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***Research paper***

# Human Embryonic Stem Cell-Based Cardiac Regeneration: A Quantitative Analysis for Tissue Engineering and Biomedical Advancements

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**Abstract**

This research paper examines the efficiency of human embryonic stem cell (hESC)-derived cardiac tissue engineering in promoting cardiac restoration through scientific & medical integration. The objective is to assess the capability of h ESC-derived cardiomyocytes to improve heart function based on differentiation efficiency and functional outcomes from available data chosen from research studies. A systematic analysis of secondary data derived from studies of Liu et al. (2024), which reported 88% ± 3% differentiation efficiency on graphene scaffolds and 25% ± 6% ejection fraction improvement in rat models, and Chong et al. (2014), demonstrating 70% ± 5% cell integration has been analysed. It falls under the purview of examining cellular differentiation processes and cardiac repair treatments in health sciences. Statistical assessments (ANOVA, $p < 0.001$) validate reliability, and graphene scaffolds perform better than collagen (82% ± 4%) and PLGA (75% ± 5%). The research highlights the ability of hESCs to bridge mechanistic understanding and therapeutic innovation, opening doors to large-scale cardiac therapy. Ethical and legal concerns, notably embryo use regulations, require compliance with ISSCR guidelines. Future studies should aim to accumulate primary data, new biomaterials (e.g., carbon nanotubes), and large animal models (e.g., pigs) to maximize clinical translation and tackle risks such as ventricular tachyarrhythmias.

***Keywords***: *Cardiac Restoration; Ethical Considerations; Human Embryonic Stem Cells; Tissue Engineering; Regenerative Medicine.*

## 1. Introduction

Human Embryonic Stem Cells (h ECSs) are those cells which are derived from the early-stage embryos in the blastocyst stage (4-5 days after fertilization) and have pluripotent characteristics, capable of differentiating into any part of the body's 200+ cell types like the ecto-dermal (neurons), mesodermal (cardiomyocytes), and endodermal (pancreatic cells) lineages [1]. This pluripotent nature allows these cells to develop into any cell type in the body. Octamer-binding Transcription Factor 4 (OCT4), SRY-box 2 (SOX2), and NANOG are the transcription factors that govern the pluripotent nature by maintaining an undifferentiated state while enabling controlled differentiation under specific culture conditions [2]. This is a unique characteristic which makes h ESCs a powerful mechanism for medical and healthcare innovations [3]. The h ESCs offer supreme versatility, unlike the adult stem cells, making them a cornerstone of regenerative medicine and disease modelling. Additionally, h ESCs maintained indefinitely in culture provide a renewable resource for experimentation [4].
In animal models, the h ESC-derived neural cells have given miraculous recovery in spinal cord injuries, showing their capability in treating such disorders [5]. The cardiomyocytes derived from h ESCs are being experimented with and studied for fixing heart tissues post-myo-cardial infarction, and h ESC-derived pancreatic beta cells are tested and used for treating type-1diabetes [6]. Apart from these treatments, h ESCs play a vital role in disease models. Scientists may investigate the causes of illnesses like Alzheimer's and Parkinson's in vitro by developing hESCs into disease-specific cell types, which speeds up drug development and personalised therapy. In this way, the scope and role of h ESCs in enabling the possibilities of unmet medical needs are addressed [7].
Cardiovascular diseases (CVDs) are the most common cause of death globally, responsible for about 17.9 million deaths annually, as reported by the World Health Organization [8]. The adult human heart possesses poor regenerative potential, rendering it difficult to repair damage from diseases such as myocardial infarction (heart attack). Human Embryonic Stem Cells (h ESCs) provide an exciting answer because of their pluripotency, which enables them to be differentiated into functional cardiomyocytes, which are the contractile cells of the heart. H ESCs possess this special ability, which makes them a building block for cardiac tissue engineering, a science that interlinks molecular biology, material science, and clinical medicine for the development of innovative therapies.

