Chapter 10

Embryonic Stem Cells in Cardiac Tissue Regeneration; Concept and Application

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Abstract

Due to their unique pluripotent properties, ESCs[1] are a crucial component of regenerative therapy. ESCs, which are derived from the inner cell mass of blastocysts, can develop into any cell, providing unheard-of possibilities for organ regeneration and tissue repair. With a focus on myocardial regeneration, this review examines the theoretical underpinnings and therapeutic uses of ESCs. Cardiovascular disorders, especially myocardial infarction, are a leading cause of morbidity and mortality globally and result in the irreparable loss of cardiomyocytes. A promising approach to cardiac tissue engineering is provided by ESC-derived cardiomyocytes, which allow injured myocardium to be replaced and heart function to be restored. Significant obstacles still exist, nevertheless, such as immunogenicity, teratoma development risk, and host tissue integration. These restrictions are being addressed by ongoing research using techniques including genome editing, scaffold-based delivery systems, and guided differentiation. Finally, ESC-based cardiac therapies have the potential to completely transform the way heart disease is treated and represent a new frontier in customized regenerative medicine.

Keywords: Embryonic Stem Cells; Regenerative medicine; Myocardial disease

1. Introduction

ESCs are undifferentiated pluripotent cells that are produced from the inner cell mass of a blastocyst in early-stage embryos and have an extraordinary capacity to differentiate into any type of cell found in the body [1]. Since their discovery in the 1980s, ESCs have emerged as a key

[1]. Embryonic stem cells

component of developmental biology and regenerative medicine because of their capacity to treat genetic abnormalities, replace or repair damaged tissue, and further our knowledge of human development. These cells can self-renew (the process by which stem cells divide to produce more stem cells with the same capabilities as the parent cell) and differentiate into specialized cells like neurons, cardiomyocytes, and hepatocytes (**Figure 1**). Research on ESCs sits at the nexus of several scientific domains and has the potential to significantly advance our knowledge of development, transform medicine, and answer challenging biological issues. The impact of ESC research on developmental biology, regenerative medicine, and therapeutic applications provides insight into the field's larger context. Repairing, replacing, or regenerating damaged tissues and organs is the goal of regenerative medicine. Because of their pluripotency, ESCs are regarded as a fundamental component of this discipline [1, 2]. In developmental biology, ESCs research has been helpful in understanding the mechanics of early human development and can be used as a model to investigate processes such as embryonic development, gene regulation, and cell differentiation. Cell treatment, drug testing, toxicity screening, and hereditary disorders such as Parkinson's disease, type 1 diabetes, spinal cord injuries, and myocardial infarction are important therapeutic applications of ESCs (**Figure 2**). The goal of regenerative medicine, a groundbreaking area of biomedical research, is to replace or repair damaged tissues and organs by either using created biological substitutes or the body's natural repair processes. The use of pluripotent stem cells, especially ESCs, which have the rare capacity to develop into every type of adult cell and demonstrate unlimited self-renewal, is essential to this research [3]. Therefore, it is essential to investigate the function of ESCs in the field of regenerative medicine, paying particular

attention to their distinct pluripotent traits, therapeutic potential in organ regeneration and tissue repair, and the contemporary ethical and scientific issues that affect their clinical use [4].

