes such as nuclear reprogramming and somatic cell nuclear transfer.[34]

Somatic cell cloning, also known as nuclear transfer or simply cloning, is an innovative method in which the nucleus-containing DNA of a somatic cell is placed within an oocyte in metaphase-II after its nucleus has been removed. This procedure results in the formation of a new individual that is a precise genetic replica of the donor somatic cell. The incredible achievement of cloning Dolly, an entire organism from a matured adult mammary epithelial cell, has ignited a revolution within the scientific community. It showcased that genes that were turned off during tissue differentiation can be fully reactivated through a phenomenon called nuclear reprogramming - a technique that reverses a differentiated nucleus to a totipotent state. [35]

Somatic cloning holds unique possibilities, including the potential to produce multiple copies of genetically superior farm animals, generate transgenic animals for pharmaceutical protein production or xeno-transplantation, and aid in the conservation of

endangered species. Moreover, through optimization, it offers vast biomedical potential for therapeutic cloning and allo-transplantation.[35]

In addition to its practical applications, cloning has become an indispensable tool for studying gene function, genomic imprinting, genomic re-programming, developmental regulation, genetic diseases, gene therapy, and various other research areas.[35]

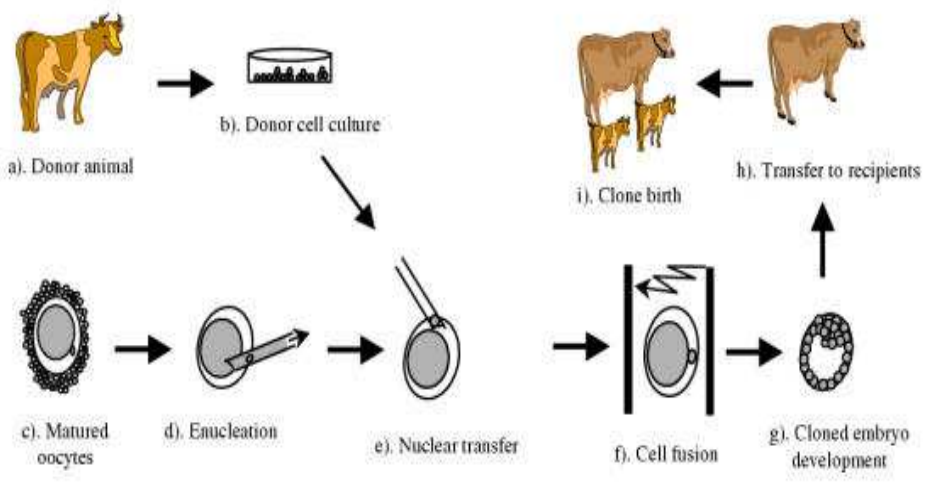

Figure 7: The Image Illustrates Somatic Cloning Process Involving Cell Collection, Culture, Fusion, Embryo Development And Birth. (Tian Et Al., 2003; Online)[54]

- The somatic cloning process involves several steps. Firstly, cells are gathered from the donor
- The cells are cultivated outside the body in a controlled environment.
- Next, a fully developed oocyte has its nucleus removed
- A donor cell is inserted into the oocyte from which the nucleus has been removed
- The somatic cell and the oocyte are subsequently merged
- The resultant embryos are cultured in a controlled environment until they mature into blastocysts.
- Later, the blastocysts can be transplanted into a host, leading to the birth of cloned animals after the gestation period has concluded. [35]

Pluripotency is the capacity of cells to develop into diverse types of cells, this can be attained through reprogramming. This procedure entails triggering the dedifferentiation of somatic cells, which is thought to happen by eliminating the epigenetic imprints of the cells, resulting in an elevation of their potential. In the past, a significant portion of reprogramming research was carried out through nuclear transfer methods. [36]

Nonetheless, in more recent times, multiple research teams have demonstrated that reprogramming can also be accomplished by introducing pluripotency genes. The effective reprogramming of somatic cells results in their "dedifferentiation," converting

them into a pluripotent state and facilitating the creation of pluripotent cell lines. The application of reprogramming to produce pluripotent cells enables the establishment of cell lines that possess the same genetic makeup as the donor cells. [36]

Somatic cell nuclear transfer (SCNT) capitalizes on an exclusive characteristic of the oocyte cytoplasm, facilitating the transformation of somatic nuclei into a state of pluripotency. In this process, the nucleus of a mature cell is transplanted into an oocyte that has had its nucleus removed. Following that, the nucleus of the somatic cell undergoes reprogramming, and there exists a potential for partial progression to the ICM stage while in a cultured environment. Subsequently, two options can be followed: implanting into a readied uterus to produce cloned animals, or extracting the ICM to establish lines of embryonic stem cells (ESCs).[36]

In 2005, a South Korean research team asserted the creation of human embryonic stem cells (ESCs) from blastocysts specific to patients using somatic cell nuclear transfer (SCNT). Unfortunately, this study was subsequently revealed to be fraudulent, and to date, there have been no documented instances of successfully generating human embryonic stem cell lines using somatic cell nuclear transfer (SCNT). [36]

