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(REVIEW ARTICLE)

Origin and Evolution of Induced Pluripotent Stem Cells (IPSCs)

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Abstract

The discovery of induced pluripotent stem cells (iPSCs) revolutionized cell biology, offering groundbreaking potential for regenerative medicine and therapeutic applications. By reprogramming adult somatic cells into a pluripotent state through the introduction of embryonic transcription factors, iPSCs provided unprecedented opportunities to study human diseases and develop autologous cell-based therapies.

The development of iPSCs Technology stems from three pivotal advancements: (a) Nuclear Reprogramming, which demonstrated that differentiated cells retain genetic information essential for development (b) Master Transcription Factors where MyoD was shown to convert fibroblasts into muscle cells, introducing the concept of "master regulators" capable of defining cell fates.(c) ESC Culture Advances: The establishment of embryonic stem cells (ESCs) in 1981 and 1998 provided insights into pluripotency and its maintenance, laying the groundwork for identifying key transcription factors required for iPSC generation. Synthesizing these advancements, Scientists identified four transcription factors (Oct4, Sox2, Klf4, and c-Myc) to generate mouse iPSCs in 2006 and human iPSCs in 2007.The above development promoted research in modeling development and disease, drug discovery, cell therapy, complex tissue model system.

Despite advances, iPSCs face challenges, including the immature state of derived cells, which limits their utility for age-related disease modeling. Researchers are addressing this through methods like mitochondrial stress induction and direct trans differentiation. Advances in CRISPR/Cas9 editing, multiomics, and automation of organoid production are also enhancing iPSC applications. .iPSCs have revolutionized developmental biology, disease modeling, and regenerative medicine, driving progress toward personalized and precision medicine. With continued advancements, iPSC technology holds the promise to deliver tailored therapies for complex diseases, transforming the future of healthcare.

Keywords: Induced Pluripotent cells; Disease modeling; Differentiation; Regenerative medicine; Therapy for complex diseases; Healthcare

1. Introduction

1.1. Origins and Evolution of Induced Pluripotency

1.1.1. The Foundation of Induced Pluripotent Stem Cells

The discovery of induced pluripotent stem cells (iPSCs) represents the culmination of decades of scientific inquiry and technological advancement. Central to this breakthrough were three key developments [1]

- Genetic Information Retention: Somatic cell nuclear transfer (SCNT) demonstrated that differentiated cells retain the same genetic information as embryonic cells.

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- Culturing Techniques: The establishment of methods to derive, culture, and maintain pluripotent stem cell lines opened new avenues for stem cell research.
- Role of Transcription Factors: Research revealed that transcription factors are critical determinants of cell fate, capable of reprogramming one mature cell type into another.

These milestones collectively paved the way for the groundbreaking discovery of iPSCs by Shinya Yamanaka in 2006 [2,3].

1.1.2. Historical Advances Leading to iPSCs

The path to iPSC technology is rooted in several pivotal moments in biological research. In 1981, embryonic stem cells (ESCs) were successfully derived from the inner cell mass of mouse blastocysts [3-8]. By 1998, human ESCs were isolated. These cells demonstrated the ability to self-renew, or proliferate indefinitely, and also exhibited pluripotency, meaning they could differentiate into various cell types derived from all three embryonic germ layers (ectoderm, mesoderm, and endoderm. Concurrently, studies on somatic cell nuclear transfer, notably Ian Wilmut's cloning of Dolly the sheep, reaffirmed John Gurdon's earlier findings that somatic nuclei could be reprogrammed under the right conditions [9,10]

In another breakthrough, the discovery of master regulator genes revealed how transcription factors could direct cell fate. For instance, MyoD expression in fibroblasts converted them into muscle cells, suggesting that similar mechanisms could enable broader reprogramming.[8]

1.1.3. iPSC Breakthrough: Shinya Yamanaka's Contribution

By introducing four transcription factors—Oct3/4, Sox2, c-Myc, and Klf4—into mouse fibroblasts, Yamanaka successfully reprogrammed adult cells into pluripotent stem cells. This method, initially using retroviral vectors, established iPSCs that resembled ESCs in their ability to self-renew and differentiate (2). In subsequent studies, human iPSCs were developed, marking a new era in regenerative medicine (Figure 1).

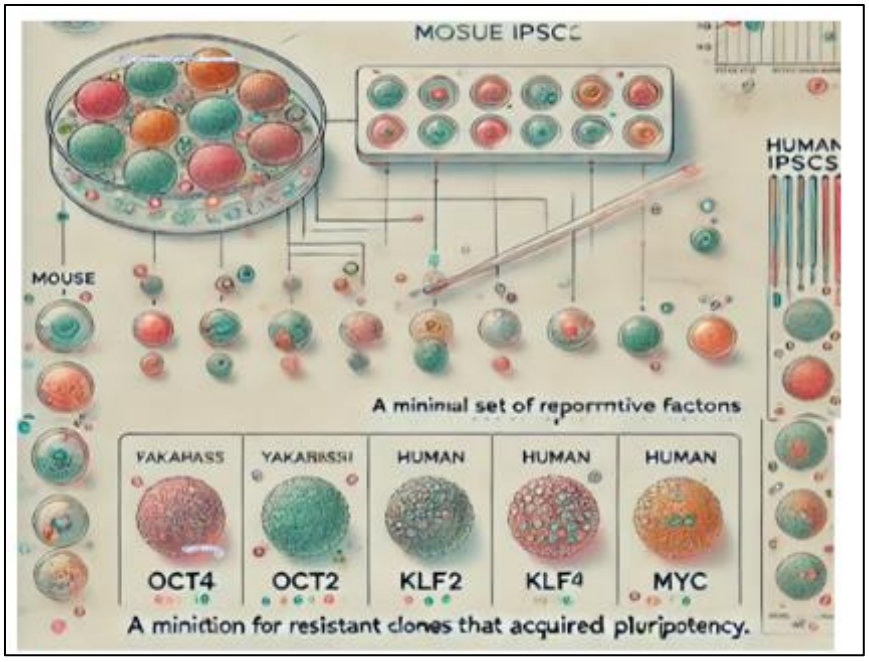

Figure 1 Somatic Cells Reprograming to Pluripotency. Fibroblasts carrying a neomycin resistance gene under a PSC promoter that acquired pluripotency

1.1.4. Refining iPSC Technology

Early methods of reprogramming, while groundbreaking, posed risks due to the integration of viral vectors, which could lead to insertional mutagenesis and potential tumorigenesis (7). Advances in reprogramming technologies addressed these challenges:

Non-Integrative Methods: Techniques involving adenoviruses, plasmids, piggyBac transposons, and Sendai viruses were developed to eliminate the risks associated with permanent genetic modifications (8-15).

Chemical Reprogramming: Recent approaches demonstrated that mouse fibroblasts could be reprogrammed using chemical compounds alone, offering a safer alternative to transcription factor introduction (16).

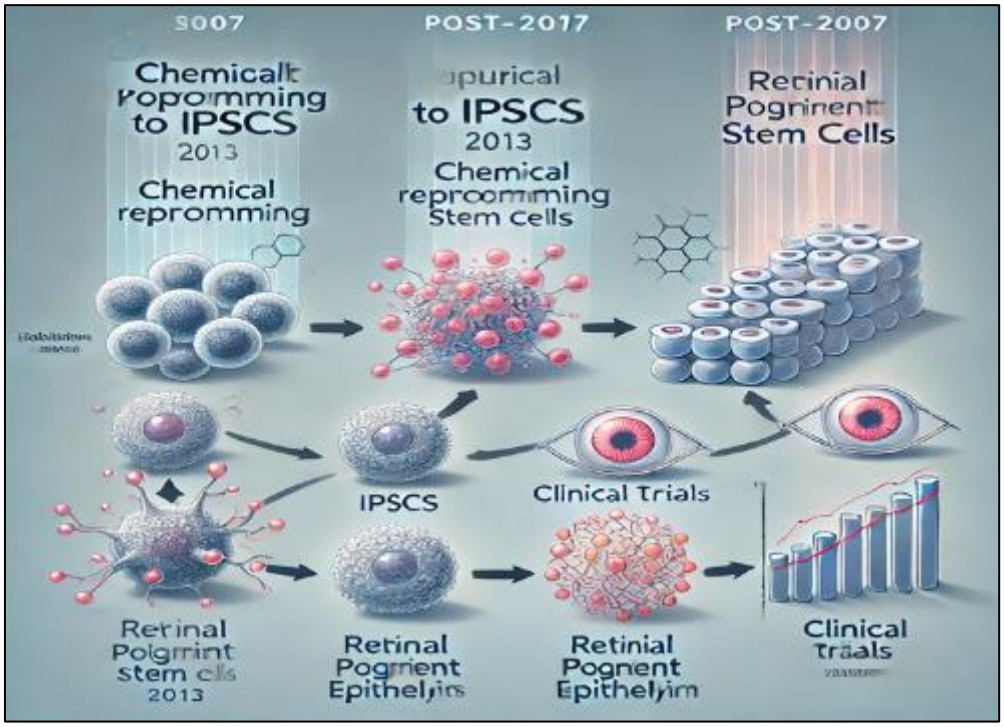

Figure 2 IPCS technology

1.2. Mechanisms of Reprogramming

The reprogramming process hinges on the interplay between key transcription factors:

- Oct3/4 and Sox2: Essential for maintaining pluripotency, with Oct3/4 regulating stem cell identity and Sox2 modulating Oct3/4 expression.(17-20)
- c-Myc: A proto-oncogene that enhances transcription but can be replaced by L-Myc to reduce oncogenic risk. (21- 23)
- Klf4: Functions contextually as either a tumor suppressor or oncogene and helps regulate Sox2. (24).

These factors operate synergistically, resetting the somatic cell's identity and reinstating a pluripotent state. Despite their effectiveness, reprogramming efficiency has historically been low but has improved with chemical enhancers, such as valproic acid and histone deacetylase inhibitors (25-27). Environmental conditions, like low oxygen levels, also contribute to enhanced efficiency (28).