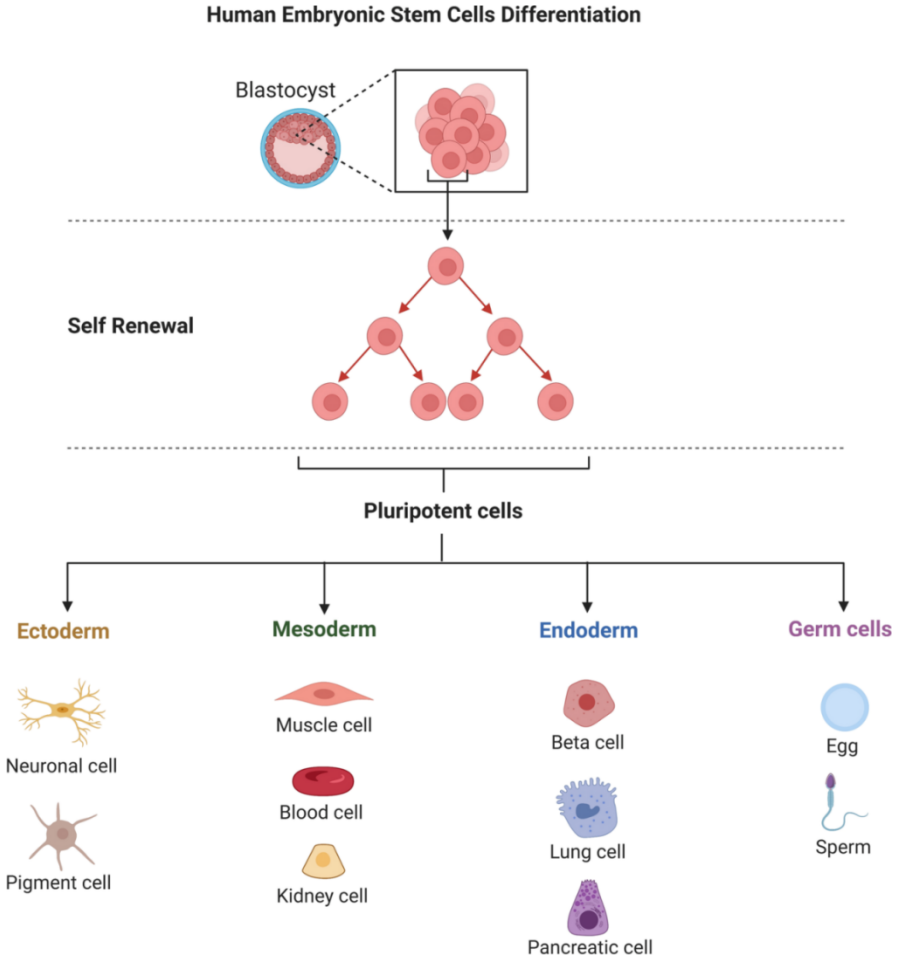

Figure 1. Human ESC differentiation. This illustration depicts how human ESCs with self-renewal and differentiation capacity can mature into various cell types, including myocytes, blood cells, and neuronal cells, highlighting the eligibility of these cells in human regenerative medicine, drug testing, disease modeling, and various therapeutics. Reproduced with permission [5]. Stem Cell Research & Therapy. 2025.

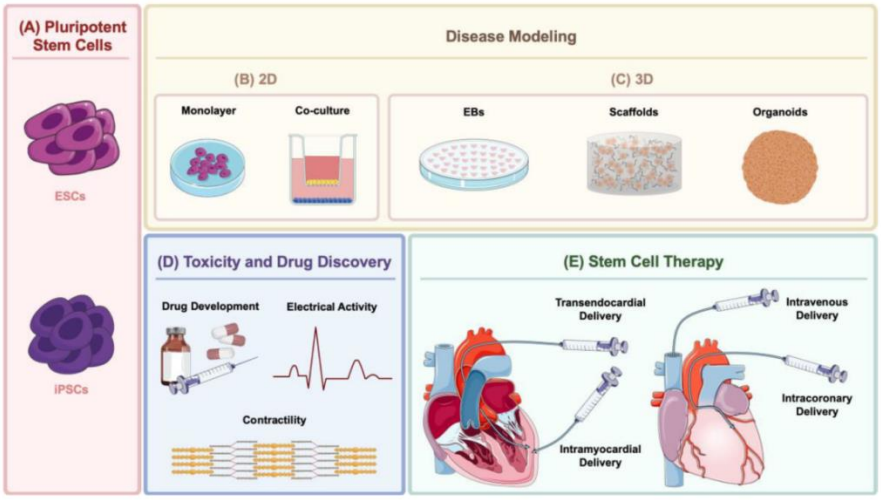

Figure 2. Application of stem cells in disease modeling, toxicity studies, drug discovery, and cardiovascular diseases. Reproduced with permission. [6]. Cells. 2023.

2. Application of ESCs in toxicological studies

Accurately predicting toxic reactions in humans is still a major difficulty in toxicology, which affects both chemical safety evaluation and pharmaceutical development. In vitro cell-based tests and in vivo animal models have long been the mainstays of traditional toxicological assessment. However, the creation of more human-relevant and morally acceptable testing systems has become necessary due to ethical concerns around animal experimentation and interspecies differences that limit the translatability of animal data to people [7]. ESCs are a possible substitute because of their special biological characteristics, which make them ideal for toxicological applications. The ability of ESCs to self-renew indefinitely and develop into any of the three germ layers' cell types—ectoderm, mesoderm, and endoderm—is two of its distinguishing traits. These characteristics make ESCs an excellent platform for simulating early developmental processes and evaluating the potential effects of