2. **Induced Pluripotent Stem Cells (iPSCs):** Induced pluripotent stem (iPS) cells represent a category of pluripotent stem cells derived from adult somatic cells that undergo genetic reprogramming, resulting in them attaining a state resembling that of embryonic stem (ES) cells. This reprogramming involves the controlled activation of specific factors and genes crucial for maintenance of the characters of ES cells. Notably, human induced pluripotent cells were initially generated independently in late 2007 by two separate research teams, namely Yamanaka's and Thomson's groups, using human fibroblasts as the source of somatic cells.[37]

The revolutionary revelation of iPS cells has offered researchers a means to acquire pluripotent stem cells without resorting to contentious embryo usage. This novel and potent technique enables the "de-differentiation" of cells, overturning the traditional belief that their developmental destinies were fixed. Additionally, tissues derived from iPS cells bear a striking resemblance to the donor's cells, making them invaluable in disease modeling and drug screening research. Anticipations are high that iPS cells will provide insights on reprogramming cells to mend damaged tissues within the human body.[37]

The prevailing notion was that the genetic material of a fully developed cell remained permanently fixed in a mature non-reproductive state, incapable of reverting to an embryonic stem cell (ESC)-like state. However, Sir John B. Gurdon shattered this belief by successfully creating a completely operational tadpole emerging from an egg that hasn't been fertilized that contained the nucleus of a differentiated intestinal epithelium cell from a mature frog. This groundbreaking experiment demonstrated that differentiated cells retained the crucial genetic information necessary for an organism's development. Later, more than three decades after Gurdon's work, the cloning of Dolly the sheep using nuclear transfer technology further affirmed that differentiated cells preserved their genetic memory. This remarkable discovery revealed that oocytes

contained factors capable of reprogramming the nuclei of mature cells, allowing them to exhibit pluripotent capabilities akin to ESCs.[38]

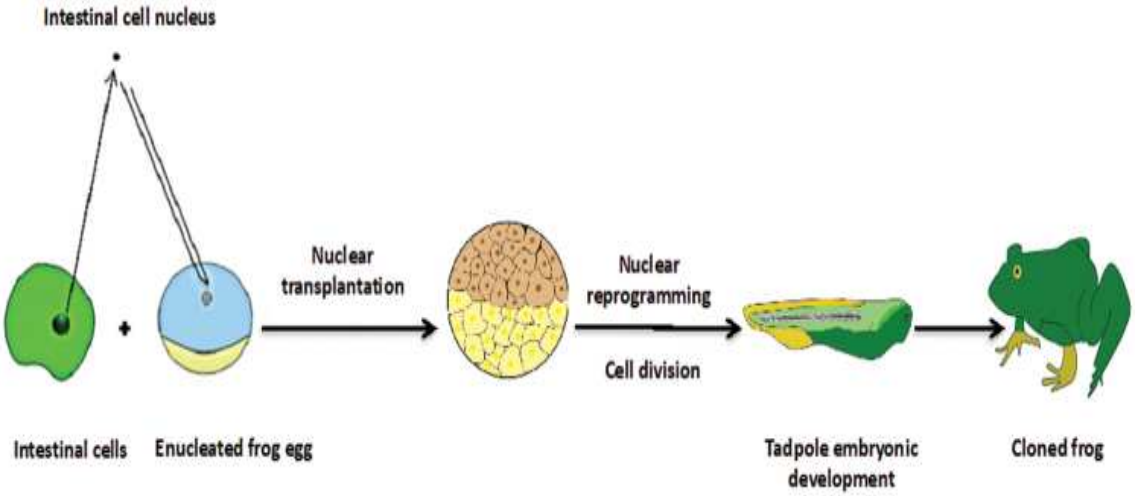

Figure 8: Illustrates Nuclear Reprogramming. (Abbar Et Al.,2020; Online) [55]

During the process of nuclear transfer, the nucleus from a differentiated cell is transferred into an egg from which the nucleus has been removed during meiotic metaphase. This transplanted genome undergoes reprogramming, transforming it into a state of pluripotency. As a result, the egg experiences cellular division, leading to the production of a cloned animal.[38]

It was postulated that the factors responsible for maintaining the identity of embryonic stem cells (ESCs) might also have a crucial role in inducing pluripotency in somatic cells. Substantial efforts have been devoted to identifying these factors. Takahashi and Yamanaka were pioneers in demonstrating the generation of pluripotent stem cells from adult fibroblasts through the introduction of four distinct transcription factors: Oct3/4, Sox2, Klf4, and c-Myc (OSKM). Their groundbreaking work paved the way for further research in this area.[38]