1.3. Sources of Pluripotent Stem Cells

Pluripotent stem cells (PSCs) share two defining characteristics (29,30)

- Self-Renewal: The ability to proliferate indefinitely.
- Potency: The capacity to differentiate into specialized cells from the ectoderm, endoderm, and mesoderm germ layers.

Pluripotent stem cells (PSCs) are characterized by their ability to self-renew and differentiate. Self-renewal refers to their continuous division and proliferation, while potency denotes their capability to develop into specialized cells originating from the three primary germ layers: ectoderm, endoderm, and mesoderm. Aoi (2016) described three key in vivo assays used to evaluate the potency of pluripotent stem cells in mouse models.

The first, the teratoma formation assay, involves transplanting PSCs into immunodeficient mice to observe whether they spontaneously differentiate into tissues representing all three germ layers. Second, the chimera formation assay tests the integration of PSCs into developing embryos by injecting them into diploid (2N) blastocysts. This method assesses their ability to contribute to embryonic development, achieve germline transmission, and maintain full pluripotency with chromosomal stability. The third, the tetraploid (4N) complementation assay, evaluates whether PSCs can generate a complete organism. In this method, cells are introduced into 4N blastocysts, and their capacity to support development is examined by tracking extra-embryonic lineage formation.

- Stem cells are classified into five major types: embryonic stem cells (ESCs), very small embryonic-like stem cells (VSELs), induced pluripotent stem cells (iPSCs), nuclear transfer stem cells (NTSCs), and adult stem cells. Each of these types is derived from distinct sources, as described herein brief:

Different types of PSCs have been derived and studied, each with unique origins and applications.

1.3.1. Embryonic Stem Cells (ESCs)

ESCs are derived from the inner cell mass of blastocysts at the early embryonic stage (4-5 days post-fertilization). These cells were the first pluripotent stem cells to be studied extensively and remain a cornerstone of stem cell research. ESCs demonstrate robust self-renewal and pluripotency, making them valuable for clinical trials and fundamental research (31)

1.3.2. Very Small Embryonic-Like Stem Cells (VSELs)

First identified by Ratajczak et al. in 2006 [39] and later confirmed by more than 20 independent research groups [31-35], the existence of VSELs remains a topic of debate, with some researchers questioning their validity [36]. These cells are found in adult tissues, are remarkably small, and express markers associated with pluripotency. Their morphology is primitive, resembling inner cell mass cells of the blastocyst, with a size of 3-5 μm in mice and 5-7 μm in humans, making them smaller than red blood cells.

VSELs exhibit pluripotency markers such as SSEA, nuclear Oct-4A, along with markers associated with migrating primordial germ cells (PGCs) [36]. Single-cell cDNA analysis of murine bone marrow has identified VSEL biomarkers, particularly Sca-1+lin-CD45- cells [37]. It has been suggested that VSELs originate from primordial germ cells that become embedded in developing organs during embryogenesis [37,38].

In 2019, Ratajczak [31,32] proposed that VSELs give rise to three primary cell types: mesenchymal stem cells (MSCs), hem angioblasts (which include subtypes such as hematopoietic stem cells [HSCs] and endothelial progenitor cells [EPCs]), and tissue-committed stem cells (TCSCs). Because of their pluripotent nature, VSELs can differentiate into various germ layer-derived cell types within adult organisms. This makes them a promising alternative to tissue-committed adult stem cells. Additionally, VSELs could potentially overcome some of the ethical concerns surrounding embryonic stem cells (ESCs) and the teratoma formation risks associated with induced pluripotent stem cells (iPSCs), making them a valuable focus for future stem cell research and clinical applications [38].

1.3.3. Nuclear Transfer Stem Cells (NTSCs)

The somatic cell nuclear transfer (SCNT) technique, first developed in 1996, has evolved significantly, enabling the generation of nuclear transfer stem cells (NTSCs). SCNT involves transferring the nucleus from a fully differentiated somatic cell (e.g., a fibroblast) into an enucleated oocyte (an egg cell with its nucleus removed). The recipient oocyte then reprograms the donor nucleus, allowing it to undergo mitotic division and eventually form a blastocyst consisting of approximately 100 cells at an early embryonic stage. This technique results in an organism that is genetically almost identical to the nuclear donor, essentially creating a clone. While the nuclear donor contributes most of the genetic material, the cytoplasmic donor also influences the resulting organism by providing certain genetic and phenotypic characteristics. SCNT is used in both therapeutic and reproductive cloning [39,40]

A major breakthrough in this field occurred in July 1996 with the birth of Dolly the Sheep, the first successfully cloned mammal, achieved in Scotland, UK [41-43]. Since then, scientists have cloned nearly two dozen species [43]. More recently, in January 2018, Chinese researchers in Shanghai successfully cloned two female macaque monkeys using SCNT with fetal fibroblasts, marking the first instance of primate cloning with this technique [44-46].

The ability to clone primates has the potential to revolutionize research into human diseases [44-46]. Genetically identical non-human primates could serve as valuable animal models for biomedical studies, allowing for more precise

investigations into disease mechanisms and drug development without the confounding factor of genetic variability. This could reduce the number of laboratory animals required for research [44-46]. Additionally, combining SCNT with CRISPR-Cas9 gene editing could lead to the development of genetically modified primate models for studying diseases such as Parkinson's disease and specific cancers. The pharmaceutical industry has shown considerable interest in cloned primates for drug testing [45,46]. In response to this growing demand, Shanghai has allocated funding to establish an International Primate Research Center, aimed at producing cloned primates for global scientific use [43-46].

Unlike other stem cell technologies, SCNT has the unique capability of generating an entire living organism, whereas embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can only produce cell sheets, tissues, or organ components. This fundamental distinction gives SCNT certain advantages over ESCs and iPSCs in both basic research and potential clinical applications

1.3.4. Induced Pluripotent Stem Cells (iPSCs)

iPSCs are created by reprogramming adult somatic cells using transcription factors such as Oct3/4, Sox2, c-Myc, and Klf4. They offer a patient-specific approach to regenerative medicine and disease modeling. Although iPSCs are a major milestone in stem cell research, they can present challenges, such as variability in differentiation capacity and the risk of introducing epigenetic anomalies during reprogramming.[47].

1.3.5. Challenges

Understanding the molecular mechanisms governing epigenetic memory, clonal variability, and differentiation capacity is essential for the reliable application of iPSCs. Advances in profiling techniques and the development of optimized reprogramming methods continue to improve the quality and utility of iPSCs for therapeutic purposes.

2. Characterization of iPSC Lines: Exploring the Frontiers of Stem Cell Research

2.1. Reprogrammed Stem Cells

2.1.1. Reprogrammed Stem Cells (RSCs): A Milestone in Cellular Technology

Since Yamanaka and his team successfully generated induced pluripotent stem cells (iPSCs) in 2006, reprogramming technologies have significantly progressed [2, 3]. This advancement is particularly evident in direct reprogramming techniques, both *in vitro* and in vivo, which enable the conversion of specific cell types without passing through the iPSC stage. By employing lineage-specific transcription factors, RNA signaling modifications, and chemical agents, these methods allow the direct formation of specialized cells, such as induced neural progenitor cells (iNPCs), which can further differentiate into neural and motor neurons. Reprogrammed stem cells (RSCs) are created by modifying the genetic programming of donor cells, excluding somatic cell nuclear transfer (SCNT) [48-54]

iPSCs have emerged as a valuable alternative to human embryonic stem cells (hESCs), addressing ethical concerns and reducing immune rejection risks. These cells are derived from adult somatic tissues, including accessible sources like blood, skin, and urine. Since iPSCs can be generated from a patient's own cells, they offer a promising solution for personalized medicine by minimizing the risk of immune rejection in autologous transplantation. They can theoretically be derived from various mature human cell types, such as umbilical cord blood, bone marrow, peripheral blood, fibroblasts, keratinocytes, or urine, and then differentiated into specific target cells as required [55-58]. It is important to clarify that the term "mature stem cells" refers to the differentiated state of the original cells rather than the donor's age. Certain stem cells, like those from umbilical cord blood or bone marrow, can be directly transplanted without requiring reprogramming, a topic explored further in the next section.

2.1.2. Autologous Stem Cells and Urine-Derived iPSCs

Stem cells harvested from a patient's own tissues—such as skin, hair, or urine—eliminate the immune rejection issues associated with non-autologous cells. This makes urine an especially attractive source for fragile patients or those with severe injuries, such as spinal cord trauma or heart disease, due to its ease of collection. While research on urine-derived cells remains limited, their potential as a valuable stem cell source is gaining interest.

To optimize iPSC differentiation protocols for clinical applications, it is crucial to use non-invasive and reproducible cell sources. Urine samples provide an abundant, easily obtainable supply of autologous cells with strong reprogramming potential. In 2011, Zhou and colleagues developed a technique to derive human iPSCs (hiPSC) from renal tubular cells

in urine, refining the protocol in 2012 [56]. This cost-effective, rapid approach requires only a 30-mL urine sample, with high-efficiency iPSC generation occurring after two weeks of culture and 3-4 weeks of reprogramming. These urine-derived iPSCs, produced via the Sendai virus system from midstream urine samples, exhibited normal karyotypes and the ability to differentiate into all three germ layers in teratoma assays [57,58].

Further studies by Zhang and colleagues identified a specific subpopulation of progenitor cells in urine expressing markers such as c-Kit, SSEA4, CD105, CD73, CD91, CD133, and CD44. These markers are associated with various bladder cell types, including urothelial, smooth muscle, endothelial, and interstitial cells [59]. Such progenitor cells could be utilized in urinary tract tissue engineering and regenerative medicine. Additionally, cells from the upper urinary tract have demonstrated the capacity to expand and differentiate into urothelial and myogenic cells, which may have applications in bladder reconstruction for patients requiring cystoplasty [60]. Notably, these studies bypassed the iPSC stage before differentiation.