hazardous chemicals on cellular survival, proliferation, or differentiation. These characteristics are especially helpful in developmental and reproductive toxicology, where it's crucial to comprehend how toxicants affect early embryogenesis. The use of ESCs in toxicological research is in line with the NAMs[1] paradigm shift, which aims to decrease or eliminate animal testing while increasing the effectiveness and relevance of safety assessments. Because ESCs may be utilized to develop high-throughput, mechanistically informative experiments that are more representative of human biology, their usage is particularly beneficial in this situation. Furthermore, human ESC lines offer a special chance to simulate developmental pathways unique to humans, overcoming the drawbacks of cross-species extrapolation. Over the past 20 years, ESCs have found several important uses in toxicology [8]. The EST[2], one of the most well-known methods, was created to determine a compound's potential to harm embryos by analyzing how it affects the differentiation of murine ESCs into beating cardiomyocytes. Although the EST was initially developed using mouse ESCs, efforts have been made to adapt it to human ESCs to improve its predictive relevance. In parallel, ESC-derived models have been used to study organ-specific toxicity, such as hepatotoxicity, neurotoxicity, and cardiotoxicity, by instructing ESCs to differentiate into lineage-specific cells that recapitulate key features of their in vivo counterparts. This assay combines endpoints for cytotoxicity and differentiation, providing a comprehensive measure of developmental toxicity [9]. Furthermore, more intricate 3D[3] culture methods and organoid models made from ESCs have been made possible by developments in

[1]. New Approach Methodology
[2]. Embryonic Stem Cells Test
[3] three-dimensional

stem cell biology and bioengineering. Compared to conventional two-dimensional cultures, these systems provide better physiological relevance, allowing for a more precise evaluation of the effects of toxicants on tissue architecture, cellular connections, and metabolic function. ESC-based toxicological investigations have made use of transcriptome, proteomic, and biomarkers linked to toxicant exposure at the molecular level [10]. These omics techniques offer a system-level comprehension of how chemical agents affect developmental programs and gene regulatory networks. Finding AOPs[1] and strengthening the mechanistic underpinnings of predictive toxicology requires such data. Furthermore, regulatory toxicology and chemical risk assessment frameworks have benefited from the usefulness of ESC-based assays in screening vast chemical libraries for harmful effects. The use of ESCs in toxicology has numerous benefits, but there are drawbacks as well. One significant problem is the moral debate over using human ESCs, which has led to the creation of substitute pluripotent models like iPSCs[2] [11]. Although iPSCs and ESCs have many characteristics in common, such as pluripotency and self-renewal, they may differ in their ability to differentiate or in their epigenetic memory, which could limit their use in toxicological research. The standardization and repeatability of ESC-based assays present another technical difficulty. To guarantee uniformity across labs, meticulous control of culture conditions, differentiation procedures, and test outcomes is necessary. Moreover, ESC-derived models may not accurately replicate the intricacy of in vivo systems, especially when it comes to metabolism, immunological responses, and systemic interactions, even while they provide a human-relevant substitute for

[1]. Adverse outcome pathways
[2] induced pluripotent stem cells

animal research [12]. In order to overcome these drawbacks, attempts are being made to combine ESC-based models with other NAMs, like computational modeling and microphysiological systems, to produce more thorough and accurate testing platforms. These combined methods have a lot of potential to decrease the need for animal testing while increasing the precision and effectiveness of toxicological evaluations. To sum up, ESCs are a revolutionary instrument in toxicological research that offers a biologically significant, mechanistically instructive, and morally forward-thinking approach [13].

3. Potency of ESCs in differentiation into vascular cells

The two distinguishing traits of ESCs, which are produced from the inner cell mass of blastocyst-stage embryos, are pluripotency and self-renewal. Because of these special characteristics, ESCs can continue to divide into derivatives of the ectoderm, mesoderm, and endoderm layers of the embryonic germ layer indefinitely under the right in vitro conditions (**Figure 3**). The ability of ESCs to differentiate into vascular cells, specifically ECs[1] and VSMCs[2], is one of the many possible differentiation pathways [14]. This holds great promise for tissue engineering, regenerative medicine, and the treatment of ischemic and vascular diseases. In addition to reflecting an important component of embryonic biology, ESCs' ability to aid in the production of functional vasculature offers a convincing method for restoring vascular integrity in diseased conditions. The movement of oxygen, nutrients, metabolic waste, and signaling chemicals is made easier by the vasculature, an important organ system that maintains homeostasis. Endothelial cells, which make up the

[1]. Endothelial cells
[2]. Vascular smooth muscle cells

inner lining of blood vessels, are essential for controlling vascular tone, coagulation, inflammation, and permeability. Vascular smooth muscle cells, which surround the endothelium layer, control vessel contractility and offer structural support. Many clinical diseases, such as peripheral artery disease, diabetes mellitus, hypertension, and atherosclerosis, are characterized by the loss or dysfunction of various vascular cell types. With the potential for both structural and functional vascular regeneration in addition to symptomatic treatment, stem cell-based therapies represent a paradigm change in this regard [15]. One of ESCs' main advantages over adult stem cells, like MSCs[1] or iPSCs[2], is their strong differentiation potential and genetic stability. ESCs offer a more stable and widely distributed source of progenitors, whereas MSCs have limited lineage potential, and iPSCs may have genetic and epigenetic defects resulting from reprogramming. Notably, several techniques that replicate embryonic development by sequentially exposing ESCs to growth factors like BMP4, VEGF, and FGF2 have been used to drive differentiation of ESCs into vascular cell lineages. Mesodermal identity is frequently first induced in these protocols, and then endothelial or smooth muscle fates are specified [16]. Numerous investigations conducted both in vitro and in vivo have confirmed that ESC-derived vascular cells may integrate into host vasculature and aid in neovascularization. ESC-derived endothelial progenitor cells, for example, have been demonstrated to contribute to vascular healing after ischemia injury in animal models and to generate functional capillary-like structures in Matrigel experiments. Likewise, VSMCs produced from ESC have shown contractile qualities and the capacity to stabilize immature vasculature. Crucially, co-transplanting

