Chapter 11

Embryonic Stem Cells in Human Medicine; Terminology and Application

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Abstract

hESCs[1] possess remarkable self-renewal and pluripotency, enabling differentiation into cell types from all three germ layers. Since their isolation in 1998, hESCs have offered significant potential for tissue repair and regenerative medicine, disease modeling, drug testing, and genetic engineering. However, challenges persist, including tumorigenicity, immune rejection, ethical concerns surrounding embryo destruction, and regulatory hurdles. The document reviews derivation methods, culture systems (including feeder-dependent and feeder-free, xeno-free and GMP-compatible conditions), and strategies to maintain genomic stability, as well as clinical applications across tissue engineering, disease modeling, and personalized medicine. It also discusses developmental

[1] . Embryonic stem cells

biology context, directed differentiation protocols, quality control, and the regulatory/ethical landscape, highlighting ongoing advances such as naive pluripotency, organoid models, CRISPR-based genome editing, and scalable bioprocessing for translational therapies.

Keywords: Embryonic stem cells, Inner cell mass, Tumorigenicity and safety, Regenerative medicine, Disease modeling, Drug testing and toxicology.

1. Introduction

The field of hESCs has greatly influenced stem cell research since their first isolation in 1998. These cells have attracted considerable interest because of their remarkable self-renewal ability and pluripotency, which enable them to differentiate into a wide range of cell types derived from all three embryonic germ layers [1]. This potential offers promising possibilities for repairing damaged tissues in diseases where current treatment options are limited [2]. Nonetheless, hESC research faces several challenges, such as the risk of tumor development and immune rejection, as well as ethical and political debates owing to the destruction of human embryos necessary for cell isolation [3, 4].

The therapeutic applications of hESCs involve both systemic and localized administration techniques, including intravenous or intramuscular injections and surgical placements, occasionally involving bioscaffolds [5]. These methods can be divided into temporary interventions and permanent installations designed for long-term tissue regeneration [2, 6, 7]. Despite significant progress, maintaining consistent properties of hESCs across different experimental settings remains a

major challenge for clinical applications. Compliance with GLP[1] and GMP[2] standards is crucial to minimize variability and contamination risks in both research and clinical settings. GLP offers a structured approach that ensures the reliability and reproducibility of preclinical studies involving hESCs. While iPSCs[3] present a feasible alternative, hESCs still hold distinct advantages in exploring genetic diseases and human development [8]. Although the ethical concerns and a slower pace of related clinical research compared to iPSCs, hESCs continue to be an essential resource in biomedical research, enhancing our comprehension of early human development and genetic disorders. This understanding is vital for developing effective regenerative medicine strategies and personalized therapies [9]. Additionally, it aids in refining iPSC-based methods, ultimately advancing the field of regenerative medicine [10]. This synergy between hESC and iPSC research is crucial for addressing existing challenges and realizing the full potential of stem cell-based therapies [11]. The collaborative approach accelerates the translation of basic research into clinical applications, paving the way for innovative treatments and improved patient outcomes. However, further research is needed to fully unlock their therapeutic potential.

In practical laboratory settings, the derivation of hESCs generally begins with the extraction of the ICM[4] from blastocysts. This process can be achieved through immunosurgery, a technique in which antibodies and complement agents selectively induce lysis of trophectodermal cells, thereby preserving the integrity of the ICM [12], or through mechanical microsurgery, which avoids the use of exogenous serum. More recently,

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1. Good Laboratory Practice
2. Good Manufacturing Practice
3. Induced pluripotent stem cells
4. Inner cell mass

derivation methods have also been conducted utilizing morula-stage embryos or even individual blastomeres; however, these methodologies have lower efficiency [13]. hESCs are sustained either in feeder-dependent systems, where MEFs[1] supply the ECM[2] and growth factors, or in feeder-free environments using specified matrices like Matrigel, vitronectin, or laminin-521, along with a chemically defined medium [2]. In clinical settings, xeno-free media formulations are being increasingly adopted to remove animal-derived components, thereby minimizing variability and the risk of contamination [14]. Strategies for maintenance and expansion vary: manual passaging maintains chromosomal stability but is time-consuming, whereas enzymatic passaging (using collagenase, trypsin, EDTA[3]) is more scalable but may lead to chromosomal instability [15]. Recent advancements integrate enzymatic dissociation with ROCK[4] inhibitors, facilitating the clonal survival of single cells in suspension cultures, which supports scalability and workflows compatible with automation [16].