A method for generating iPSCs from urine-derived cells without using the oncogene c-Myc has been established, leading to the creation of hiPSCs stored under feeder-free, virus-free, and serum-free conditions. This resulted in the production of 93 hiPSC lines from 20 diverse donors [61]. Urine-derived iPSCs have been shown to differentiate into multiple cell subtypes across various biological systems. In cardiovascular research, cardiomyocytes derived from urine cells could generate action potential both *in vitro* and in vivo [62]. Similarly, in metabolic disease research, iPSCs were successfully derived from a patient with a mitochondrial DNA mutation [63]. For diabetic wound healing, urine-derived stem cells were found to promote angiogenesis and tissue repair [64].

Urine-derived iPSCs have also been explored in neuroendocrine research, with iPSCs created from patients with multiple endocrine neoplasia type 1 (MEN1) using episomal plasmids that exclude c-Myc [65][66]. In psychiatric research, urine-derived iPSCs were generated from a patient with obsessive-compulsive disorder using a CytoTune®-iPS 2.0 Sendai kit [56,67,68].

2.1.3. Adult Stem Cells and Their Clinical Potential

The discovery of adult stem cells generated significant excitement due to their potential applications in regenerative medicine, though their full therapeutic utility remains under evaluation. These stem cells, found in organs such as the brain, spinal cord, and heart, may offer new opportunities for cell therapy. Research suggests that adult stem cells respond to their microenvironment, detecting tissue damage and regenerating only the necessary cell types. Tissue-resident adult stem/progenitor cells are an attractive option for regenerative therapies, as they possess robust self-renewal capabilities and differentiation potential, allowing them to repair damaged tissues without immune rejection. Additionally, adult stem cells from mature tissues can be reprogrammed into iPSCs, as discussed earlier. Furthermore, small molecules can be employed to stimulate endogenous stem cells, encouraging them to proliferate and differentiate into specialized cell types (69-71).

Among the most extensively studied adult stem cells are mesenchymal stem cells (MSCs), which are currently the most widely used in research and clinical applications. Initially discovered in bone marrow, MSCs have since been identified in other tissues [72-74]. Key sources of human MSCs include umbilical cord blood, bone marrow, adipose tissue, placenta, amniotic fluid, and menstrual blood. Umbilical cord blood, collected at birth, presents practical challenges such as safe banking, potential contamination, and maintaining long-term viability [75]. There are established protocols for collecting and storing umbilical cord blood, ensuring its future use under ethical guidelines that emphasize informed consent and financial transparency [75-78]. While bone marrow-derived stem cells have been well-documented in laboratory and animal studies, clinical trials have yielded mixed results.

The discovery of adult stem/progenitor cells in the brain and heart [79] has fueled optimism that these cells might one day be used to repair damage caused by strokes or heart attacks. However, for effective therapeutic applications, MSCs must be accurately identified using specific biomarkers. The International Society for Cellular Therapy defines human MSCs (hMSCs) by their expression of surface markers CD105, CD73, and CD90 (present in ≥95% of cells), while lacking CD45, CD34, CD14 or CD11b, CD79a or CD19, and the human leukocyte antigen–DR isotype (≤2% presence) [75].

Optimizing signaling pathways can enhance the efficiency of stem cell transformations, whether using externally sourced or endogenous cells. While ESCs and iPSCs can be cultivated to generate specific cell types for transplantation, another approach involves delivering growth factors and biomolecules directly into damaged tissues to stimulate resident adult stem cells into differentiating. This strategy is particularly relevant for regenerating neurons, glial cells, and other structures affected by spinal cord injuries or strokes. However, while promising in animal models, these

methods have yet to demonstrate consistent success in clinical applications, necessitating further research to refine and validate their therapeutic potential [80].

2.1.4. Second Generation: Direct In Vivo Cellular Reprogramming

This section explores the potential of second-generation cellular reprogramming through direct in vivo techniques, which could overcome challenges commonly associated with *in vitro* approaches, such as disrupted cellular organization, contamination risks, and lengthy processing times [81]. Unlike traditional first-generation *in vitro* methods, direct in vivo reprogramming occurs entirely within a living organism, targeting native tissues like the brain or heart in animal models (e.g., mice). By leveraging the body's natural microenvironment, this method facilitates the production of essential biological components and promotes localized tissue repair and regeneration.

In direct *(in vivo*) cellular reprogramming, transcription factors and microRNAs play a crucial role in converting support cells into specific functional cell types. This technique is distinct from *in vitro* reprogramming as it directly transforms cells in their native environment using early-stage factors like OSKM, which can differentiate into any cell type. Instead of reverting cells into a stem or progenitor state, transcription factors and microRNAs enable the direct conversion of local somatic cells into desired cell types. While the exact biological mechanisms remain unclear, they likely involve cellular memory and interactions within the surrounding tissue environment.

Recent advancements in this field have been particularly notable in the reprogramming of cells into cardiomyocytes and neurons. In 2008, Zhou and colleagues demonstrated that pancreatic exocrine cells could be transformed into beta cells using the transcription factors NGN3, PDX1, and MAFA [82]. This pioneering study showed that cellular reprogramming could occur without first inducing pluripotency. Similarly, transcription factors such as FOXa3, GATA4, HNF1a, and HNF4a have been successfully used to reprogram myofibroblasts into hepatocyte-like cells in fibrotic mouse livers, reducing fibrosis and suggesting potential therapeutic applications for chronic liver diseases [83].

In cardiovascular research, mouse cardiac fibroblasts have been reprogrammed into cardiomyocyte-like cells using key cardiac transcription factors such as Gata4, Mef2c, and Tbx5, sometimes supplemented with HAND-2 [84,85]. These transformed cells integrated electrically into heart tissue and contributed to improved cardiac function post-injury. This approach holds promise for clinical applications that would utilize the heart’s abundant fibroblast population for targeted regenerative therapy.

Within the nervous system, endogenous astrocytes in mice have been directly converted into neurons through reprogramming genes like Ascl1, Brn2a, and Myt1l [86]. Notably, Ascl1 alone has been sufficient to turn brain astrocytes into functional neurons in vivo [87] and has also been employed to direct retinal Müller glia towards neuronal differentiation [88]. In the adult mouse brain, Sox2 was found to induce astrocytes into proliferative neuroblasts, which matured into functional neurons when supported by factors like brain-derived neurotrophic factor, noggin, or histone deacetylase inhibitors [80]. Additionally, Sox2 has been used to convert brain pericytes into neurons [89]. These discoveries highlight the remarkable plasticity of astrocytes in vivo and illustrate the feasibility of reprogramming cells within their native environment to promote tissue regeneration.

2.1.5. Types of Differentiated Cells and Genetic Memory

Stem cells can undergo reprogramming and differentiation to generate specific cell types. Key areas of research focus on the differences between iPSCs and ESCs, the concept of cellular genetic "memory," and the techniques used for both *in vitro* and in vivo applications.

Studies comparing the neural differentiation abilities of human iPSCs and ESCs indicate that iPSCs can generate neuroepithelial structures and functional neurons similar to those derived from ESCs under the same conditions [90]. However, iPSCs tend to be less efficient and more variable than ESCs, though advancements in culture methods have helped mitigate these limitations [90]. Some iPSC lines exhibit epigenetic traits that predispose them toward certain cell lineages [90]. When a mature cell type, such as an adult fibroblast, is reprogrammed into iPSCs, it may retain a genetic "memory" of its original identity, potentially complicating full reprogramming [91]. This epigenetic memory could also influence the lineage-specific tendencies observed in some hiPSC lines [10,11, 91]. Requires more research to understand how genetic memory varies across different cell types and tissues.

Specific cell types can also be generated directly *in vitro* or in vivo without transitioning through a stem cell stage [92]. For example, a combination of the genes Ascl1, Brn2, and Myt1l has been shown to efficiently convert mouse ESCs and postnatal fibroblasts into functional neurons *in vitro*. These neurons express neuron-specific markers, exhibit action potentials, and form synapses [93].

2.2. Inducing Pluripotency through Genetic Modifications

In 2006, Yamanaka and his team made a groundbreaking discovery that only four out of 24 known pluripotency transcription factors were needed to reprogram adult mouse fibroblasts into embryonic stem cell-like states, termed iPSCs (2,3). These four factors, Oct4, Sox2, Klf4, and c-Myc (OSKM)—demonstrated a remarkable ability to induce pluripotency, theoretically enabling cells to differentiate into any of the body's 220 cell types through reversible epigenetic modifications. This OSKM method was later adapted to successfully generate human iPSCs [94-96]. More recently, Kilens and colleagues developed a protocol for simultaneously deriving both primed and naive human iPSCs [93]. Their study showed that naive iPSCs could be directly generated from somatic cells using OSKM overexpression under specific culture conditions, streamlining the process and extending cell viability compared to older methods requiring a priming phase [97-100].

2.3. Reprogramming Factor Delivery Methods

There are four primary methods for delivering reprogramming factors. Initially, integrating viral systems were used to introduce transcription factors for stem cell induction, but these carried risks of genetic integration, which could lead to teratoma formation. More recent advancements using non-integrating vectors, self-excising vectors, and non-viral methods have improved the safety and efficacy of iPSC generation, making them more viable for research and clinical use.

Oct4 is considered the most essential factor in pluripotent stem cell reprogramming, with Nanog and Lin28 acting as functional substitutes for Klf4 and c-Myc. Additionally, the Oct4 complex (comprising Oct4, Sox2, Nanog, and Esrrb) has been identified as a key player in reprogramming [82,101]. In 2007, Yu and colleagues proposed an alternative protocol using Oct4, Sox2, Nanog, and Lin28, achieving similar reprogramming efficiency while reducing oncogenic risks [102]. Given concerns regarding the tumorigenic potential of Klf4 and c-Myc, subsequent methods explored alternative factor combinations, such as Oct4, Sox2, and Esrrb, which demonstrate enhanced reprogramming efficiency in mouse embryonic fibroblasts (MEFs) [104]. Other studies confirmed that c-Myc was not essential for reprogramming fibroblasts [105,106], and subsequent research even reduced the number of required factors to two or a single factor like Oct3/4 alone [106-108].