[1]. Mesenchymal stem cells
[2]. Induced pluripotent stem cells

ESC-derived ECs and VSMCs has produced better results in engineered tissues and vascular grafts, demonstrating the importance of both cell types for effective vascular regeneration. Despite these developments, there are still obstacles in the way of fully utilizing ESCs for vascular regeneration. Heterogeneous populations with differing levels of maturity and function are frequently produced using differentiation methods. To reduce the chance of teratoma formation and unfavorable immunological responses, it is essential to confirm the identity and purity of vascular progenitors [17]. For clinical use, ESC-derived vascular cell production's scalability and repeatability also need to be maximized. To manage the moral aspects of stem cell research, scientists, ethicists, and legislators must continue to discuss ethical issues about the use of human ESCs. The molecular processes controlling ESC differentiation into vascular lineages have been clarified by recent developments in single-cell transcriptomics, epigenetic profiling, and lineage tracing. While the myocardium and SRF coordinate the differentiation of smooth muscles, important transcription factors like ETV2, SOX17, and NOTCH signaling components, have become key regulators of endothelial specification. Refining differentiation techniques and improving the fidelity of ESC-derived vascular cells requires an understanding of the temporal dynamics and signaling networks involved in vascular development. Furthermore, bioengineering techniques such as bioreactor culture, three-dimensional scaffolds, and microfluidic systems are being used to enhance the maturation and functionality of ESC-derived vascular cells by simulating the vascular niche. All things considered, embryonic stem cells are a powerful and adaptable source of vascular cell production, with significant ramifications for vascular biology and regenerative medicine. Researchers can fully utilize these cells for medicinal purposes by clarifying the processes of

ESC differentiation and refining differentiation methods. Transforming laboratory results into clinical reality will require interdisciplinary cooperation between stem cell scientists, bioengineers, doctors, and regulatory bodies as the field develops [18].

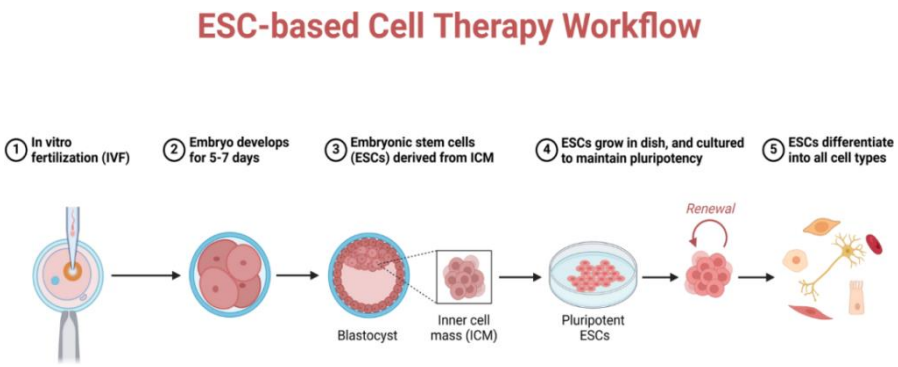

Figure 3. Developing ESC-based therapies. This illustration outlines the isolation of ESCs, conducting quality control, differentiating into specific cell lineages, and transplanting them into patients. It was demonstrated that ESCs can alleviate degenerative diseases via the regeneration of damaged cells, while addressing critical issues such as safety, immune rejection, and treatment effectiveness. Reproduced with permission. [5]. Stem Cell Research & Therapy. 2025.