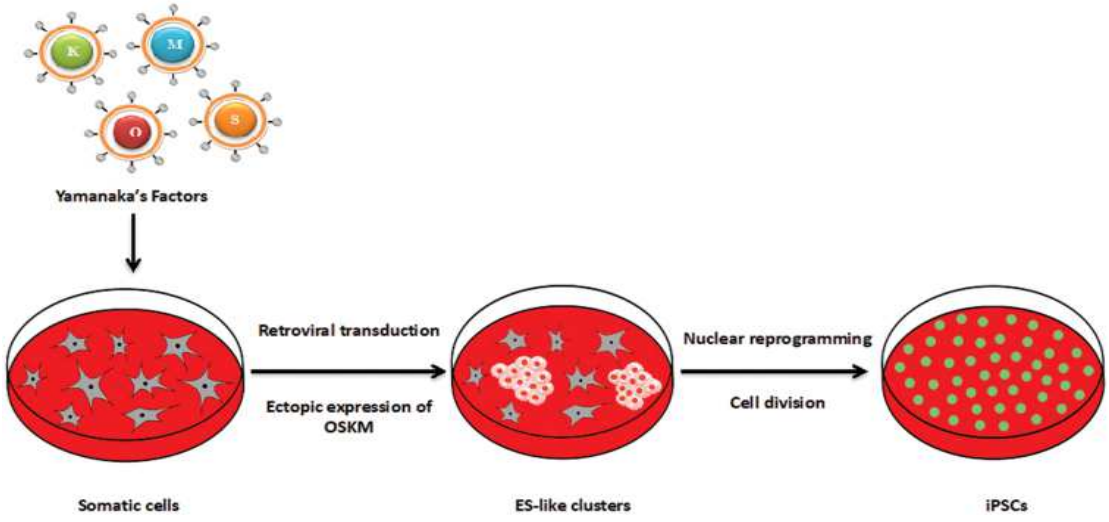

Figure 9: The Image Illustrates Nuclear Reprogramming Strategy using Specific Transcription Factors to Induce Pluripotency. (Abbar Et Al.,2020; Online) [56]

The approach of nuclear reprogramming entails the overexpression of four particular transcription factors associated with pluripotency, namely Oct4, Sox2, Klf4, and c-Myc. By introducing these factors, the unipotent state of cells can be reversed, transforming them into a pluripotent state. To clarify, c-Myc stands for cellular-Myelocytomatosis, Klf4 for Krüppel-like factor 4, Oct4 for octamer-binding transcription factor 4, and Sox2 for SRY (sex determining region Y)-box 2.[38]

Embryonic stem (ES) cells exhibit the capability to induce reprogramming in somatic cells through cell fusion. Resultant hybrids of these cells display properties similar to pluripotent cells. Additionally, it has been illustrated that alterations in transcription can take place within a heterokaryon even in the absence of nuclear fusion. Nevertheless, it remains uncertain if these alterations can persist after the dominant ES nucleus is removed.[39]

Through the merging of cells with embryonic stem cells, somatic cells can be transformed into a state of pluripotency. This reprogramming can be achieved experimentally through various methods, including the use of chemicals like electrical currents, PEG and viruses. Typically, this process yields a hybrid cell with a tetraploid configuration.[39]

Cell fusion-induced reprogramming entails combining a fusion of a somatic cell and a pluripotent cell, leading to the formation of a tetraploid cell hybrid. Despite ES-somatic cell hybrids exhibiting many attributes of pluripotent stem cells, their tetraploid state poses certain limitations.[39]

ES-somatic cell hybrids, due to their tetraploid state, are limited in their ability to significantly contribute to late gestation epiblast and chimeras. In order to establish cell fusion-based reprogramming of somatic cells as a feasible technique for producing autologous pluripotent cells, it becomes essential to eliminate the ES cell-derived DNA following the reprogramming process.Our laboratory has proposed a method involving the reprogramming of a somatic cell in a heterokaryon before nuclear fusion.Consequently, the removal of the ES nucleus through enucleation holds the potential to yield successful creation of autologous pluripotent cells. [39]

V. APPLICATIONS OF CELL FUSION IN REGENERATIVE MEDICINE AND DISEASE TREATMENT

1. **Cell-Based Therapies:** Cell replacement can be used in cell therapies to repair the covert mechanisms that cause disease onset and progression. Such therapies can make use of stem cells, progenitor cells, or primary cells [40]. It has been experimentally observed that the formation of improved blood vessels, transformation to cardiac monocytes, and alternatively or additionally providing proangiogenic and antiapoptotic factors upgrade tissue restoration in a paracrine manner after ischemia by a variety of cell types, which increases the functional restoration of the heart. Cell-based therapy has been shown to be an effective treatment for ischemic diseases. Adult bone marrow-derived cells can be used preferentially to treat patients who have acute cardiac infections. Most research suggest that cell treatment improves myocardial contractile function and reduces infarct size.

Ischemic illnesses continue to be one of the leading causes of death and morbidity in the modern world, despite the introduction of several therapeutic approaches. There are several types of stem cells with the potential to promote heart regeneration, repair, and neovascularization, but only two types should be distinguished: Embryonic stem cells and adult stem cells. While adult stem cells are more specific (i.e., lineage committed is greater) and can only be used to a limited extent for organogenesis, embryonic stem cells have the capacity to produce tissues and organs by evolving into a variety of cell types. The bone marrow-derived stem cells, the circulating pool of stem or progenitor cells, and tissue-resident stem cells are among the more than three distinct categories seen in adult cells.