2. Developmental Biology Context

The development of embryoids is similar to that of embryos, characterized by the formation of structured multicellular formations through coordinated cellular processes, such as pattern formation, morphogenesis, differentiation, and growth [17]. This chapter starts with early development processes in mouse and human embryos, which transition from a zygote to phases like morula, blastula, and gastrula. Both species share a similar pre-implantation development program, leading to the formation of the blastocyst, which

[1]. Mouse embryonic fibroblasts
[2]. Extracellular matrix
[3]. Ethylenediaminetetraacetic acid
[4]. Rho kinase

consists of an outer trophectoderm layer surrounding a cavity and an ICM. As the blastocyst matures, the ICM differentiates into two groups: the EPI[1] and the PE[2] [18]. The onset of blastocyst implantation is not the same across cases, taking place at E5.0 in mice and E7.0 in humans, with significant differences in morphogenesis and lineage development after implantation. In mice, the EPI forms a cup shape alongside the extraembryonic ectoderm derived from the trophectoderm, whereas in humans, the EPI undergoes a process to form the pro-amniotic cavity, differentiating into the amniotic ectoderm and embryonic disc. Early development includes crucial signaling pathways like WNT and NODAL, which establish the anterior-posterior axis during gastrulation, transforming EPI into three germ layers (ectoderm, mesoderm, endoderm) that interact to drive organ development [19]. In mice, PGCs[3] emerge early in the gastrulation process, but data on human PGC specification remain limited. Both species' gastrulation phases lead to the establishment of definitive ectoderm, mesoderm, and endoderm structures. Following gastrulation, the ectoderm undergoes neurulation, contributing to the formation of major organ systems and the primitive gut tube [20]. hESCs, derived from blastocysts, exhibit a transcriptome different from that of pre-implantation human embryos, indicating that standard culture conditions do not fully replicate early development. Instead, hESCs resemble the post-implantation EPI of other primates. Although they can initiate PGC formation, differences remain between hESCs and mouse EPI stem cells [21]. Recent advancements have led to the creation of naive pluripotent hESC lines, but functional validation of these cells remains limited due to ethical constraints. hiPSCs[4], derived from somatic cells, show similarities to hESCs in terms of

[1]. Pluripotent epiblast
[2]. Primitive endoderm
[3]. Primordial germ cells
[4]. Human induced pluripotent stem cells

molecular and functional characteristics [22]. However, hiPSCs exhibit epigenetic memory from their tissue of origin, influencing their differentiation potential and limiting their utility in certain developmental studies [23]. Emerging studies have also focused on establishing hEPSCs[1] and hTSCs[2], which have unique developmental potentials, signaling the need for further characterization and authentication [24]. Biological research on human embryogenesis is critical for improving assisted reproductive technologies, preventing pregnancy complications, and understanding birth defects, yet it is often hindered by ethical limitations on embryo access. The recent ability to culture human embryos *in vitro* has expanded research possibilities but is still subject to regulatory limits that restrict studies beyond the onset of gastrulation [25]. This restriction poses challenges for studying later stages of human development and necessitates innovative approaches to model these processes *in vitro*. These approaches include the use of stem cell-based models and advanced imaging techniques to overcome the limitations of studying intact human embryos. While hESCs reflect a primed state, mESCs[3] are considered naïve. Protocols have been developed to transition hESCs to a naive state using various small molecules, producing different naïve cell populations with distinct characteristics [26]. Ongoing investigations into the nature of naïve pluripotency and its correspondence to *in vivo* epiblast conditions highlight the need for comparative studies to deepen our understanding of pluripotency transitions [27, 28]. These studies are crucial for refining cell culture protocols and improving the fidelity of *in vitro* models of early human development. These developmental comparisons highlight the significant impact of *in vitro* culture conditions on the phenotype of hESCs.

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[1]. Human extraembryonic stem cells
[2]. Trophoblast stem cells
[3]. Mouse embryonic stem cells

Traditional hESCs tend to resemble the post-implantation epiblast more than the truly naïve preimplantation state, leading to efforts aimed at refining culture systems to better mirror developmental stages (**Figure 1**) [26].