4. Application of ESCs in ischemic heart disease

The world's top cause of morbidity and mortality is still IHD[1], also known as coronary artery disease. If left untreated, IHD, which is characterized by decreased blood flow to the myocardium as a result of coronary artery blockage or constriction, can result in myocardial ischemia, infarction, and eventually heart failure. IHD has a huge worldwide health burden, as seen by the estimated 9 million deaths it causes each year, according to the World Health Organization. None of the available treatments can completely regenerate the damaged myocardium or restore the heart's contractile function following an infarction, despite notable advancements

[1]. Ischemic heart disease

in pharmacological therapy, percutaneous coronary procedures, and coronary artery bypass grafting [19]. As a result, interest in stem cell-based treatments for myocardial repair has increased due to the need for novel regenerative techniques. ESCs are pluripotent cells that can differentiate into any somatic cell type, including cardiomyocytes, endothelial cells, and vascular smooth muscle cells. They are derived from the inner cell mass of pre-implantation blastocysts. Because of their proliferative potential and intrinsic adaptability, ESCs are a very attractive cell source for heart regeneration (**Figure 4**). ESCs have the potential to produce fully functional, contractile cardiomyocytes and vascular elements that could structurally and functionally replace infarcted tissue, in contrast to adult stem cells like MSCs[1] or bone marrow-derived mononuclear cells, which have demonstrated limited regenerative efficacy in clinical trials because of their limited differentiation potential and poor engraftment [20]. The use of ESCs in ischemic heart disease includes a number of therapeutic objectives, including enhancing overall cardiac function, stimulating neovascularization to restore perfusion, replacing damaged cardiomyocytes, and regulating the inflammatory milieu. ESC-derived CM[2] (ESC-CMs) have been shown in preclinical experiments in both small and large animal models to be able to engraft into damaged myocardium, create gap junctions with host cardiomyocytes, and help to enhance the left ventricular ejection fraction. It has also been demonstrated that vascular progenitors and endothelial cells produced from ESC contribute to neovascularization and prevent detrimental remodeling. These results demonstrate the diverse reparative potential of ESCs in the context of ischemic myocardium [14]. However, there have been significant obstacles

[1]. mesenchymal stromal cells
[2]. Cardiomyocytes

in putting ESC-based treatments into clinical practice. Because ESCs are pluripotent, one of the main worries is the possibility of teratoma formation, particularly when undifferentiated cells are implanted. Therefore, before transplantation, strict procedures are needed to guide ESCs into particular lineages, especially cardiomyocytes. In order to replicate embryonic cardiogenesis in vitro, directed differentiation protocols have advanced significantly in recent years. These protocols use a variety of signaling molecules, including Activin A, BMP4[1], and Wnt[2]modulators (**Figure 5**). Beyond differentiation, the integration and survival of transplanted cells remain major hurdles. The ischemic microenvironment—marked by inflammation, hypoxia, and oxidative stress—impairs cell retention and viability. Numerous approaches, such as scaffold-based tissue engineering, pro-survival cocktails, and preconditioning cells before transplantation, have been investigated to solve this. Moreover, ESC-derived cells must be supplied in a manner that promotes mechanical and electrical interaction with host myocardium to minimize arrhythmogenic consequences. Tissue engineering techniques, like the use of ESC-CMs to create cardiac patches, offer a promising way to enhance cell alignment and connectivity [21]. Immunogenicity also provides a considerable hurdle to clinical translation. After transplantation, human ESCs, which are produced from genetically different embryos, may trigger immunological reactions that demand immunosuppression or the creation of hypoimmunogenic cell lines. The development of universal donor ESC lines with decreased MHC[3] expression has been made easier by developments in genome editing methods, such as CRISPR/Cas9. As an

[1]. Bone morphogenetic protein 4
[2]. Wingless-related integration site
[3]. Major histocompatibility complex

alternative, patient-specific iPSCs provide a customized strategy, although they have drawbacks of their own, including epigenetic memory and mutations linked to reprogramming [22]. The use of human embryos is still controversial because of ethical concerns, which have resulted in regulatory limitations in many nations. Although hESCs are still considered the "gold standard" for pluripotent stem cell research because of their stability, reproducibility, and well-characterized differentiation potential, these worries have prompted the creation of alternate pluripotent sources, such as iPSCs. To guarantee responsible and open progress in this area, scientists, ethicists, and regulatory agencies must have constant communication. Despite the difficulties, there has been continuous progress in the direction of clinical application. A crucial step in applying preclinical results to human patients was taken in 2015 when the French biotechnology company Cellectis launched the first clinical trial using hESC-derived cardiac progenitor cells (the ESCORT trial). Although the trial's scope and duration were constrained, it showed that transplanting ESC-derived cardiac cells into patients suffering from severe heart failure was feasible and, thus far, safe. More sophisticated trials that take into account advancements in cell production, distribution, and safety monitoring have been made possible by these preliminary results [23]. In conclusion, by facilitating genuine myocardial regeneration, embryonic stem cells present a hitherto unheard-of chance to overcome the basic drawbacks of existing treatments for ischemic heart disease. Even though there are still many ethical, technical, and scientific obstacles to overcome, developments in immunomodulation, bioengineering, and stem cell biology are quickly coming together to make ESC-based treatments a feasible choice soon. by combining the body of knowledge already in existence and determining important topics for further study [24].