The complex mixture of progenitor cells found in bone marrow also includes mesenchymal stem cells, multipotent adult progenitor cells, and hematopoietic stem cells, also known as "side population cells." Various studies have demonstrated how these bone marrow-derived cells collaborate to repair damaged tissue. The blood's circulating progenitor cells also exhibit therapeutic potential. Circulating progenitor cells were discovered originally while looking for proangiogenic cells for therapeutic vasculogenesis. After ischemia, the as yet unidentified endothelial progenitor cells by Asahare and Isner promote neovascularization and produce new blood vessels. According to the assumptions, these cells may be adult hemangioblasts; they were identified by the expression of at least two hematopoietic stem cell markers, the endothelial marker VEGF-receptor 2 and the CD133+ OR CD34+. The marker combination CD34+CD133+KDR+ is used to identify circulating progenitor cells that are clonally expandable and have a strong capacity to adopt an endothelial phenotype that has recently been challenged. While in vitro research indicates that CD34+/CD45- cells play a prominent role in acquiring an endothelial phenotype, CD34+CD133+KDR+ cells do not evolve into endothelial cells. Numerous studies have shown that circulating progenitor cells encompass a variety of cell populations, especially when cultured in vitro. However, it is unclear how these findings apply to the in vivo situation, where ischemic or necrotic tissue may create a unique environment influencing cell destiny. While certain cells are capable of cloning and show stem cell traits, other cells provide proangiogenic substances, promote vessel maturation, or work as a group as pericytes, ultimately leading to neovascularization. For instance, a subset of myeloid and myeloid precursor cells can be found in these cultivated or naturally circulating cells. These cells have the capacity to develop into endothelial cells and have been identified to potentially serve as proangiogenic cells. Myeloid subpopulations may help in muscle regeneration in addition to neovascularization because it has been shown that myeloid cells can fuse with skeletal muscle myotubes. As a result, the term "endothelial progenitor cells" probably refers to a variety of cell types that cooperate to help save ischemic tissue. Consequently, this word refers to a particular functional feature within a variety of cell populations that can trigger neovascularization.

Multiple tissues produce additional stem cell types that have shown therapeutic promise in ischemic situations. These include tissue-resident cardiac stem cells, mesoangioblasts, and mesenchymal and endothelial progenitor cells generated from adipose tissue. Mesoangioblasts, multipotent progenitors connected to blood arteries, express the VEGF-receptor 2, a crucial indicator of angiogenic progenitors. From hematopoietic endothelial progenitor cells, they are distinct. Mesoangioblasts have the

ability to develop in vitro into a variety of mesodermal cell types, such as smooth, cardiac, and striated muscle, bone, and endothelium. In vivo investigations have demonstrated their capacity to restore heart function and boost skeletal muscle function in a muscular dystrophy modelAdipose tissue is an important source of many stem/progenitor cell subsets that have the potential to promote neovascularization and mend the heart. Both mesenchymal stem cells and endothelial progenitor cells have been successfully extracted after enzymatic digestion of adipose tissue and have shown positive benefits in experimental trials.

It is now possible to induce the multiplication and development of tissue-resident stem cells, also referred to as "cardiac stem" cells, in vivo. These cells are ideal for heart repair because they are predisposed to have a cardiac phenotype. Several different populations of cardiac stem cells, including as c-Kit+ cells, Sca-1+ cells, side population cells (SP), and cells expressing the protein Islet-1, have been discovered and characterized. Islet-1 expressing cells have so far only been found in neonatal hearts, despite the fact that c-Kit+, Sca-1+, and cardiac SP cells have been recovered from adult hearts. It is currently unclear to what extent c-Kit+, Sca-1+, and cardiac SP cells represent three different cell types. The cultivation of self-adherent clusters known as "cardiospheres" obtained from subcultures of murine or human biopsy tissues has led to the identification of a novel class of cardiac stem cells. This technique, which was modified from the formation of neurospheres, suggests that cardiac neural crest cells may have contributed to the cardiac side population's (SP) cells. Heart-specific cell types such as myocytes and vascular cells that express endothelium or smooth muscle cell markers can be differentiated from cardiac stem cells formed from cardiospheres and c-Kit+. These cells also have the ability to sustain long-term self-renewal. The mechanisms governing the preservation of the cardiac stem cell pool as well as the precise origin of these c-Kit+, Sca-1+, SP, Islet-1+, or cardiosphere-derived cardiac stem cells are yet unknown. Recent research suggests that bone marrow may be the source of c-Kit+ and cardiac SP cells. These studies do not completely rule out the possibility that particular subpopulations of cardiac stem cells originate from the heart, suggesting that these cardiac stem cells could be leftovers from embryonic development found in particular niches within the heart. This is an important point to remember. Various autologous adult progenitor cells are currently undergoing preclinical testing to determine their potential for heart repair. The most often used source in clinical settings is bone marrow. This preference is explained by the long 30-year clinical track record and superior safety profile of bone marrow-derived mononuclear cells (BMCs), which are utilized for bone marrow restoration. With the exception of the BOOST experiment, which used a sedimentation methodology, the mononuclear cell fraction is typically collected using density gradient centrifugation after bone marrow aspiration. A variety of cells, including various percentages of hematopoietic stem cells, endothelial progenitor cells, mesenchymal stem cells, and side population cells, make up the mononuclear fraction. Right now, isolated bone marrow-derived cells are administered straight to the heart without being expanded ex vivo. Studies have concentrated on isolating particular progenitor cell subpopulations, such as a subset of hematopoietic and endothelial progenitor cells that express the marker protein CD133+. Progenitor cells from peripheral blood are also used in therapeutic settings to treat peripheral ischemia and heart repair. These cells, which come from mononuclear blood cells, are selectively enriched ex vivo by being cultured for three days in an "endothelium-specific" medium. Another strategy