Advances in direct cellular reprogramming bypass the stem cell stage entirely. For instance, transcription factors like Ascl1, Brn2, and Myt1l have been used to convert fibroblasts directly into functional neurons, producing cells that generate action potentials and form synapses. These techniques reduce reprogramming complexity and may offer faster clinical applications [73]

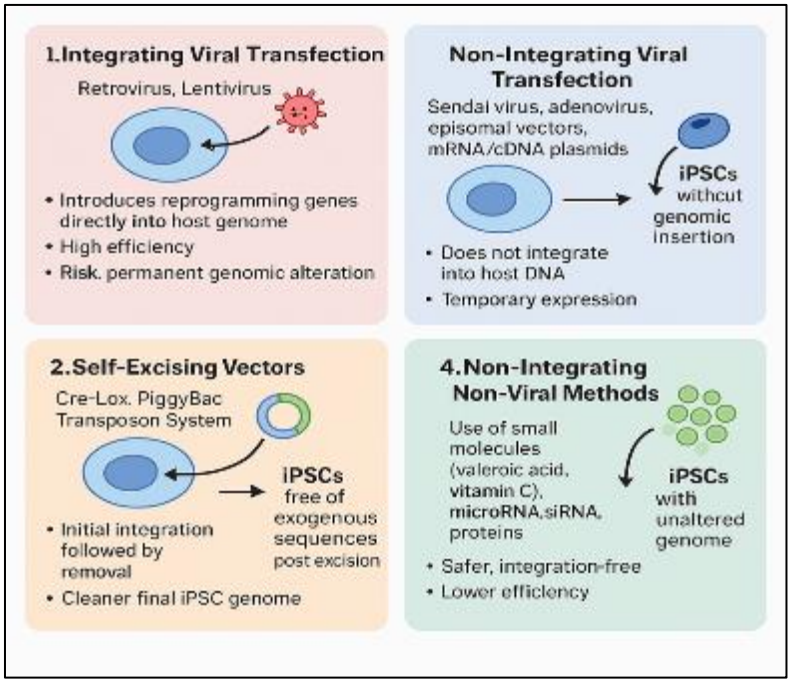

Figure 3 Reprogramming Factor Delivery Methods

3. Inducing Pluripotency: A Revolution in Reprogramming Methods

There are four main methods for delivering reprogramming factors. Initially, integrating viral systems were used to introduce transcription factors for stem cell generation, but these carried the risk of genetic integration that could lead to teratoma formation. Newer approaches using non-integrating vectors, self-excising vectors, and non-viral vectors have improved the safety and efficacy of iPSC generation, making them more suitable for scientific and clinical applications.

3.1. OSKM Factors: The Core of Reprogramming

Yamanaka's discovery that OSKM factors—Oct4, Sox2, Klf4, and c-Myc—could reprogram somatic cells into pluripotent states revolutionized stem cell research (2,3). These factors enable reversible epigenetic changes, allowing cells to develop into any of the 220 human cell types. Recent protocols have refined these methods, introducing safer and more efficient reprogramming techniques [62,80].

3.2. Safer Reprogramming Factor Delivery

Initial methods relied on viral vectors, posing risks of genetic integration and oncogenesis. Advances now include non-viral vectors, self-excising systems, and small molecule-based methods. The introduction of Nanog and Lin28 as alternatives to Klf4 and c-Myc has further improved reprogramming safety [101-105].

Different combinations of transcription factors can vary in their efficiency, producing specific cell subtypes at various developmental stages. For example, while the OSKM method can reprogram early-stage non-terminal B cells, mature late-stage B cells require additional factors, such as the C/EBP-α transcription factor or suppression of PAX5, a B cell transcription factor [109].

Early reprogramming research utilized retroviral and lentiviral vectors, which enhanced reprogramming efficiency [24], but the integration of viral genes posed risks of significant genomic changes, including oncogenic transformations in Klf4 and c-Myc, making these protocols unsuitable for clinical use [10].

Advances in iPSC technology have addressed challenges such as incomplete reprogramming and genetic alterations induced by viral integration [110]. Recent developments have focused on using non-integrating reprogramming techniques and maximizing reprogramming efficiency with non-viral vectors, genetic and signaling molecules, small bioactive compounds, microRNAs, and chemicals, as discussed in the following section.

4. Applications of iPSCs: Transforming Medicine

The rapid evolution of iPSC technology has transformed *in vitro* research and therapeutic development [109]. iPSCs possess an exceptional ability to proliferate indefinitely and differentiate into diverse human cell types, offering a significant ethical advantage over human ESCs [110,111] Consequently, iPSC-derived cells are widely employed for modeling human diseases, conducting high-throughput drug screenings.

4.1. Disease Modeling and Drug Discovery

iPSCs provide unparalleled opportunities to model human diseases and conduct large-scale drug screenings. Their ability to replicate a patient's genetic makeup allows for personalized approaches to understanding disease mechanisms and testing therapies [109].

4.2. Cell Therapy and Regenerative Medicine

iPSC-derived cells offer significant promise for both autologous and allogeneic therapies. Their potential to regenerate damaged tissues, such as cardiomyocytes for heart repair or neurons for spinal injuries, represents a breakthrough in regenerative medicine [110,111].

Second-generation *in vivo* reprogramming is reshaping the boundaries of regenerative medicine. By addressing the challenges of traditional methods and harnessing the natural microenvironment, these advancements bring us closer to personalized, effective, and safer therapies. As research continues to refine these approaches, the potential for clinical applications grows, promising transformative outcomes for patients worldwide.

5. IPSC-Derived Cellular Models: A Revolution in Biomedical Research

5.1. The Need for Accurate Cellular Models

Creating reliable *in vitro* cellular models for human development and disease requires cells that closely mimic human biology. While primary cells from sources like skin or blood are readily accessible, cells from complex tissues such as the brain or heart are harder to obtain. Animal models, particularly rodent cells, are often used as substitutes but fail to fully replicate human-specific characteristics due to species differences. iPSCs overcome these challenges by providing human-derived cells with the genetic fidelity needed for accurate biological modeling [112,113]

5.2. Differentiation Techniques for iPSCs

Protocols for differentiating iPSCs into specific cell types have become increasingly sophisticated. The process often involves simulating natural developmental signals using targeted proteins, small molecules, or transcription factors. For instance:

- Neurons and Cardiomyocytes: Derived within a week using straightforward techniques.
- Oligodendrocytes and T Cells: Require complex, multi-step differentiation processes spanning months [114-116]

Tools like CRISPR/Cas9, transcription factor mapping, and temporal profiling have been instrumental in refining these protocols. For example, hematopoietic cells are derived through mesoderm and hemogenic endothelium specification, followed by cytokine-driven differentiation into myeloid and lymphoid lineages. Similarly, neural cells are generated through dual SMAD inhibition, with additional morphogens guiding specialization, such as WNT pathway inhibition for forebrain neurons. [114,117-120]

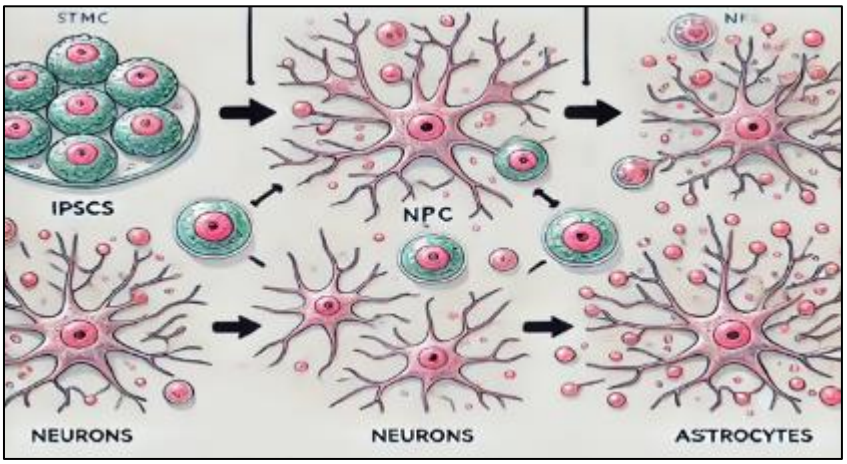

Figure 4 Complex Cellular Models and Organoids

iPSC technology facilitates the creation of diverse *in vitro* models, ranging from basic 2D cultures to more intricate 3D structures. These models can be used for various purposes, such as studying specific cellular responses and conducting high-throughput screening assays [121,122]. However, traditional monocultures lack the complex cell-cell interactions present in living organisms. To replicate more realistic tissue interactions, iPSC-derived cells can be cultured together. For example, co-culturing neurons with astrocytes promotes neuronal growth and survival due to astrocytic support. Even tri-culture setups incorporating neurons, astrocytes, and microglia can produce more sophisticated *in vitro* brain models [112.,123].

Three-dimensional organoids are a significant advancement, offering a better representation of human tissues by self-organizing under suitable conditions. These organoids often consist of multiple cell types, enabling the study of organ development, cell function, and disease [124-126]. Brain organoids, for instance, have shown human-like regional specifications, and kidney organoids exhibit structures mimicking nephron segments. Different organoid types, such as those representing specific brain regions or stomach linings, can be derived using various patterning techniques [127]. More complex structures called assembloids can be created by fusing different organoids to study interactions like neural innervation [128].