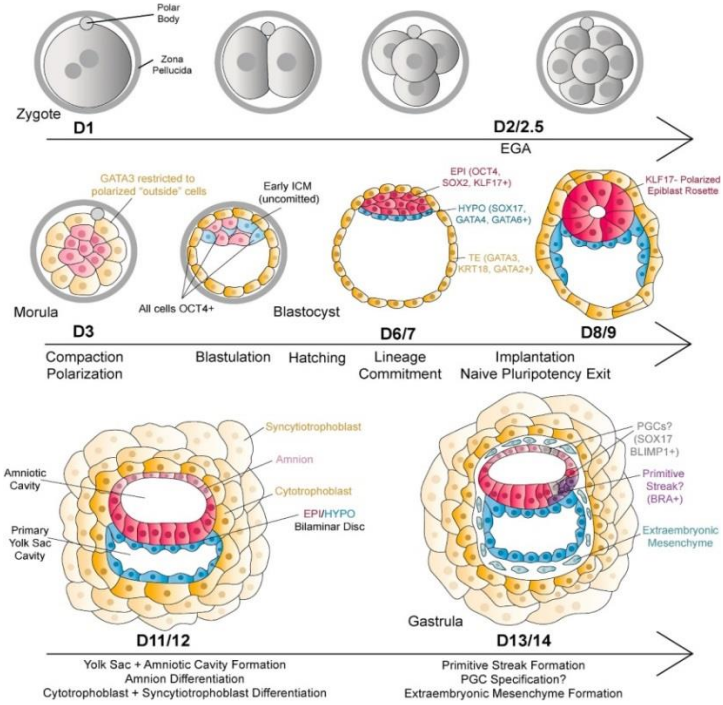

Figure 1. Pre-gastrulation step in the human embryo. After fertilization, the zygote undergoes cleavage divisions and genome activation between the 4- and 8-cell stages. In the embryonic stage with 8 to 16 cells, compaction and polarization start to generate a morula. In this step, outer cells form the trophectoderm, and inner cells can produce the ICM[1]. On day 5, the blastocyst stage is evident, and by the late blastocyst stage, the lineages—epiblast, hypoblast, and trophoblast—have become specified. The mature blastocyst implants into the maternal uterus, the epiblast loses pluripotency and initiates lumen formation to create the amniotic cavity, while the hypoblast expands to form the visceral endoderm around the yolk sac cavity. Epiblast cells contacting the trophoblast contribute to the amniotic membrane, and those contacting the hypoblast contribute to the embryo proper. The trophectoderm differentiates into cytotrophoblast and multinucleated syncytiotrophoblast, which together generate the embryonic portion of the placenta. Around day 13–14, primordial germ cells are thought to be specified, extraembryonic mesenchyme appears, and the primitive streak forms in the posterior epiblast. Abbreviations: ICM = Inner Cell Mass; EPI = Epiblast; HYPO = Hypoblast; TE = Trophectoderm; PGCs = Primordial

[1]. Inner cell mass

Germ Cells. Reproduced with permission (Bailey A.T. Weatherbee et al., 2021. Developmental Biology. 2021.) [28].

3. Research and Medical Applications

As above-mentioned, ESCs are pluripotent cells derived from the ICM of the blastocyst stage embryo, characterized by their ability to self-renew indefinitely and differentiate into nearly all cell types of the human body [1, 12]. This unique combination of properties has positioned ESCs at the forefront of biomedical research and regenerative medicine, offering unprecedented opportunities for understanding human development, modeling diseases, and developing novel therapeutic strategies.

Regenerative medicine represents one of the most promising applications of ESCs. Their pluripotency allows for the generation of specific cell types that can replace or repair damaged tissues in a variety of diseases. For instance, ESC-derived cardiomyocytes have been used to remuscularize infarcted myocardium, offering hope for patients with ischemic heart disease [29]. Similarly, neuronal precursor cells derived from ESCs show potential in repairing central nervous system injuries such as spinal cord damage and neurodegenerative diseases, including multiple sclerosis and amyotrophic lateral sclerosis [30, 31]. ESCs can also differentiate into insulin-producing pancreatic beta cells, which could revolutionize treatment for diabetes mellitus by restoring endogenous insulin production [32]. These applications are supported by the ability of ESCs to integrate into host tissues and functionally contribute to tissue regeneration, as demonstrated in various preclinical models [2, 33, 34].

Disease modeling is another critical area where ESCs have made significant contributions [35]. By directing ESC differentiation into specific cell types affected by diseases, researchers can create in vitro models that recapitulate human pathophysiology more accurately than traditional

animal models. This is particularly valuable for studying genetic, metabolic, and neurodegenerative disorders. For example, ESC-derived neurons have been used to model AD[1] and PD[2], enabling the investigation of disease mechanisms at the cellular and molecular levels. These systems support high-throughput evaluation of potential therapeutics, accelerating drug discovery and tailoring treatments to individuals [36, 37]. The ability to genetically modify ESCs using CRISPR/Cas9 technology further enhances disease modeling by allowing precise introduction of disease-causing mutations or correction of genetic defects to study their effects in a controlled environment [34].

Genetic engineering and gene therapy applications leverage the stable karyotype and pluripotency of ESCs to explore gene function and develop novel therapeutic interventions [38]. ESCs serve as an ideal platform for genome editing techniques, enabling the correction of mutations responsible for inherited diseases before differentiation into therapeutic cell types. This approach holds promise for treating monogenic disorders such as Duchenne muscular dystrophy, sickle cell anemia, and cystic fibrosis. Additionally, ESCs can be used to study gene regulatory networks critical for development and disease, providing insights that inform gene therapy strategies [32, 33, 35].