uses enriched hematopoietic progenitor cells CD34+ from whole blood following G colony-stimulating factor (CSF)-mediated movement of these cells from the bone marrow into the bloodstream.

Initial experimental studies have demonstrated that the infusion of bone marrow-derived mononuclear cells or CD34+ cells have no detectable adverse effects; nevertheless, in recent years, questions have been raised about possible problems with cell therapy. The potential effects of cell treatment on electrical stability, an increase in restenosis, or the development of atherosclerotic disease are some of these worries. But unlike some trials using myoblasts, none of the clinical investigations using bone marrow mononuclear cells (BMCs) have found a higher incidence of arrhythmias. Furthermore, just one study using CD133+ cells found a rise in restenosis, which was first thought to be a potential adverse effect coming from progenitor cell-mediated plaque angiogenesis or inflammation. This result is unexpected because it was anticipated that isolating specific progenitor cells—while excluding proinflammatory cells—would lower the likelihood of restenosis and the progression of atherosclerotic disease. Although there were no systemic anti-mouse antibodies found in the patients, it is important to note that CD133+ cells were extracted using a mouse antibody, and it is likely that the leftover antibody may have caused a local proinflammatory reaction. Contrarily, none of the other studies found an elevated risk of restenosis; rather, the REPAIR-AMI study showed a lower requirement for revascularization treatments. Direct infusion of unpurified bone marrow cells or mesenchymal stem cells has been shown to cause intramyocardial calcification in mice models of myocardial infarction. However, based on MRI imaging, no clinical trials undertaken to date have reported the incidence of calcifications. This disagreement may be related to the density gradient centrifugation method used in the majority of clinical research to enrich mononuclear cells, which was the cause of the enrichment. Only the injection of unfractionated bone marrow cells and mesenchymal stem cells, but not the injection of pure hematopoietic progenitor cells, caused pathological defects and calcification in experimental models, according to a side-by-side comparison.

Based on the already available clinical data, it can be said that cell therapy using cells obtained from bone marrow is both possible and secure for the duration of the available follow-up period (up to 5 years for the initial studies). The proangiogenic capability of endothelial progenitor cells (EPCs) and its potential connection with enhanced tumour vascularization have been discussed. As most clinical trials have excluded patients with established tumours, it is still unclear if a single application of EPCs is enough to induce tumour growth. Patients receiving treatment with bone marrow-derived cells did not have an increased incidence of cancer over the follow-up period of the conducted research. However, given the rarity of such occurrences, careful attention must be paid to this element in subsequent investigations [41].

2. **Cancer Research and Treatment:** Stem cell-based therapies are proving to be effective in combating cancer. When modified to carry therapeutic substances, stem cells are potent delivery vehicles that can effectively target malignant tissues. Various types of stem cells have demonstrated natural ability to target tumours. An overview of stem cell-based cancer treatments is presented here, along with the need to translate the most promising findings from preclinical research into clinical trials. Anti-tumour agents are created to target cancer cells with minimal harm to healthy cells as a primary objective of cancer

therapy. There is currently a major drawback to conventional treatments in terms of their inability to select healthy tissue, leading to significant tissue loss. It has been common practice to treat non-surgical cancer with chemotherapy and radiation for a significant period of time. The use of many of these treatments is not successful in treating some cancers, and these cancers eventually develop resistance to them as well [42].