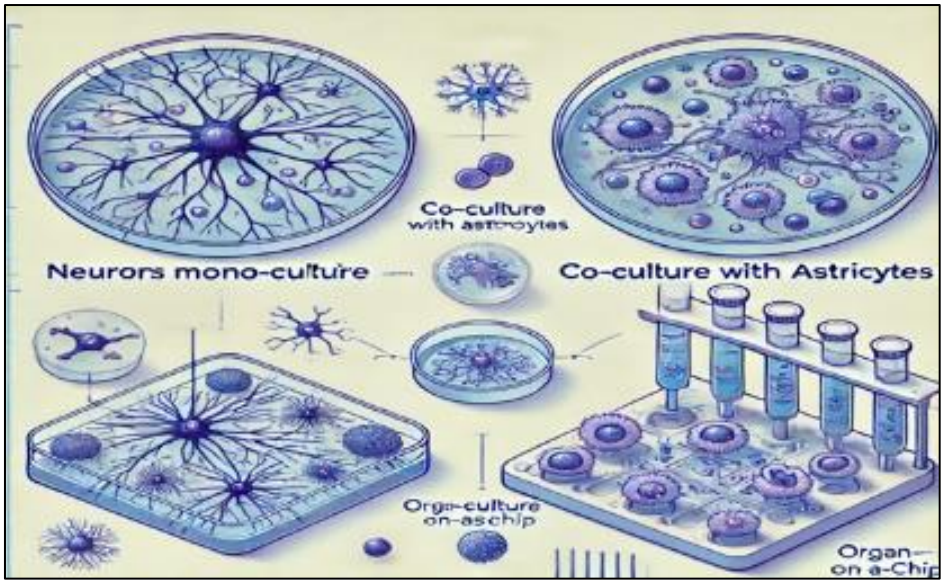

Figure 5 Organ-on-a-Chip and In Vivo Applications

The organ-on-a-chip (OoC) system presents another method to model tissue architecture. By integrating iPSC-derived cells into a microfluidic device, these platforms simulate the cellular environment and functions of tissues, including important barriers like the blood-brain barrier (BBB). For example, a BBB-on-a-chip constructed with neural cells and endothelial-like cells mimics the electrical resistance and permeability of in vivo conditions [129]

Additionally, iPSC-derived cells and organoids can be transplanted into animal models, combining human cellular characteristics with animal physiology for more complex studies [130,131]. This approach has been used to integrate microglia into mouse brains and vascularize blood vessel organoids, leading to models that better represent human tissues and diseases. Overall, iPSC-derived models offer versatile platforms for studying cell functions, interactions, and organ-level behaviors [130,131] .

6. Modeling Human Development with iPSC-Derived Cells

6.1. Harnessing iPSCs to Study Early Human Development

- Induced pluripotent stem cells (iPSCs) closely mimic embryonic stem cells (ESCs), enabling differentiation into somatic cells and organoids that replicate early embryonic and fetal stages. This makes iPSCs invaluable for studying human developmental [132] processes such as:
- Epiblast Formation and Embryogenesis: iPSCs simulate critical events like the formation of the epiblast lumen, embryonic sac, primitive streak, and primordial germ cells [133].
- Somitogenesis: Differentiation into presomitic mesoderm models human segmentation and the "segmentation clock."[134].
- Germline Development: Primordial germ cell-like cells (PGCLCs) derived from iPSCs exhibit unique gene expression patterns crucial for understanding germline development [135].

6.2. Exploring Pre-implantation and Post-implantation Embryogenesis

Although iPSCs represent the post-implantation epiblast, they can be reprogrammed into naïve iPSCs that replicate the pre-implantation stage, aiding in the study of earlier embryonic events.

- Naïve iPSCs: First reported in 2009, these cells are created using transcription factors and signaling molecules, facilitating studies on:
  - X Chromosome Inactivation
  - Transposable Element Regulation
  - Extraembryonic Lineage Differentiation
- Blastoid Organoids: Developed from naïve iPSCs, these models mimic blastocyst development and implantation, offering insights into trophoblast differentiation and placenta formation [136,137].

6.3. iPSCs in Cell Specification and Maturation

iPSCs enable the exploration of cell specification and maturation:

- Dopaminergic Neuron Differentiation: Single-cell RNA sequencing highlights transcription factors like ASCL1 in neuron specification [138]
- Organoid Models: Temporal profiling of brain organoids uncovers transcriptional and epigenetic pathways in brain development, while spinal cord organoids replicate neurulation processes [139].
- Cardiac Organoids: Co-culture with epicardial-like cells models heart formation, particularly the myocardium-epicardium interaction [139].

6.4. Assembloids: Complex Tissue Interaction Models

Assembloids combine organoids from different regions to replicate complex tissue interactions. For example:

- Gut Assembloids: Combine anterior and posterior gut spheroids to model hepato-biliary-pancreatic development using retinoic acid signaling. [140]
- Brain Assembloids: Replicate neuronal migration and long-range connections, reflecting brain development processes.[141].

7. Enhancing Maturity in iPSC-Derived Cells

The differentiation of iPSCs into various cellular models, particularly in monoculture, often takes place with limited exposure to a tissue microenvironment that is physiologically relevant and at a faster pace compared to natural in vivo cell development. [142]. This rapid process results in iPSC-derived cells frequently being immature, which poses a significant challenge for using iPSC technology in disease modeling and cell therapy. Immature cells lack the full functionality of their *in vivo* equivalents, potentially obscuring critical disease phenotypes or reducing their effectiveness compared to primary cells in therapeutic applications [143]. For instance, iPSC-derived spinal motor neurons may display characteristics similar to fetal neurons, whereas the gene expression patterns associated with conditions like amyotrophic lateral sclerosis (ALS) align with the maturation and aging of motor neurons. This implies that immature iPSC-derived neurons might not fully replicate ALS pathology, making the maturation of these cells a crucial step before moving forward with applications [144].

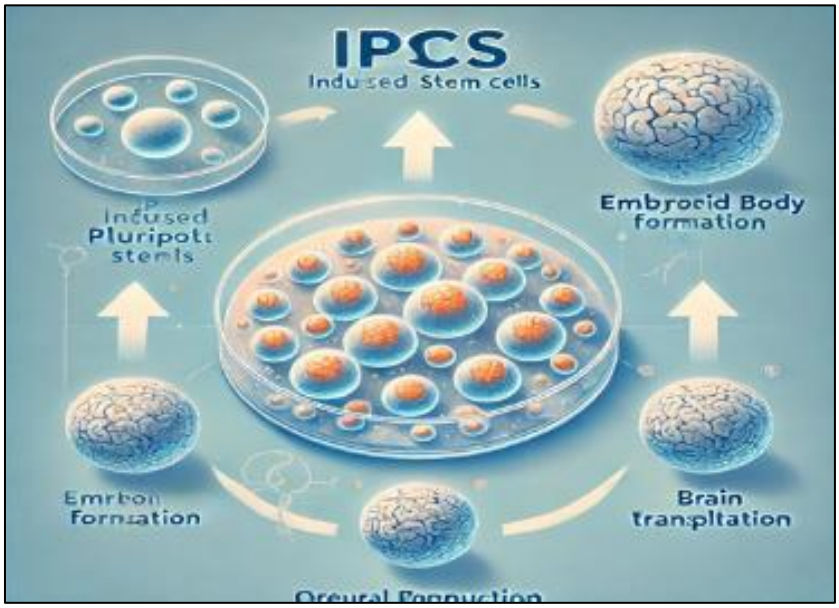

Figure 6 Enhancing Maturity in iPSC-Derived Cells

In their natural context, somatic cells mature through interactions within their tissue environment, which provides necessary signals, metabolites, and cell-to-cell communication. Simulating these conditions *in vitro* can support the maturation of iPSC-derived cells [145] For example, using an artificial extracellular matrix with biomimetic nanofibers has been shown to enhance the structural and functional development of cortical neurons. Additionally, co-culture

systems that allow multiple cell types to interact can replicate relevant paracrine signaling. [146-148]. For instance, co-culturing cardiomyocytes with mesenchymal stem cells can enhance myofibril organization and gap junction formation, partly due to extracellular vesicles released by the mesenchymal stem cells, highlighting the importance of cell communication that is difficult to replicate with chemically defined media alone. The influence of cell-to-cell interactions is also evident in 3D structures like organoids and organ-on-a-chip (OoC) models, which typically show greater maturity than 2D models. Incorporating cardiac fibroblasts into spheroids containing cardiomyocytes and epithelial cells can lead to enhanced sarcomere development and improved cardiomyocyte function and electrical maturation. Similarly, BBB-on-a-chip models display metabolic coupling between neurons and endothelial cells [146-148].

Organoid maturity can be further enhanced through *"in vivo"* transplantation, which promotes vascularization, better nutrient exchange, and exposure to systemic factors relevant to physiological function (149). For example, lacrimal gland organoids transplanted into their natural anatomical location can mature to produce tear proteins and mimic primary human tissue (129).

Cells also undergo tissue-specific mechanical and environmental conditioning in vivo, which can be partially replicated *in vitro*. Mechanical stress, such as stretching applied to iPSC-derived cardiomyocytes, can improve their gene expression and functional maturity (150). Pulsatile stretching has been shown to aid the development of vascular grafts from iPSC-derived smooth muscle cells, improving their mechanical properties and reducing vessel dilation. Shear stress can enhance the development of multiciliated airway cells, while electrical stimulation has been found to support further cardiomyocyte maturation (150).

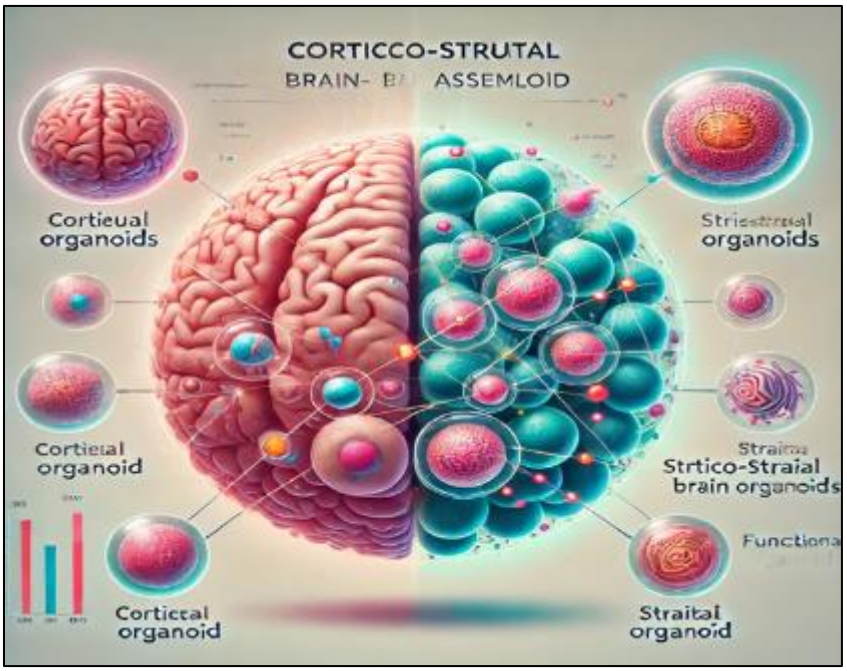

Figure 7 Model human development and disease

Ultimately, for iPSC-derived cells to effectively model human development and disease, they must closely replicate the biology and function of their in vivo counterparts. Extensive omics data from primary human tissues can serve as a benchmark for assessing the maturity of iPSC-derived cells. For instance, research by Shin et al. used spatial mapping of single-cell transcriptomes to compare iPSC-derived thalamic organoids with primary human brain tissue, showing a significant similarity between the organoids and the primary thalamus (151). Multi-omics datasets, like those from the Human Cell Atlas Project, can be invaluable for iPSC research, providing reference points for the molecular profiles of mature, functional cells and tissues (152).