Tissue engineering combines ESCs with biomaterial scaffolds to create three-dimensional constructs that mimic native tissues [39]. This approach aims to repair or replace damaged organs, offering solutions for conditions where organ transplantation is currently the only option. ESC-derived cells seeded onto biodegradable scaffolds have been used to engineer cardiac patches, neural tissue constructs, and pancreatic islets. These engineered

[1]. Alzheimer’s disease
[2]. Parkinson’s disease

tissues not only restore function but also integrate with the host's vascular and nervous systems, improving long-term outcomes [33, 34]. The potential to generate patient-specific tissues using ESCs could reduce transplant rejection and the need for immunosuppression.

Drug testing and toxicology benefit from ESC-derived cells as they provide a physiologically relevant human cell platform for evaluating drug safety and efficacy [40, 41]. ESC-based models can replicate human tissue responses more accurately than animal models, reducing the incidence of late-stage drug failures due to toxicity or lack of efficacy. For example, ESC-derived cardiomyocytes are used to assess the cardiotoxicity of new compounds, while hepatocytes differentiated from ESCs serve to evaluate drug metabolism and hepatotoxicity. This application not only improves drug development pipelines but also aligns with ethical considerations by reducing reliance on animal testing [34, 40, 42].

At the molecular level, ESCs are defined by the expression of key pluripotency markers such as OCT4, SOX2, and NANOG, which regulate self-renewal and prevent differentiation [43]. OCT4, encoded by the Pou5f1 gene, is essential for maintaining the ICM and preventing premature differentiation into trophectoderm. Its expression, in concert with SOX2 and NANOG, forms a regulatory network that sustains pluripotency and coordinates cell fate decisions [32]. Other markers such as BMP4, GDF3, and REX1 contribute to maintaining the undifferentiated state and regulating differentiation pathways. Understanding these molecular signatures is critical for ensuring the quality and safety of ESC-derived products for clinical use.

4. Quality Control and Characterization

In addition to transcription factors, hESCs are characterized by surface antigens like SSEA[1] (SSEA-3 and -4) and TRA-1-60, TRA-1-81, which are commonly employed in their characterization [2]. Immunocytochemistry offers qualitative evidence of marker expression, whereas flow cytometry allows for more accurate quantification and analysis at the population level. Quality control also includes testing for genomic stability, such as G-banded karyotyping, SNP[2] arrays, and comparative genomic hybridization, in addition to regular mycoplasma and pathogen testing [7]. Global initiatives like the ISCI[3] aim to standardize these practices worldwide to ensure the uniformity of hESC lines used in both research and therapeutic applications. Directed differentiation protocols aim to replicate human embryogenesis by utilizing specific combinations of signaling modulators. In this regard, cardiomyocytes are produced through a process involving sequential WNT activation and inhibition, resulting in cells that beat spontaneously and express cardiac markers like NKX2.5 and cTnT. Despite this, these cells retain a fetal-like state, characterized by immature calcium handling and action potentials, highlighting the ongoing pursuit of achieving adult-like maturation [44, 45]. Hepatocyte-like cells derived from hESCs are induced in stages, progressing through definitive endoderm and hepatic progenitors. These cells produce albumin and urea and exhibit liver enzyme expression. However, their cytochrome P450 activity, crucial for drug metabolism, is not as effective as that of primary hepatocytes [46]. β-like insulin-producing cells are created through a multistage differentiation process that replicates pancreatic development. Recent methodologies have successfully produced insulin-

[1]. Stage-specific embryonic antigens
[2]. Single Nucleotide Polymorphism
[3]. International Stem Cell Initiative

secreting cells that respond to glucose, which are now the focus of ongoing clinical studies [47]. Unlike spontaneous embryoid body protocols, directed differentiation offers greater efficiency, though challenges remain in achieving fully mature and clinically useful phenotypes [48]. Despite these advances, the use of ESCs raises significant ethical and regulatory challenges. The derivation of ESCs involves the destruction of human embryos, which has sparked moral debates and led to varying regulations worldwide. Some countries have imposed strict limitations on ESC research, while others have developed frameworks to balance scientific progress with ethical considerations. Embryos used for ESC derivation are typically surplus from in vitro fertilization procedures or donated explicitly for research, emphasizing the importance of informed consent and ethical oversight. Comprehensive regulatory standards are essential for responsible application, ensuring patient safety and preserving public trust as ESC-derived therapies move into clinical settings [2, 49].