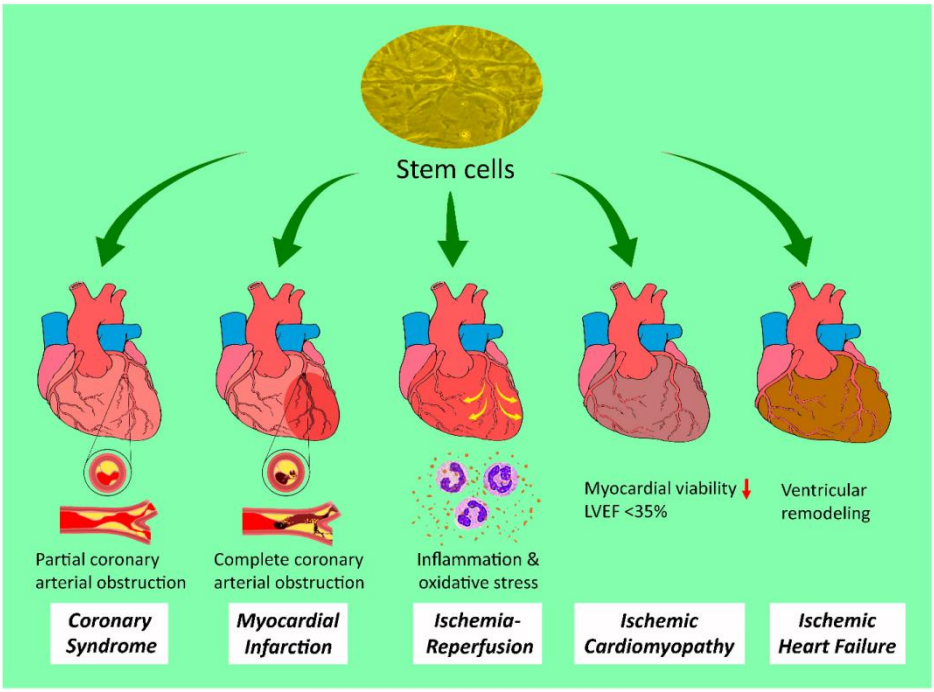

Figure 4. Therapeutic potential of stem cells against a spectrum of IHDs. Reproduced with permission. [25]. Journal of Molecular Sciences. 2024

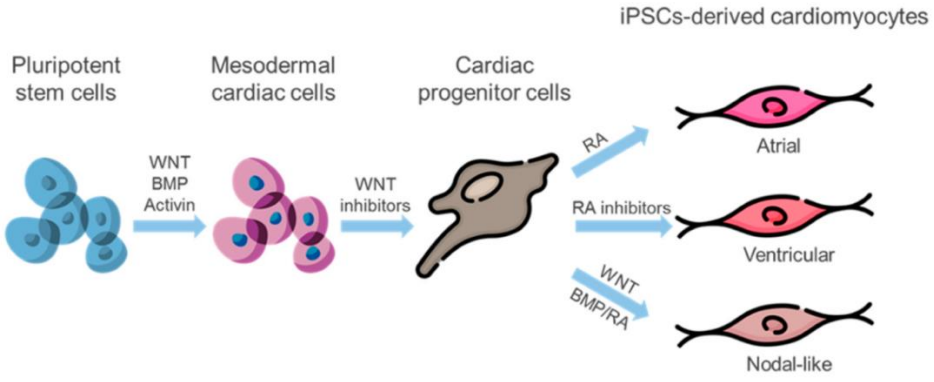

Figure 5. The orientation of pluripotent stem cells into different cardiomyocytes. For this purpose, stem cells should first be guided toward cardiac mesoderm by activating Wnt and BMP signaling pathways. Using Wnt inhibitors, it is possible to commit mesodermal cells

to cardiac progenitors. The fate of progenitors is tuned atrial cells in the presence of RA[1]. The inhibition of RA promotes ventricular cell orientation of CPCs[2]. The combined activity of RA and BMP can dictate progenitors into becoming nodal-like pacemaker cells, a process also aided by Wnt signaling. Reproduced with permission [26]. Journal of Molecular Sciences. 2023.