8. Modeling Human Development with iPSC-Derived Cells

iPSCs, once reprogrammed, share similarities with an embryonic stem cell (ESC)-like state, allowing their differentiation into somatic cells or organoids to primarily mimic early embryonic and fetal-like cell stages. This makes iPSCs highly suitable for studying early human development. (143). Controlled differentiation of iPSCs replicates crucial events in

early embryogenesis, such as the formation of the epiblast lumen, the development of the embryonic sac, and the formation of the primitive streak and primordial germ cells. iPSC-derived primordial germ cell-like cells (PGCLCs) display unique germline-specific gene expression patterns and can be utilized to investigate germline development (144). Additionally, guiding iPSCs to form presomitic mesoderm replicates human somitogenesis and the segmentation clock process. There have also been recent advancements in creating post-implantation human embryo models from ESCs, and it is anticipated that similar models will soon be derived from iPSCs (145).

Although iPSCs resemble the post-implantation epiblast, they can also be reprogrammed into naïve iPSCs that mimic the pre-implantation epiblast, which facilitates the study of embryogenesis before blastocyst implantation. The first reports of creating naïve human iPSCs from somatic cells appeared in 2009, typically involving combinations of transcription factors and small molecules that influence different signaling pathways(146) . These naïve iPSCs are useful for exploring X chromosome inactivation, the regulation of transposable elements, cell fate changes, differentiation into extraembryonic lineages, and other key pre-implantation events. Recently, blastoid organoids have been developed from naïve iPSCs for the study of blastocyst development and implantation. Additionally, trophoblast stem cells can be derived from iPSCs to model the development of the placenta (147).

Differentiating iPSCs into various cell types helps elucidate the processes involved in cell specification and maturation. For example, single-cell RNA sequencing (scRNA-seq) of dopaminergic neuron differentiation has highlighted the importance of the ASCL1 transcription factor in specifying these neurons(148). Differentiating multiple iPSC lines allows for broader population-level analyses, such as quantitative trait loci (QTL) studies, revealing key gene regulatory mechanisms involved in development. Organoid models can also be used to explore the development of specific organs. Temporal profiling of brain organoid differentiation, for instance, uncovers the transcriptional and epigenetic networks that guide human brain development and the regional formation of different brain areas (149). Spinal cord organoids demonstrate aspects of neural tube development through neurulation-like processes, while cardiac organoids co-cultured with epicardial-like cells model the epicardium's coverage of the myocardium during heart formation(150,151).

Assembloids offer a way to model complex tissue interactions that drive developmental processes through paracrine signaling and cell migration. For example, combining anterior and posterior gut spheroids can create structures that resemble the hepato-biliary-pancreatic region, driven by retinoic acid signaling (130). Brain assembloids, such as cortico-striatal combinations, replicating the migration of interneurons and the development of long-range neuronal connections observed in brain development. Overall, modeling development with iPSC-derived cells offers significant insights into human-specific developmental pathways and informs cell differentiation strategies for various applications (131).

8.1. Disease Modeling Using iPSC-Derived Cells

Patient-derived iPSCs can be used to study genetic diseases like Alexander's disease. Tissue samples taken from patients are reprogrammed into iPSCs, which are then edited to correct mutations (152,153). These iPSCs can be differentiated into specific cell types, such as astrocytes, to study disease-related characteristics. For example, in Alexander’s disease, affected astrocytes show increased GFAP expression and impair oligodendrocyte function, which can be examined through co-culture studies and transcriptomic analysis (153). Such models help validate findings in human tissues and animal experiments.

8.2. Modeling Sporadic and Infectious Diseases

Sporadic diseases, like Alzheimer’s, can be modeled using patient-derived iPSCs that contain genetic risk factors. These cells can also be exposed to external risk elements to simulate disease conditions. For instance, exposing brain organoids to human serum can mimic blood-brain barrier disruptions and induce Alzheimer’s-like pathology, showing amyloid peptide buildup and tau hyperphosphorylation with impaired neuron activity [154]. Infectious diseases like COVID-19 can also be studied using iPSC-derived cells to observe virus-specific interactions, entry mechanisms, and impacts on human cells [154, 155].

8.3. Challenges and Advantages of iPSC Disease Models

Due to the extensive applications of iPSC-derived models in disease research, a single review cannot capture all relevant studies. The following sections explore how iPSC models are used to study neurodevelopmental, psychiatric, and neurodegenerative diseases, which are difficult to replicate in animal models due to species differences and human brain complexity [156]. iPSC-based models are also useful for studying early cancer development and have been rapidly adapted to study emerging infectious diseases like COVID-19.

8.4. Neurodevelopmental and Psychiatric Disease Modeling

Neurodevelopmental and psychiatric disorders are challenging to study because they involve cognitive symptoms that animal models alone cannot fully replicate (157). While animal models can show some cognitive traits, they do not capture the cellular and molecular details of these disorders due to differences between human and animal brains (157-161). However, iPSC-derived models provide valuable insights into disease mechanisms, and advanced techniques like brain organoid transplantation and machine learning are opening new avenues for studying complex brain functions (158).

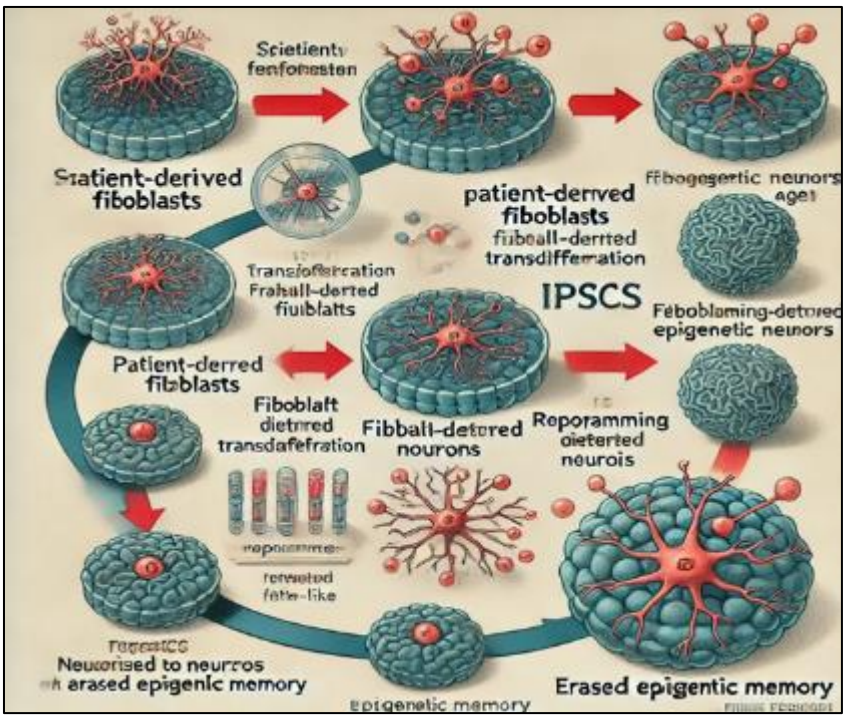

Figure 8 Neurodevelopmental and Psychiatric Disease Modeling

iPSC-derived neural cells from patients with neurological conditions often show functional impairments. For example, studies of schizophrenia have shown altered proliferation and movement of neural progenitors, abnormal neuron structure, and disrupted glutamate uptake by astrocytes (162). Autism spectrum disorder (ASD) models derived from iPSCs show increased proliferation, impaired migration, and chromatin changes at the molecular level (163). Electrophysiological tools like multi-electrode arrays (MEA) help study neuronal activity and connectivity, revealing differences in synaptic density and function. Schizophrenia models have shown reduced synaptic density and impaired synaptic transmission, while ASD models often show increased synaptic density and hyperactive neuronal firing. Machine learning combined with MEA has also been used to simulate learning environments and synaptic adaptations *in vitro*, potentially highlighting differences in patients' neural cell remodeling (164).

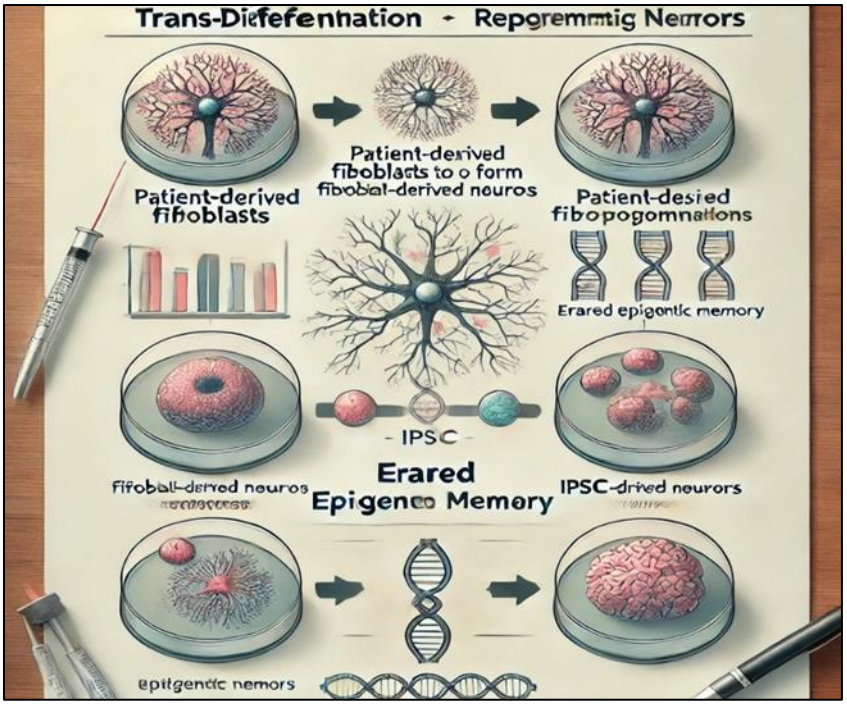

Figure 9 Trans differentiation

Brain organoids offer a three-dimensional view of cell interactions and complex phenotypes in neurological disorders. For instance, organoids derived from patients with Down syndrome or ASD show abnormal neural progenitor cell behavior and excessive production of inhibitory neurons (165). These models benefit from advanced electrophysiological properties and a 3D structure that mimics the brain more accurately. Transplanting iPSC-derived cells into animal brains enables the study of cell behavior in a more complex environment, aiding in the examination of cognitive and behavioral deficits. For example, iPSC-derived glial cells from schizophrenia patients show early migration into the cortex and myelination issues in animal models, which reflect anxiety-like behaviors (166).