5. Clinical Trials and Applications

Cell therapies utilizing hPSCs are gaining traction as a potential treatment for degenerative diseases [50]. Clinical trials involving pluripotent stem cell derivatives are either underway or imminent for various medical conditions. Currently, hPSCs are being investigated in clinical trials for several ailments, including type 1 diabetes mellitus, spinal cord injuries, macular degeneration, PD, and heart disease. These advancements highlight the significant progress made in stem cell research and its promising implications for treating previously challenging health issues (**Table 1**). In addition to these core applications, emerging research explores the use of ESCs in immunotherapy, where ESC-derived immune cells could be engineered to target cancers or infectious diseases. Furthermore, the potential to generate organoids, miniature, simplified

versions of organs, from ESCs offers new avenues for studying organ development, disease mechanisms, and personalized medicine [33, 50]. These advances underscore the expanding versatility of ESCs in biomedical science. In conclusion, embryonic stem cells are a cornerstone of modern biomedical research and regenerative medicine. Their pluripotency, self-renewal capacity, and molecular characteristics enable diverse applications ranging from tissue regeneration and disease modeling to drug testing and genetic engineering [2]. While ethical and regulatory issues remain, ongoing scientific innovation and responsible governance are paving the way for ESCs to transform the treatment of many debilitating diseases, offering hope for improved health outcomes worldwide.

Table 1. Clinical trials with ESCs and their derivatives based on data from http://clinicaltrials.gov/

NCT NUMBER	STUDY TITLE	STUDY STATUS	CONDITIONS	STUDY TYPE
NCT04232592	Clinical Safety Study of Human Embryonic Stem Cell-Derived Mesenchymal Cells in the Treatment of Moderate and Severe Intrauterine Adhesions	Unknown	Intrauterine adhesion	Interventional
NCT05068674	Human Embryonic Stem Cell-Derived Cardiomyocyte Therapy for Chronic Ischemic Left Ventricular Dysfunction	Recruiting	Chronic ischemic left ventricular dysfunction	Interventional
NCT01165918	Derivation of New Human Embryonic Stem Cell Lines: Identification of Instructive Factors for Germ Cell Development	Unknown	Normal healthy embryos	Observational
NCT00353197	Derivation of New Human Embryonic Stem Cell Lines for Clinical Use	Recruiting	Infertility	Observational
NCT03046407	Treatment of Dry Age-Related Macular Degeneration Disease With Retinal Pigment Epithelium Derived From Human Embryonic Stem Cells	Unknown	Dry age-related macular degeneration	Interventional
NCT03167203	A Safety Surveillance Study in Subjects With Macular Degenerative Disease Treated With Human Embryonic Stem Cell-derived Retinal Pigment Epithelial Cell Therapy	Enrolling by invitation	Macular degenerative disease	Interventional
NCT03944239	Safety and Efficacy of Subretinal Transplantation of Clinical Human Embryonic Stem Cell-Derived Retinal Pigment Epitheliums in Treatment of Retinitis Pigmentosa	Unknown	Retinitis pigmentosa	Interventional
NCT03482050	A Study to Evaluate Transplantation of Astrocytes Derived From Human Embryonic Stem Cells, in Patients With Amyotrophic Lateral Sclerosis (ALS)	Completed	Als (amyotrophic lateral sclerosis)	Interventional

NCT02590692	Study of Subretinal Implantation of Human Embryonic Stem Cell-Derived RPE Cells in Advanced Dry AMD	Unknown	Dry macular degeneration/geographic atrophy	Interventional
NCT01633359	The Association Between Very Small Embryonic-like Stem Cells and the Prognosis of Coronary Artery Disease Patients	Unknown	Coronary artery disease	Interventional
NCT01469832	Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (hESC-RPE) Cells in Patients With Stargardt's Macular Dystrophy (SMD)	Completed	Stargardt's macular dystrophy	Interventional
NCT02749734	Clinical Study of Subretinal Transplantation of Human Embryo Stem Cell-Derived Retinal Pigment Epitheliums in Treatment of Macular Degeneration Diseases	Unknown	Macular degeneration/Stargardt's macular dystrophy	Interventional
NCT02941991	A Follow-up Study to Determine the Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (hESC-RPE) Cells in Patients With Stargardt's Macular Dystrophy (SMD)	Completed	Stargardt's macular dystrophy	Observational
NCT03482050	A Study to Evaluate Transplantation of Astrocytes Derived From Human Embryonic Stem Cells, in Patients With Amyotrophic Lateral Sclerosis (ALS)	Completed	Als (amyotrophic lateral sclerosis)	Interventional
NCT00353210	The Derivation of Human Embryonic Stem Cell Lines From PGD Embryos	Recruiting	Infertility	Observational
NCT00353197	Derivation of New Human Embryonic Stem Cell Lines for Clinical Use	Recruiting	Infertility	Observational
NCT03167203	A Safety Surveillance Study in Subjects With Macular Degenerative Disease Treated With Human Embryonic Stem Cell-derived Retinal Pigment Epithelial Cell Therapy	Enrolling by invitation	Macular degenerative disease	Interventional
NCT02122159	Research With Retinal Cells Derived From Stem Cells for Myopic Macular Degeneration	Withdrawn	Myopic macular degeneration	Interventional