Due to their outstanding antigen-presenting abilities, dendritic cells (DCs) have taken centre stage in the planning of the immune system's response to cancer. Innovative strategies in this conflict, including cancer vaccination, combination therapy, and adoptive cellular therapy, have resulted from fundamental studies into the molecular biology of DCs. With the development of immunotherapy, which strengthens the body's anti-tumour immune response, during the past ten years, the treatment of cancer has experienced a revolutionary transition. In several patient populations and for various tumour types, this strategy has produced potent and sustained immune responses. Targeting T-cell inhibitory checkpoint proteins like CTLA-4, PD-1, or PD-L1, immune checkpoint inhibitors (ICIs) have been instrumental in this advancement. They have been approved to treat a variety of cancers, including melanoma, non-small-cell lung cancer, head-neck cancer, bladder cancer, renal cell cancer, hepatocellular carcinoma (HCC), and other tumour types. Despite these inhibitors' amazing success, ICI therapy has only been beneficial for a small subset of cancer patients. The success of adoptive chimeric antigen receptor (CAR)-T-cell transfer against solid tumours has also been less prominent, despite the fact that it has been licensed for treating hematological cancers. In order to activate naive T cells and urge them to develop into cytotoxic T lymphocytes (CTLs), dendritic cells (DCs), which operate as very potent antigen-presenting cells, are crucial in the development of anti-tumour immune responses. DCs help immune responses to infections and tumours while preserving tolerance to the body's own tissues. Different human malignancies have been connected to the development of DC function deficiencies. The creation of efficient cancer vaccines has been difficult since they target tumour antigens, some of which are self-antigens.

Dendritic cells (DCs) develop from bone marrow progenitors of the myeloid lineage. The comparable human progenitor for lymphoid DCs has not yet been identified, despite data from mice suggesting the significance of a lymphoid DC compartment in sustaining peripheral tolerance. Approximately 0.5% of DCs are found in the bloodstream, however they are primarily found at sites of entry such the skin and mucosae. DCs are discovered in these peripheral tissues in an immature stage of development. They can be drawn from blood monocytes or DCs produced from colony-forming units (cfu) to injury sites in response to stress or inflammatory signals (TNFa, IL-1, MIP-3), where they capture antigens and start the processing of exogenous antigens. They move towards the T cell-enriched regions of lymphoid organs by afferent lymph and eventually transform into "interdigitating DCs" through chemokine-mediated signalling (6-Ckine, MTP-3). Dendritic cells (DCs) convert into fully developed antigen-presenting cells (APCs) during this stage of development. The primary skill of immature DCs is the ability to engulf foreign antigens by a variety of processes including macropinocytosis, phagocytosis, and receptor-mediated absorption (e.g., mannose and FcR). They consequently express MHC class I, MHC class II, and costimulatory molecules at relatively low quantities on their cell surface. Notably, MHC class II molecules are immature because of their location in late endocytic compartments.

Langerin, CD1a, CD68, and CCR6 (a receptor for MIP3a) are phenotypic markers for "immature DCs" in humans that are widely acknowledged. A mature morphology with distinct dendrites develops in contrast when DCs are subjected to inflammatory stimuli (such as LPS, CD40L, TNF, IL-1, PGE2, and double-stranded RNA). The surface expression and half-life of MHC class I, MHC class II, and costimulatory molecules are also improved throughout this process. This collection of antigen-presenting molecules on the plasma membrane makes it easier for T cell receptors to be sequentially activated. Mature DCs express particular markers such CCR7 (the MIP3P receptor), p55, DC-LAMP, and CD83 and are essential for priming naive T cells. DCs release IL-12, a Th1 cytokine important for delayed-type cellular immunity, in response to particular T cells. Through the signalling of members of the TNF receptor family, these interactions between DCs and T cells improve both cell types' chances of survival. Mature DCs secrete "recruitment chemokines," such as the macrophage-derived chemokine (MDC), and express certain adhesion molecules, such as DC-SIGN, which start cell/cell membrane contacts (ICAM3 ligation), to make interactions with activated T cells easier. The ability of DCs to cross-present foreign antigens in combination with MHC class I molecules and to move to lymph nodes is one of the fundamental biological characteristics that sets them apart from macrophages. These characteristics are essential for delivering antigens at the ideal dose and time, at the correct location, to effectively prime CD4+ T helper cells and CD8+ cytotoxic T cell-mediated immune responses. The tendency of typical adjuvants used in immunizations to encourage antibody responses rather than cytotoxic T lymphocyte (CTL) responses is an intriguing finding. DCs are therefore seen as selective natural adjuvants that favour the induction of cellular immunity. Intriguingly, dendritic cell (DC) infiltrates have been linked in pathology reports to poor tumour prognosis, underscoring the significance of DCs in anti-tumour immunity.

Dendritic cells (DCs) generated from bone marrow or spleen that have been cultivated ex vivo in the presence of GM-CSF with or without IL-4 and subsequently loaded with pertinent CTL-defined tumour epitopes have been shown to have the ability to immunize. In mice with established immunogenic palpable tumours, these DC-mediated anti-tumour immune responses led to notable tumour growth decreases or even total tumour elimination. In prophylaxis experiments, DCs effectively shielded the host against a deadly cancer challenge when they were pulsed with the proper tumour peptides.