Although *in vitro* brain organoids have advanced disease modeling, they often lack proper blood vessel formation, which can limit nutrient flow and lead to cell stress and incomplete maturation. However, transplanting organoids into live hosts supports vascularization and improves cell characteristics like neuron development and microglia survival. For example, Timothy syndrome models show abnormal neuronal shapes and heightened synaptic activity, while ASD models indicate microglia activation. Overall, iPSC-based models shed light on complex molecular and cellular changes related to neurological diseases (137,138). .

9. Modeling Neurodegenerative Diseases with iPSC-Derived Cells.

Neurodegenerative diseases like Alzheimer's, Parkinson's, and ALS are age-related conditions characterized by a strong association with aging, alongside genetic mutations and risk factors (167,168). Aging itself is a significant risk factor, intricately tied to the molecular mechanisms driving disease progression. However, iPSC-derived cells resemble a fetal-like state and typically do not show age-related characteristics naturally (169). This is due to the reprogramming process that rejuvenates cells, effectively erasing signs of aging, which do not reappear upon differentiation (170). This limitation poses a challenge for using iPSC-derived cells in modeling age-associated diseases. Despite this, iPSC-based models of neurodegenerative diseases have been developed, and strategies to replicate aging traits in these cells are evolving (171,172).

9.1. Inducing Aging Phenotypes in iPSC-Derived Cells

One major limitation of using iPSC-derived cells for modeling is their young, fetal-like state, lacking features linked to aging. The process of reprogramming somatic cells into iPSCs removes aging markers and characteristics. To address this, different methods have been developed to induce aging-like features in iPSC-derived cells (172, 173). One approach involves exposing these cells to compounds that disrupt cellular equilibrium, causing traits like mitochondrial stress or senescence. For instance, treatment with rotenone affects mitochondrial electron transfer, leading to increased reactive oxygen species (ROS), mitochondrial damage, and cellular senescence (174). Another method is the ectopic expression

of progerin, a shortened version of lamin A, which causes Hutchinson-Gilford progeria syndrome—a disorder marked by accelerated aging. Progerin expression in iPSC-derived neurons can lead to age-related characteristics such as neurite damage and changes in gene expression. However, it is important to note that progeria differs from typical human aging, and the induced traits may not fully represent normal aging (175).

9.2. Direct Trans differentiation for Aging Traits

A promising way to maintain aging-associated features is through direct trans differentiation, bypassing the iPSC stage (176). This process involves converting patient-derived fibroblasts directly into neurons using specific transcription factors or microRNAs, along with small molecules (177). These transdifferentiated neurons retain the donor fibroblast's epigenetic age and aging traits, making them suitable for studying age-related aspects of neurodegenerative diseases. For example, neurons directly converted from fibroblasts of elderly Alzheimer's patients show distinct characteristics such as altered metabolism, increased senescence, and incomplete maturation that are not observed in typical iPSC-derived neurons (178).

9.3. Modeling Genetic and Sporadic Disease

Neurodegenerative diseases can be modeled using iPSC-derived cells with known genetic mutations. For instance, iPSC-derived cortical neurons with PSEN1 mutations replicate Alzheimer's amyloid β pathology, while dopaminergic neurons with SNCA mutations show α-synuclein accumulation typical of Parkinson's disease. Motor neurons with TDP-43 mutations exhibit features seen in ALS [152]. On the other hand, most neurodegenerative cases are sporadic, with no clear genetic cause. Identified genetic risk factors from genome-wide association studies (GWAS) can be modeled in iPSCs to observe their impact on disease progression [153]. For example, the APOE4 variant, a strong risk factor for Alzheimer's, has been studied in iPSC-derived neurons, astrocytes, and microglia, all showing disrupted cellular function. Additionally, non-genetic factors such as the peripheral immune system and environmental toxins also play roles in neurodegenerative diseases. For instance, co-culturing iPSC-derived dopaminergic neurons with T cells from Parkinson's patients has demonstrated increased neuron death driven by IL-17 secretion [156].

9.4. Age-Related Modeling and Future Directions

Understanding how aging interacts with genetic and environmental risk factors in neurodegenerative diseases is crucial. Aging at the molecular level can be linked to epigenetic changes and DNA damage that disrupt gene expression, leading to cell dysfunction and senescence. Techniques that induce aging-like changes, such as mitochondrial stress or exposure to progerin, help mimic age-related dysfunction. For example, exposing iPSC-derived neurons to human serum can simulate blood-brain barrier (BBB) breakdown, triggering Alzheimer's-like symptoms, including amyloid and tau accumulation and reduced neuron activity.

Direct trans differentiation methods offer valuable alternatives for creating aged cell models, retaining original cellular aging markers. This approach has been used to highlight how aging impacts Alzheimer's pathology in neurons derived from older patients' fibroblasts. Additionally, iPSC-derived brain organoids exposed to conditions mimicking BBB breakdown have shown rapid onset of Alzheimer's-like pathology, underscoring how these models can simulate age-related disease mechanisms.

Advances in modeling aging-related phenotypes using iPSC-derived cells are expected to deepen our understanding of both neurodegenerative and broader age-related diseases, revealing insights into disease mechanisms and potential therapeutic targets.

9.5. Modeling Cancer Initiation with iPSC-Derived Cells

Primary cancer cells obtained from tumor biopsies are commonly used to study tumor biology and evaluate responses to treatments due to their inherent ability to proliferate (181). However, these cells have already undergone transformation, a pivotal process that disrupts normal cellular regulation and leads to cancer formation. iPSC technology provides a valuable approach to explore how somatic mutations and other factors alter the molecular and cellular behavior of normal cells, leading them toward cancerous states[181].

For instance, iPSC-derived neural stem cells containing the H3.3K27M mutation, linked to diffuse intrinsic pontine glioma (a pediatric brain tumor), display abnormal gene expression patterns that drive increased proliferation and stem cell properties [182]. Similarly, iPSC-derived colonic organoids from patients with mutations in the APC gene, known to be involved in familial colorectal cancer, show heightened WNT pathway activity and increased proliferation of epithelial cells compared to normal controls [183].

Environmental factors also contribute to cancer initiation. For example, chronic infection with *Helicobacter pylori* has been linked to a higher risk of gastric cancer, likely due to long-term inflammation of the stomach lining. When *H. pylori* is introduced into iPSC-derived gastric organoids, the epithelial cells respond with a marked increase in proliferation [184]

Genetic modifications in iPSCs followed by differentiation into relevant cell types can be used to model the step-by-step progression of cancer, simulating how somatic mutations accumulate and how cancer cells expand clonally [185]). For example, introducing key mutations associated with acute myeloid leukemia (AML) into iPSCs and differentiating them into hematopoietic progenitor cells allows researchers to trace the transformation from a pre-cancerous state to fully developed leukemia. Comprehensive gene expression profiling during this process highlights unique molecular pathways, such as disrupted inflammatory signaling, that facilitate cancer development [185].

10. iPSC-Derived Cells in Drug Development and Therapy: Unlocking the Future of Medicine

10.1. iPSCs in Drug Development: A New Paradigm

10.1.1. Human-Specific Models for Drug Testing

iPSC-derived cells provide an invaluable platform for drug development by offering human-specific models to test drug efficacy and toxicity [180,181]. Unlike animal models, which may fail to replicate human molecular mechanisms, iPSCs derived from disease-specific genetic backgrounds allow researchers to observe the precise effects of drugs on human cells. For example, iPSCs carrying disease-related mutations can predict how a drug interacts within specific genetic contexts, enabling personalized and targeted therapy approaches [182]

10.1.2. High-Throughput Drug Screening

iPSCs facilitate large-scale drug screening by enabling the differentiation of various cell types that are otherwise difficult to access. For instance:

- Anti-Apoptotic Screening: Gu et al. screened 4,500 compounds to identify agents reducing cell death in iPSC-derived endothelial cells from pulmonary arterial hypertension patients [182-185].
- Alzheimer's Drug Discovery: Park et al. used tissue-clearing techniques to evaluate amyloid β and tau pathology in iPSC-derived brain organoids from Alzheimer's patients, advancing therapeutic strategies. [183, 184-188].

10.1.3. Integration with Computational Tools

Combining iPSC-based drug testing with machine learning and computational methods streamline drug discovery. For example:

- Repurposing Drugs: Taubes et al. identified bumetanide as a potential Alzheimer's treatment through computational models and validated its efficacy using iPSC-derived neurons [185].
- Gene Expression Correction: Machine learning has enabled the identification of compounds like ERRα inverse agonists, which reverse gene abnormalities in calcific aortic valve disease.

10.1.4. Toxicity Assessments

One of the leading causes of drug failure is unexpected toxicity. iPSC-derived cells address this by providing human-relevant platforms for toxicity screening:

- Nephrotoxicity: Podocytes in a glomerulus-on-a-chip system replicate kidney damage caused by drugs like Adriamycin [189].
- Cardiotoxicity: iPSC-derived cardiac tissues reveal structural damage and halted contractions induced by doxorubicin [190].