NCT01469832	Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (hESC-RPE) Cells in Patients With Stargardt's Macular Dystrophy (SMD)	Completed	Stargardt's macular dystrophy	Interventional
NCT02941991	A Follow-up Study to Determine the Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (hESC-RPE) Cells in Patients With Stargardt's Macular Dystrophy (SMD)	Completed	Stargardt's macular dystrophy	Observational
NCT03046407	Treatment of Dry Age-Related Macular Degeneration Disease With Retinal Pigment Epithelium Derived From Human Embryonic Stem Cells	Unknown	Dry age-related macular degeneration	Interventional
NCT02749734	Clinical Study of Subretinal Transplantation of Human Embryo Stem Cell-Derived Retinal Pigment Epitheliums in Treatment of Macular Degeneration Diseases	Unknown	Macular degeneration/Stargardt's macular dystrophy	Interventional
NCT02590692	Study of Subretinal Implantation of Human Embryonic Stem Cell-Derived RPE Cells in Advanced Dry AMD	Unknown	Dry macular degeneration/geographic atrophy	Interventional

6. Challenges and ethical considerations

6.1. Technical and Biological Challenges

6.1.1. Differentiation Control

One of the primary challenges in ESC research is directing these cells to mature specific, functional cell types with high levels of efficiency and purity [51]. While ESCs have the potential to transform into all cell types, effectively utilizing this capability necessitates careful control of signaling pathways and gene expression patterns. Protocols typically involve sequential exposure to growth factors, small molecules, and sometimes genetic alterations to replicate developmental signals [16, 52]. The efficiency of differentiation can significantly differ based on the target lineage, and small groups of undifferentiated or inadequately differentiated cells may remain. These leftover cells can increase the risk of tumorigenesis and diminish the therapeutic effectiveness of products derived from ESCs [53]. Moreover, incomplete differentiation can lead to functional deficits in the transplanted tissues, limiting clinical success [15].

6.1.2. Tumor Risk

One of the primary safety issues associated with ESC-based treatments is the potential for tumor development, especially teratomas, which are tumors comprising various differentiated tissue types [54]. Both undifferentiated ESCs and certain differentiated derivatives have the potential to become tumorigenic following transplantation [55-57]. The development of tumors is affected by genetic mutations that occur during cell culture or reprogramming, as well as by epigenetic irregularities and defects in chromatin remodeling [53]. The immune system of the host, particularly NK[1] cells, is capable of destroying undifferentiated ESCs and

[1]. Natural killer

lowering the risk of tumors in recipients with a functioning immune system. Nonetheless, cells that have undergone differentiation might contain tumorigenic subpopulations that are resistant to being cleared by the immune system [56]. Strategies to reduce these risks involve thorough purification to eliminate undifferentiated cells, implementation of suicide gene systems, and adjustment of epigenetic regulators to maintain stable differentiation states [53, 57].

6.1.3. Immune Rejection and Immunogenicity

When ESC-derived tissues undergo differentiation, they begin to express major MHC[1] molecules, which makes them susceptible to rejection by the host's immune system [58, 59]. Typically, allogeneic ESC grafts necessitate the use of immunosuppressive therapy, which poses risks such as infection and cancer [55]. Autologous iPSCs present an alternative, yet they can still provoke immune reactions due to abnormal gene expression or genomic instability [15, 52]. Recent advancements involve modifying ESC-derived cells to express immune checkpoint molecules like PD-L1 or CTLA4Ig, which promote localized immunotolerance without the need for systemic immunosuppression or an increased risk of tumors [60]. These approaches are promising for overcoming immune barriers while maintaining safety.

6.1.4. Scalability

Producing clinically significant amounts of ESCs and their differentiated forms presents a significant challenge. The efficiency of deriving these cells from blastocysts is low, and isolating pure, stable stem cell populations involves intricate protocols [15]. Furthermore, maintaining

[1]. Histocompatibility complex

genomic stability and differentiation potential during large-scale expansion is difficult. Progress in bioprocessing techniques, such as suspension cultures, microcarrier systems, and bioreactors, is enhancing scalability and reproducibility [16]. Nonetheless, achieving consistent quality and safety on a large scale remains a significant obstacle for clinical application [53].