It was discovered that CD4+, CD8+, and Th1 cytokines were necessary for these DC-induced anti-tumour immune responses. Surprisingly, these DCs could also cause sizable tumours and metastases in the brain to recede. Recombinant tumour cells that expressed potential tumour antigens (PGAL, OVA) were used in animal models, and DCs were either loaded with certain peptides from these recombinant proteins or genetically altered to express such peptides or proteins. The use of C3-HPV16/18-generated sarcoma to mimic cervix carcinomas was one method used to simulate virally induced carcinogenesis, including cervical cancer. Dendritic cells (DCs) were loaded with E6/E7 peptides in these animals, and it was remarkable how well they protected the host from tumours. Other studies concentrated on p53 (wild-type or mutant epitopes) to show the immunogenicity of such peptides, especially in p53 knock-out mice. These animal models, however, were unable to prove with certainty whether or not DCs can get rid of

in vivo self-tolerance. According to research, peripheral self-tolerance may be overcome by selectively stimulating DCs with the cognate signal CD40L to promote DC maturation. Investigations have shown that DCs can directly stimulate CD8+ T cells by receiving activating signals from active CD4+ T cells via CD40/CD40L interactions. Based on this, efforts to improve immune responses included treating DCs with CD40L trimers prior to in vivo injection and combining Flt3L with CD40L trimers [43].

3. **Tissue Engineering and Organ Transplant:** A multidisciplinary field called tissue engineering combines bioengineering, material science, and life sciences to develop biological replacements with the goal of restoring, maintaining, and enhancing tissue functioning following damage brought on by illness or trauma. Tissue engineering's essential concepts entail fusing living cells with an organic or synthetic framework to create a three-dimensional living structure that meets or exceeds the target tissue's functionality, structure, and mechanical qualities. End-stage illnesses or tissue loss that would otherwise be incurable have shown to benefit from artificial transplantation or the usage of transplanted organs. However, there are drawbacks to this strategy, including a lack of viable organs, the requirement for ongoing immunosuppression, and the possibility of catastrophic consequences. Tissue engineering has evolved as a solution to these problems and is now an important component of regenerative medicine. To construct biological substitutes, the interdisciplinary area of tissue engineering combines concepts and techniques from bioengineering, material science, and life sciences. These alternatives strive to repair, preserve, and enhance tissue functions following damage brought on by illnesses or traumatic events [44].

   To construct engineered tissues, tissue engineering uses two main strategies. In the first method, cells that have been sown in vitro are supported by scaffolding. The foundational matrix is subsequently produced by the cells, resulting in a transplantable tissue. The second method uses the scaffold as a delivery system for medications or growth factors. When a scaffold is implanted, growth factors are added to it, which attracts body cells to the area and causes tissue to grow on and within the scaffold. Given that the composition, topography, and design of scaffolds can interact and affect cell behaviour, it is imperative to precisely match the cell type and scaffold combination for the intended goal. It has been demonstrated that the scaffold's architecture affects how cells react and how tissues form, as seen by the development of mineralization fronts in particular scaffold regions. Nano- to microscale topography modifies cytoskeleton configurations to influence cell activity. Furthermore, differing materials cause different cell types to react in different ways; for instance, different scaffold materials can cause varied levels of glycosaminoglycans in tissue-engineered cartilage. When choosing scaffolds for tissue engineering, the selection of cell source and culture conditions is an important consideration. Tissue-engineered constructions can now be made by fusing different cell types with scaffolds. Using cells to populate matrices and create a matrix that resembles that of the natural tissue is essential for the successful in vitro generation of synthetic tissues. The primary cells taken from the patient and coupled with scaffolds to produce tissue for subsequent re-implantation have led to the greatest advancements in this discipline. However, this method is constrained by the invasiveness of cell collection and the potential for employing sick cells. Thus, the use of stem cells, such as embryonic stem (ES) cells, bone marrow mesenchymal stem cells (BM-MSCs), and umbilical cord-derived mesenchymal stem cells (UC-MSCs), has become the focus of research. These

stem cells have the potential to develop into a variety of cell types and have regenerative properties, making them promising alternatives for tissue engineering [45].

Alternative methods to repair damaged cells or restore organ function are urgently needed given the dearth of donor organs and tissues for transplantation treatment. Investigating the therapeutic potential of cell fusion is one possible path. We look at many situations where cell fusion happens naturally during mammalian development and explain the ramifications of this, especially its healing effects after tissue damage or cell transplantation. However, there are considerable obstacles that must be overcome in order to make cell fusion a practical therapeutic technique. These difficulties include selecting the most appropriate cells for reparative fusion, figuring out the best approach to introduce these cells into the target tissue, figuring out how to improve the frequency of cell fusion, and making sure that the resulting fused cells are functional. The therapeutic potential of cell fusion could be unlocked by removing these barriers, as several recent transplantation trials have shown. [46]