5. Clinical trials

With previously unheard-of potential for replacing, repairing, or regenerating human tissues and organs, the subject of regenerative medicine has become a revolutionary area within the biomedical sciences [5]. ESCs, which are pluripotent cells produced from the inner cell mass of blastocysts and have the rare capacity to develop into all cell types of the three germ layers, are at the vanguard of this progress. This inherent plasticity renders ESCs an exceptionally promising candidate for the treatment of a wide range of degenerative conditions, including but not limited to neurodegenerative disorders, cardiovascular diseases, diabetes, and spinal cord injuries in different animal models (**Table 1**). Despite their potential, the transition of ESC-based therapies from bench to bedside is complex and tightly regulated, particularly when involving human clinical trials. Clinical translation requires rigorous evaluations of safety, ethical acceptability, and long-term functionality in addition to proven efficacy and reproducibility [27]. As a result, preclinical research and the changing clinical trial environment using ESC-derived products have been the subject of an expanding corpus of literature, with a focus on therapeutic outcomes, dangers, and technical difficulties. Since their initial isolation in mice in 1981 and later in humans in 1998, ESCs have been the subject of extensive research that has clarified their molecular properties, signaling pathways, and capacity for directed differentiation. Unlike adult stem cells,

[1]. Retinoic acid
[2]. Cardiac progenitor cells

ESCs are not limited by lineage or have a reduced capacity for proliferation, which makes them ideal for large-scale cell replacement therapies [28]. Nevertheless, this same adaptability comes with hazards, including immunogenicity, tumorigenicity, and moral dilemmas related to embryo destruction—factors that still affect public policy and clinical trial design. Clinically speaking, there was initial interest in advancing ESC-based treatments into human trials in the early 2000s. Notably, the first human clinical trial employing ESC-derived oligodendrocyte progenitor cells to treat spinal cord damage was authorized by the U.S. FDA[1] in 2010 and was carried out by Geron Corporation. The trial set the stage for further research, even if it was eventually stopped for strategic and budgetary reasons rather than a lack of scientific evidence. Since then, some of the most significant and long-lasting research in this area has been conducted on ESC-derived RPE[2] cells for Stargardt's disease and age-related macular degeneration. ESC-based therapies have gradually increased in the global register of clinical trials, primarily in the fields of neurology and ophthalmology [29]. For example, studies by Advanced Cell Technology (now a division of Astellas Pharma) showed that injecting ESC-derived RPE cells was feasible and initially safe. These initiatives highlight the growing interest around the world in using ESCs for therapeutic purposes, but they also highlight significant differences in trial design and regulatory rigor between nations [30]. The creation of uniform procedures for cell derivation, differentiation, and quality control is a significant obstacle in ESC-based clinical trials. Consistent therapeutic results are severely hampered by the diversity of stem cell populations, variations in differentiation efficiency, and the possibility of remnant

1. Food and Drug Administration
2. Retinal pigment epithelium

undifferentiated cells. Furthermore, nothing is known about the long-term destiny of transplanted ESC derivatives, which makes post-trial surveillance and patient monitoring necessary. With continuous attempts to create hypoimmunogenic ESC lines using gene-editing technologies like CRISPR[1]-Cas9, immunological compatibility is still a major concern. Clinical application is made more difficult by ethical constraints [31]. The use of human embryos for ESC derivation has generated discussions over the embryo's moral standing, which has resulted in differing national regulations. While some countries strictly regulate the production and use of human embryonic stem cells, others completely forbid it or place significant limitations on it. The hunt for alternate sources of pluripotent cells, iPSCs, which have many traits in common with ESCs but do not require the loss of embryos, has been indirectly spurred by these ethical restrictions [32]. Nevertheless, ESCs continue to be the gold standard for pluripotency and are essential for both fundamental and applied research. With an emphasis on their philosophical underpinnings, therapeutic justifications, trial designs, reported results, and enduring difficulties, the current paper aims to examine and critically analyze the state of clinical studies employing embryonic stem cells. We seek to offer light on the practical use of ESCs in regenerative medicine by analyzing published data, registered trial procedures, and regulatory environments. In addition to listing recent advancements, we also aim to evaluate translational barriers and suggest future research and clinical innovation avenues. This article aims to close the gap between clinical reality and theoretical potential [33]. Even though scientists and medical professionals have long been fascinated by embryonic stem cells, their integration into mainstream medicine is still in its early stages. In addition to promoting regenerative

[1]. Clustered regularly interspaced palindromic repeats

medicine, it is crucial to comprehend the complexities of clinical trials—what has been accomplished, what is still elusive, and what lies ahead. This knowledge also informs ethical discourse, directs public policy, and establishes reasonable expectations for patients and stakeholders [3].

Table 1. Some studies related to the application of ESCs in animals and humans with cardiovascular diseases