10.2. Precision Medicine

Patient-specific iPSCs allow for individualized drug responses, enhancing precision medicine. For example:

Patient specific iPSCs may help with precision medicine by predicting individual responses to drugs. For example, transcriptomic analysis of iPSC-derived cardiomyocytes has shown variability in patient-specific sensitivity to oxidative

stress due to differing levels of NFE2L2 expression [189]. Cardiomyocytes with lower NFE2L2 expression were more vulnerable to drugs like tacrolimus and rosiglitazone [190]. Understanding such mechanisms of toxicity can inspire new strategies to counteract it; Sharma et al [190]. demonstrated that tyrosine kinase inhibitor exposure in iPSC-derived cardiomyocytes activated compensatory insulin signaling, which could be protective, and adding insulin or IGF1 enhanced cell viability.

Drug distribution and accumulation in specific tissues can also contribute to unexpected toxicity [191]. iPSC-derived organoids can assess pharmacokinetics; for example, choroid plexus organoids can form fluid-filled cysts with selective drug permeability. Intestinal epithelial cells derived from iPSCs are useful for studying drug absorption and cytochrome P450 enzyme metabolism [191.192]. Humanized animal models, created by transplanting iPSC-derived organoids, provide insights into how drugs interact with human tissue. For instance, iPSC-derived kidney organoids transplanted into rats have been used to study drug exposure in vivo. Overall, iPSC technology offers an effective way to evaluate both drug efficacy and toxicity using human-relevant models([191.192]

10.3. iPSC-Based Cell Therapy

Cell therapy has become an innovative method for repairing or replacing damaged tissues and engineering immune responses to diseases like cancer (193,194]. The success of CAR T cell therapy in treating acute lymphoblastic leukemia and large B cell lymphoma has paved the way for new cell therapies, including those using iPSC technology [191-194]. While primary cells, such as T cells, NK cells, and mesenchymal stem cells, can be harvested from patients for use in autologous therapies, other cell types, such as neurons, are not as easily obtained for transplantation. Additionally, the quality of primary cells can be affected by diseases or genetic mutations and may show unwanted variability. iPSC technology addresses these limitations as iPSCs can be genetically modified, expanded in culture, and differentiated into most somatic cell types. iPSC-based therapies also face fewer ethical issues compared to ESC-based therapies, as iPSCs are sourced from somatic cells [192].

Studies involving xenotransplantation have shown that transplanted iPSC-derived cells can improve tissue function and restore balance [194]. For instance, human iPSC-derived pancreatic islets have been shown to secrete insulin and regulate blood sugar levels in diabetic mice and macaques. Similarly, iPSC-derived oligodendrocyte progenitor cells (OPCs) have been found to restore myelination in mice with myelin deficiencies, suggesting potential use in treating white matter disorders (123,145). These examples highlight that iPSCs can be used to generate rare cell types and support tissue function through transplantation. As a result, clinical trials using iPSC-derived cells for various diseases are being initiated (145).

10.4. Autologous iPSC-Based Therapy

10.4.1. Advantages of Personalized Treatments

Autologous therapies use iPSCs derived from the patient, reducing immune rejection risks. The process involves reprogramming the patient's somatic cells, correcting genetic mutations, and differentiating them into the required cell type. Applications include :

- Hereditary Antithrombin Deficiency: Gene-corrected iPSC-derived hepatocytes normalized antithrombin levels in preclinical models [194].
- Wolfram Syndrome: Gene-edited pancreatic beta cells from patient-derived iPSCs successfully regulated blood sugar in diabetic mice [195,196]

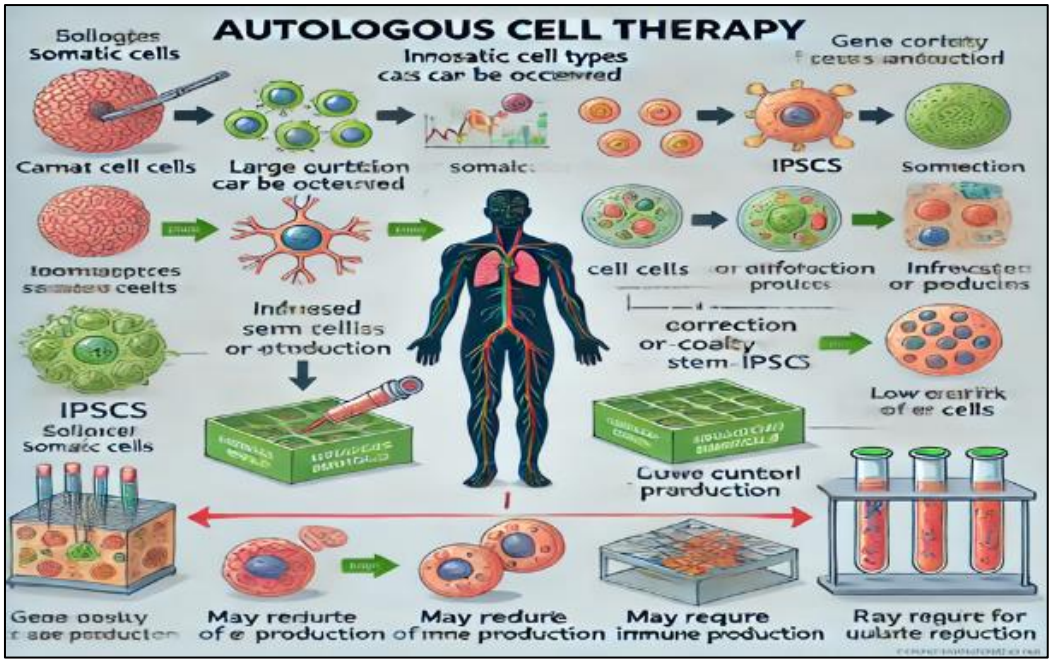

Figure 10 Autologus Cell Therapy

10.5. Allogeneic iPSC-Based Therapy

10.5.1. Universal Donor Cells

Allogeneic therapies rely on iPSCs derived from universal donors, allowing for "off-the-shelf" treatments. However, these therapies face immune rejection risks, which can be mitigated through genetic modifications [196].

- Immune Evasion Strategies: Techniques like B2M knockout and CD47 overexpression create hypoimmunogenic cells capable of avoiding immune detection [191 -195].
- HLA Compatibility: HLA-homozygous iPSC banks, such as Japan's haplobank, match patients' HLA types and cover large population segments efficiently [196-198].

Figure 11 The Allogenic Cell Therapy

11. Revolutionizing Medicine with iPSC-Based Cell Therapy

11.1. The Potential of iPSC Therapy

Induced pluripotent stem cells (iPSCs) are transforming regenerative medicine by providing a versatile platform to repair or replace damaged tissues. Unlike primary cells, iPSCs can be expanded in culture, genetically modified, and differentiated into nearly any cell type, offering solutions that bypass the ethical challenges associated with embryonic stem cells.

11.2. Preclinical Successes in Regenerative Applications

Studies using iPSC-derived cells have shown remarkable success in animal models:

- Diabetes Management: iPSC-derived pancreatic islets regulate blood sugar effectively in diabetic mice and macaques.
- Neurological Recovery: Oligodendrocyte progenitor cells derived from iPSCs restored myelination in mice, offering hope for white matter disorders.

11.3. Personalized Medicine with Autologous iPSCs

By creating iPSCs from a patient's own cells, autologous therapies reduce the risk of immune rejection. This personalized approach has shown promise in preclinical studies:

- Correcting Genetic Disorders: Gene-edited iPSC-derived hepatocytes normalized antithrombin levels in hereditary antithrombin deficiency.
- Diabetes Solutions: Pancreatic beta cells derived from gene-corrected iPSCs effectively regulate blood sugar in Wolfram syndrome models.

11.4. Streamlining Treatments with Allogeneic iPSCs

Allogeneic therapies use iPSCs from universal donors to create "off-the-shelf" treatments, reducing the time and cost of individualized cell preparation. However, these therapies must address immune rejection risks through:

- Genetic Modifications for Immune Evasion: Techniques such as knocking out B2M or overexpressing CD47 produce hypoimmunogenic cells that avoid immune detection.
- HLA-Compatible iPSC Banks: Initiatives like Japan's haplobank provide a collection of HLA-homozygous iPSC lines, covering large segments of the population and enabling efficient matching.

11.5. The Future of Regenerative Medicine

iPSC-based therapies are poised to revolutionize medicine by offering innovative solutions for genetic, metabolic, and degenerative diseases. With advancements in genetic editing and cell engineering, iPSC therapies continue to push the boundaries of personalized and universal treatment options.

Advancing iPSC Technology for the Future: The field of induced pluripotent stem cell (iPSC) research is progressing rapidly, with significant efforts dedicated to enhancing reprogramming efficiency and improving the functional maturity of derived cells. Scalable methods, such as fully chemical reprogramming, are simplifying iPSC production, while advanced culture systems are ensuring that differentiated cells mimic their natural, in vivo counterparts.

Applications in Disease Modeling and Drug Development: Innovative tools like organ-on-a-chip systems and brain organoid transplantation are transforming our ability to model complex diseases. These technologies provide platforms for evaluating drug efficacy, toxicity, and pharmacokinetics, while also enabling the exploration of disease mechanisms driven by genetic and environmental factors. By replicating human-specific developmental and pathological processes, these models are advancing our understanding of diseases such as Alzheimer's and cancer.

Towards Regenerative Medicine and Organ Replacement: The future of iPSC research extends to the creation of fully functional organs using techniques like chimeric organogenesis. These developments have the potential to address the critical shortage of organ donors by providing lab-grown alternatives for transplantation. Such breakthroughs could redefine the landscape of regenerative medicine and organ replacement therapies.

12. Conclusion: A New Era in Medicine

Since their inception, iPSCs have revolutionized biomedical science, unlocking new possibilities in disease modeling, drug discovery, and personalized cell therapies. As technology evolves, iPSCs are expected to play a leading role in precision medicine and regenerative treatments, offering tailored solutions to some of the most challenging medical conditions of our time.

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