When mouse tumour cells were inserted into the abdominal cavities of chick embryos and covered in a biocompatible polymer membrane, they demonstrated survival, which is when the idea of tissue engineering was originally proposed. Significant advancements were made over the ensuing decades, including the finding by Chick et al. that pancreatic beta cells from newborn rats, when cultivated on artificial capillaries and perfused with media, could produce insulin in response to variations in glucose concentration. Burke et al.'s successful development of artificial skin in the early 1980s, using fibroblasts seeded onto collagen scaffolds to treat severe burn injuries, was another significant development. This method is still employed in clinical settings today. Currently, efforts in tissue engineering are concentrated on creating different types of tissues and organs with a focus on using stem cells. The ultimate objective is to integrate cell implantation and matrix integration into patients to repair tissues and restore organ function. The three types of current tissue engineering techniques are histioconductive, histioinductive, and substitutive. Histioconductive techniques replace missing or damaged organ tissue with ex vivo constructs, whereas substitutive procedures replace entire organs ex vivo. Histioinductive methods, on the other hand, encourage self-healing and may involve gene therapy using growth factors or plasmid vectors to transport DNA. Several crucial requirements must be met for injured tissues to be repaired effectively and permanently. To begin with, enough cells must be produced to fill the tissue deficiency. Additionally, these cells must be able to develop into the desired cell types. Additionally, the cells should make the extracellular matrix (ECM) required for tissue structure and adapt to an appropriate three-dimensional structural support or scaffold. The created cells must show that they are structurally and mechanically compatible with the tissue's natural cells. Additionally, it is necessary to successfully integrate with native cells while reducing the chance of immunological rejection and the related biological problems. Autologous (derived from the patient), allogenic (from a human donor who is not immunologically identical), and xenogenic (from a donor of a different species) are the three types of cells that can be used in tissue engineering. Due to their low risk of immune-related problems, autologous cells are regarded as great for tissue engineering. However, for widespread clinical usage, they could not be cost-effective and offer issues with batch control. Allogenic cells, as opposed to autologous cells, offer advantages in terms of uniformity, standardized processes, quality control, and cost-effectiveness.

Mature (non-stem), adult stem (also known as somatic stem cells), embryonic stem cells (ESCs), and totipotent stem cells (zygotes) are some of the different types of cell sources used in tissue engineering. The low proliferative and differentiational potential of mature cells limits their potential. Adult stem cells, which reside in certain compartments or niches inside tissues including skin, bone, and blood, are an essential component of tissue engineering. These undifferentiated cells can be instructed to differentiate into particular cell types and are essential for preserving tissue integrity. Mammals have at least 20 major categories of somatic stem cells that can be found in the bone marrow, blood, cornea, retina, tooth pulp, liver, skin, GI tract, and pancreas, among other tissues. On the other hand, ESCs come from the pre-implantation blastocyst's inner cell mass. They are undifferentiated, immature cells that have the astonishing capacity for endless self-renewal and the capacity to develop into any form of cell in the body. With a focus on examining their therapeutic potential for regenerative medicine and diverse biological applications, stem cell research is quickly expanding. Stem cells provide exciting prospects to advance modern medicine when paired with tissue engineering. Adult stem cells have historically been thought to have few functions, principally producing a small number of cells unique to a certain germ layer origin. However, new research indicates that adult stem cells isolated from various tissues may be more malleable than previously thought. The differentiation process is assumed to be mediated by developmental cues, which are thought to have an impact on this enhanced plasticity. Current tissue engineering techniques use stem cells to create skin, blood vessels, cartilage, heart tissue, liver, pancreas, and neural tissue. Epidermal and dermal constructions are a typical method for repairing skin abnormalities. Neonatal foreskin is used to harvest and then expand in vitro dermal fibroblasts. They are then cultivated in a bioreactor system to generate a dermal layer after being seeded onto a polylactic or polyglycolic acid scaffold. The dermal layer is covered in several layers of keratinocytes to produce a bilaminate construct. The existence and functionality of engrafted skin stem cells are crucial for the success and long-term viability of these skin grafts. There are many methods for promoting neovascularization, the growth of new blood vessels. Recently, cell-based therapies using angioblasts or endothelial progenitor cells have been used more frequently for this purpose [44].

VI. CONCLUSION

Cell fusion represents a captivating biological phenomenon with diverse implications in developmental biology, cellular reprogramming, and therapeutic applications. As we delve deeper into the intricacies of membrane fusion and syncytium formation, we gain valuable insights into developmental processes like placental development and skeletal muscle formation. Furthermore, induced pluripotent stem cells and somatic cell nuclear transfer demonstrate the reprogramming potential of cell fusion, which has enormous potential for therapeutic applications.

The applications of cell fusion in regenerative medicine and disease treatment are vast and transformative. Cell-based therapies offer hope for enhancing tissue regeneration and restoring organ function. In cancer research and treatment, the fusion of tumour cells with immune cells opens doors to innovative immunotherapeutic approaches, propelling us closer to more effective cancer treatments. Additionally, cell fusion's potential in tissue engineering

and organ transplantation holds the key to creating complex, personalized organs for patients in need.

As we conclude this exploration of cell fusion's multifaceted role, it becomes evident that its study is an integral part of shaping the future of medicine. The continued investigation of cell fusion mechanisms and its applications will undoubtedly lead to groundbreaking advancements in regenerative medicine, disease treatment, and beyond. Embracing the power of fusion between cells as a unifying force in biologic processes paves the way for new frontiers in discovery and therapeutic interventions, ushering in an era of unprecedented possibilities in the realm of medicine and human health.

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