Ref	*Model/Disease*	*Cell Type*	*Delivery Route*	*Main Findings*	*Significance*	*Signaling*
Min et al., 2002 [34]	Rat MI model	Undifferentiated mouse ESCs	Intramyocardial injection post-MI	Left ventricular function improved; ESCs differentiated into cells expressing α-actin and troponin I.	The first experimental evidence showing ESCs could regenerate damaged myocardium.	Spontaneous differentiation; markers: α-actin, troponin I.
Laflamme et al., 2005 [35]	Rat MI model	Human ESC-derived cardiac progenitors	Direct intramyocardial injection	Grafts formed human myocardium in rat hearts and maintained proliferation and survival.	First in vivo confirmation that human ESCs can generate cardiac tissue.	Signaling: undefined cardiac induction; markers: α-actinin, Nkx2.5, cardiac troponin T.
Swijnenburg et al., 2005 [36]	Mouse MI model (syngeneic vs allogeneic)	Mouse ESCs	Intramyocardial injection	ESC grafts rejected in non-matched hosts; differentiation increased immunogenicity.	Highlighted immune-related challenges in ESC transplantation.	No directed signaling; immune markers: MHC-I, MHC-II.
Ménard et al., 2005 [37]	Sheep MI model (large animal)	Mouse ESC-derived cardiac-committed cells	Intramyocardial injection	Improved LV ejection fraction; no tumor formation detected.	First large-animal evidence supporting ESC-based heart repair.	Cardiac specification via BMP2 signaling; markers: α-actinin, connexin-43.
Laflamme et al., 2007 [38]	Rat MI model	hESC-derived cardiomyocytes (Activin A + BMP4)	Injection with pro-survival agents	Enhanced heart function and tissue recovery; increased survival of transplanted cells.	Landmark proof of functional benefit and improved cell viability.	Signaling: Activin A, BMP4, Wnt inhibition; markers: α-actinin, Nkx2.5, troponin T.

Caspi et al., 2007 [39]	Rat MI model	hESC-derived cardiomyocytes	Intramyocardial injection	Improved cardiac function and electrical integration; undifferentiated ESCs caused teratomas.	Established the necessity for pre-differentiation before cell transplantation.	Markers: connexin-43, troponin I, α-actinin.
Tomescot et al., 2007 [40]	Rat MI model	hESCs pre-specified to cardiac lineage (BMP2 + SU5402)	Injection two weeks post-MI	Long-term graft survival (~2 months) with functional cardiac differentiation and no tumor formation.	Demonstrated maturation and safety of lineage-specified hESCs.	Signaling: BMP2 induction + FGF inhibition (SU5402); markers: α-actinin, GATA4, troponin T.
Ye et al., 2012 [41]	Mouse MI model	hESC-derived cardiomyocytes via p38MAPK inhibition	Ultrasound-guided intramyocardial injection	Improved LVEF (≈39% vs 28% control), reduced scar tissue, and increased angiogenesis.	Validated a safer, more efficient differentiation approach for cardiac repair.	Signaling: p38MAPK inhibition; markers: α-actinin, troponin I, connexin-43.
Menasché et al., 2015a [42]	Translational GMP program	hESC-derived cardiac progenitors (SSEA-1^{+} / ISL-1^{+})	GMP-grade fibrin scaffold	The product satisfied safety and quality control criteria for clinical translation.	First GMP-certified hESC-derived cardiac progenitor product.	Signaling: Wnt/BMP balance; markers: SSEA-1, ISL1, NKX2.5.
Menasché et al., 2015b [43]	Human clinical case (severe ischemic heart failure)	hESC-derived cardiac progenitors on a fibrin patch	Surgical implantation during CABG	Clinical improvement from NYHA class III to I; LVEF increased from 26% to 36%; no arrhythmias or tumors observed.	Documented the first successful clinical case using hESC-derived cells for cardiac repair.	Markers: ISL1, α-actinin; fibrin scaffold ensured stable engraftment.
Bellamy et al., 2015 [44]	Rat MI model (immunosuppressed)	hESC-derived cardiac progenitors on a fibrin scaffold	Intramyocardial or epicardial implantation (4-month follow-up)	Long-term improvement in ejection fraction and ventricular structure with limited cell retention.	Provided strong preclinical evidence supporting the ESCORT human trial.	Signaling: BMP activation + Wnt inhibition; markers: cardiac troponin T, ISL1.

Menasché et al., 2018 (ESCORT Phase I) [45]	Patients with severe LV dysfunction undergoing CABG	hESC-derived cardiovascular progenitors in a fibrin patch	Epicardial implantation during surgery	Confirmed the feasibility, large-scale production, and clinical safety of hESC-derived progenitors; improved segmental systolic motion without tumorigenesis or arrhythmia.	First-in-human Phase I clinical trial validating safety, feasibility, and functional recovery in ischemic heart failure.	Signaling: BMP and Wnt pathway modulation; markers: ISL1, NKX2.5, α-actinin, troponin T.
HECTOR Trial (ongoing) [46]	Humans with chronic ischemic heart failure	hESC-derived cardiomyocytes (hESC-CMs)	Direct myocardial injection	Designed to evaluate safety, feasibility, and initial clinical outcomes of hESC-CM transplantation.	Represents an ongoing next-generation human clinical trial using hESC-derived cardiomyocytes.	Signaling: Wnt inhibition, BMP activation; markers: cardiac troponin T, connexin-43.

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