6.1.5. Xeno-Free and GMP Systems

The inclusion of animal-derived elements like MEF feeders and bovine serum albumin poses risks of contamination and inconsistencies between batches. Transitioning to xeno-free defined media and synthetic matrices such as laminin-521 or vitronectin enhances both safety and consistency. In the context of clinical-grade production, adherence to GMP standards is essential, necessitating controlled environments, traceable reagents, and validated standard operating procedures. These GMP-compliant hESC lines serve as the basis for regenerative medicine trials, in accordance with GCP[1] guidelines [7, 61].

6.1.6. Long-Term Monitoring and Clinical Uncertainty

Translational research involving embryonic stem cell therapies demands rigorous, comprehensive long-term patient surveillance to effectively detect potential delayed tumorigenesis and complex immunological interactions. Clinical trial protocols must incorporate sophisticated multi-modal monitoring strategies, encompassing advanced imaging techniques, targeted tissue biopsies, and detailed immunological profiling to systematically assess safety and therapeutic efficacy. The inherent biological complexity and dynamic *in vivo* behavior of embryonic

[1]. Good Clinical Practice

stem cells underscore the critical necessity for a measured, methodical approach to clinical translation, emphasizing patient safety and nuanced scientific validation [57].

6.2. Regulatory and Financial Challenges

ESC research and the development of related therapies are governed by strict regulatory frameworks aimed at ensuring patient safety and upholding ethical standards [62]. These regulations require adherence to GMP, extensive preclinical testing, and long-term monitoring, all of which collectively extend the time and increase the cost of development [50]. The necessity for specialized facilities, trained personnel, and costly reagents further raises financial hurdles. Additionally, the variation in regulations across different countries complicates international collaboration and commercialization efforts. These elements contribute to the high expenses and resource demands associated with bringing ESC-based therapies to market, thereby limiting accessibility and hindering innovation [15].

6.3. Ethical Considerations

6.3.1. Embryo Destruction

ESCs are obtained from human embryos at the blastocyst phase, and their extraction leads to the destruction of the embryo. This process raises significant ethical concerns about the moral status of embryos and when human life begins [2]. Some individuals believe that embryos have full moral status from the moment of conception, making their destruction morally unacceptable. Conversely, others argue that using excess embryos from IVF[1], with informed consent, is ethically defensible due to the potential medical advancements that could alleviate human suffering.

[1]. In vitro fertilization

These discussions have a significant impact on public policy and funding decisions globally [63-68].

6.3.2. Informed Consent

Obtaining informed consent from both embryo and gamete donors is crucial for conducting ethical research [69]. Donors should be thoroughly informed about the purpose of the research, any associated risks, benefits, and possible commercial uses. There is ongoing discussion regarding whether gamete donors should give explicit consent for the use of embryos, with some ethical viewpoints advocating for distinct consents to uphold donor autonomy. In practice, the processes for obtaining consent differ, and there are continuing concerns about donors' comprehension, the potential for deception, and the transparency concerning the use and significance of donated tissues [67, 70, 71].

6.3.3. Commercialization and Exploitation

The commercialization of ESC research brings forth significant concerns regarding the commodification of human tissues and the potential exploitation of women as sources for eggs and embryos. Ethical issues arise surrounding unequal access to therapies, the tendency to prioritize profit over patient well-being, and the potential for coercion or undue influence in egg donation [72]. It is essential to establish safeguards that ensure fair access, protect the rights of donors, and uphold public trust in stem cell research. Additionally, the commercialization aspect introduces the risk of exerting inappropriate pressure on women to donate eggs or embryos, particularly in environments with insufficient regulation. This situation raises important questions about autonomy, coercion, and the fair distribution of risks and benefits. Vulnerable groups may be disproportionately impacted, highlighting the need for ethical frameworks

that focus on donor welfare and prevent exploitation. Key elements of ethical practice in stem cell procurement include transparency, voluntary participation, and appropriate compensation [67, 70].

6.4. Technological Advances

6.4.1. Improved ESC Derivation Techniques

Scientists have made significant breakthroughs in extracting embryonic stem cells more gently and precisely. By developing culture methods that closely resemble the natural environment of early embryos, researchers can now keep these cells healthier, more genetically stable, and better able to maintain their versatile potential. These improvements mean we can create more robust stem cell lines that hold greater promise for both scientific studies and potential medical treatments [16].

6.4.2. Directed Differentiation

Recent advancements in stem cell research have significantly refined protocols for differentiating embryonic stem cells into precise cellular phenotypes, including neurons, cardiomyocytes, and pancreatic beta cells [29, 73]. By strategically implementing sophisticated combinations of growth factors, targeted small molecule interventions, and precise gene editing techniques, researchers can now more effectively recapitulate complex developmental trajectories. These methodological innovations not only enhance the yield and functional integrity of differentiated cell populations but also provide unprecedented insights into cellular programming mechanisms, bridging critical gaps between developmental biology and regenerative medicine [16, 52].

6.4.3. Genome Editing (CRISPR-Cas9 and Others)

Genome editing technologies, particularly CRISPR-Cas9, have dramatically transformed embryonic stem cell research by enabling unprecedented precision in genetic manipulation [34]. These sophisticated molecular tools now allow researchers to strategically correct pathogenic mutations, generate isogenic disease models with remarkable accuracy, and implement advanced reporter gene systems for real-time cellular differentiation tracking. By integrating CRISPR methodologies with three-dimensional organoid culture systems, scientists can now model human tissue development and pathological processes with a level of molecular fidelity previously unimaginable in biomedical research [16, 74, 75].

6.4.4. 3D[1] Organoid Technology

3D organoid systems generated from embryonic stem cells have emerged as transformative models that substantially more accurately replicate the complex architectural, functional, and molecular characteristics of human tissue microenvironments compared to conventional two-dimensional culture approaches. These sophisticated in vitro constructs provide unprecedented opportunities for investigating intricate biological phenomena, including neurodevelopmental pathologies, progressive neurological disorders, oncogenic processes, host-microorganism interactions, and personalized pharmacological screening [76]. By strategically integrating patient-specific organoid models with precise genome editing technologies like CRISPR, researchers can now develop increasingly nuanced, individualized approaches to understanding disease mechanisms and potential therapeutic interventions [76-79].

[1]. Three-dimensional

6.4.4. Xeno-Free and Defined Media

The advancement in the development of chemically defined, animal component-free (xeno-free) culture media represents a significant milestone in regenerative medicine and stem cell research [14]. By eliminating animal-derived ingredients, these media formulations substantially decrease variability between batches, thereby enhancing the consistency and reliability of laboratory results [61]. Moreover, they substantially mitigate the risk of contamination with zoonotic pathogens or other adventitious agents, which is critical for ensuring the safety of cell-based products intended for human use. Additionally, the reduction of immunogenic substances present in animal-derived components diminishes the likelihood of immune rejection upon transplantation, further improving the safety profile of ESC-derived therapeutic products [80]. These improvements not only advance the robustness and reproducibility of experimental outcomes but also support compliance with stringent regulatory standards imposed by health authorities. Consequently, such progress facilitates the translation of stem cell technologies from the research laboratory into clinical settings, ultimately accelerating the development of safe, standardized, and effective cell-based therapies for patients [14, 16].

6.4.5. Scale-Up and Bioprocessing

Recent innovations in large-scale culture technologies, particularly the refinement of suspension culture systems and the design of advanced bioreactors, have significantly enhanced the ability to produce ESCs and their differentiated progeny at scales sufficient for clinical and therapeutic applications. These technological advancements not only support the robust expansion of ESCs while maintaining their fundamental pluripotent characteristics but also facilitate precise and reproducible differentiation

into specialized cell types needed for regenerative medicine [81, 82]. The development of scalable bioprocessing platforms addresses critical challenges such as maintaining cell quality, viability, and functionality throughout the manufacturing process, which are essential for meeting stringent regulatory standards for clinical-grade products. Moreover, these improvements play a pivotal role in overcoming the bottlenecks associated with translating laboratory-scale protocols into industrial-scale production, thereby enabling the cost-effective manufacture of ESC-based therapies. As a result, such progress is indispensable for the successful commercialization and broad clinical adoption of ESC-derived treatments, ultimately advancing the promise of regenerative medicine to benefit patients suffering from a wide range of degenerative diseases and injuries [16].

7. Conclusion

In the future, both hESCs and iPSCs are anticipated to serve complementary functions. hESCs continue to be the "gold standard" for developmental research and as a reference point for pluripotency studies [7], whereas iPSCs offer patient-specific, autologous models. Increasing the genetic diversity of newly developed hESC lines could pave the way for personalized treatments, particularly for underrepresented ethnic groups. Concurrently, genome editing technologies like CRISPR/Cas9 facilitate the correction of disease alleles and the addition of "safety switches" to improve therapeutic applications [16]. The combination of hESCs with 3D organoid models provides human systems that are physiologically relevant for drug testing, toxicology, and regenerative medicine research. Collectively, these strategies continue to advance stem cell science toward safe and effective clinical applications.

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