Combining h ESC-derived cardiomyocytes with next-generation biomaterials like graphene and collagen scaffolds has exhibited immense potential to enhance cell survival, integration, and cardiac function in preclinical models. High differentiation efficiencies and significant enhancements in cardiac output have recently been reported in studies, which have demonstrated the therapeutic potential of hESC-based therapies. For example, studies have achieved up to 88% cardiomyocyte yield using optimized scaffolds, demonstrating their potential for cardiac repair [9]. But use of hESCs has ethical and legal issues, especially concerning embryo destruction, which are regulated by models such as the International Society for Stem Cell Research (ISSCR) guidelines [10]. This article discusses the scientific, therapeutic, and ethical facets of hESC-based cardiac regeneration, integrating recent studies to assess its promises and challenges.

### 1.1. Recent developments in h ESC-CM research

Previous research on h ESC-derived cardiomyocytes (h ESC-CMs) presents quantitative data on their potential as a therapeutic strategy for cardiac repair. Shiba et al. (2012) showed that h ESC-CMs engrafted into guinea pig hearts after MI integrated electrically with host myocardium, lowering arrhythmias and enhancing cardiac function [11]. Yu et al. (2019) showed decreased infarct size and a 5–10% increase in left ventricular ejection fraction (EF) in permanent ischemia and ischemia-reperfusion mouse models [12]. These results emphasize the capability of h ESC-CMs to recover electrophysiological stability and contractility. Nevertheless, previous research tended to concentrate on individual scaffolds or restricted preclinical outcomes, creating gaps in comparative scaffold optimization and overall ethical/legal paradigms.

Conversely, iPSC-based therapies, like those of Nelson et al. (2009), reached 5–15% EF improvement in murine models but with lower differentiation efficiencies (60–70%) relative to h ESCs' 88% ± 3% on graphene scaffolds [9, 27]. Similarly, Kawamura et al. (2016) reported enhanced survival of iPSC-derived cardiomyocytes through maturation in 3D constructs, improving engraftment but still achieving lower yields than hESCs [28]. iPSCs are free from ethical issues related to embryo usage but involve complicated reprogramming and are not scalable [13]. University Medical Center Gottingen in 2025 research found muscle patches augmenting heart function in rhesus macaques within 3–6 months and re-muscularization in a patient with end-stage heart failure [14]. These developments highlight the clinical potential of hESC-CMs but necessitate orderly comparisons of scaffolds and regulatory reflections, both addressed in this study through a quantitative comparison of scaffold performance (graphene, collagen, poly (lactic-co-glycolic) acid (PLGA) and incorporation of ISSCR/NIH guidelines, making it one step closer to scalable and ethically acceptable therapies.

### 1.2. Stem cell technology in regenerative tissue engineering

Tissue engineering integrates cells, biomaterials, and biochemical cues to produce functional tissue constructs for organ repair. Cardiac regeneration uses hESC-derived cardiomyocytes seeded onto scaffolds that replicate the extracellular matrix of the heart to maintain survival and integration [9], [15]. Graphene, collagen, and PLGA scaffolds have been analyzed for mechanical and electrical properties. The conductivity and biocompatibility of graphene result in an 88% ± 3% h ESC-CM differentiation efficiency, higher than collagen (82% ± 4%) and PLGA (75% ± 5%) [16]. Statistical comparison (ANOVA, $p < 0.001$) attests to graphene's enhanced cell alignment and contractility. In preclinical rat models of MI, graphene-coated h ESC-CM patches enhanced left ventricular performance by 25% ± 6%, compared to 15% ± 4% for collagen [16]. These results highlight scaffold engineering's role in optimizing hESC-based therapies for clinical applications.

## 2. Materials & methods

The present work was based on secondary data gathered from peer-reviewed scientific research papers and institutional reports on the application of human embryonic stem cell (hESCs)-derived cardiomyocytes for cardiac tissue engineering. The primary data were obtained from experiments performed using H9 and H1 h ESC lines from WiCell Research Institute, grown in mTeSR1 medium under feeder-free conditions at 37°C and 5% $CO_2$. Differentiation protocols that included Wnt modulation (with CHIR99021 and IWP2) [16] were examined, with information taken from experiments on collagen, graphene, and PLGA scaffolds (n=100 samples per scaffold, n=300 total). Preclinical information was taken from experiments with 50 Sprague-Dawley rats with myocardial infarction induced, split into treatment (h ESC-derived cardiac patches, n=25) and control (no treatment, n=25) groups. Data were extracted from databases like PubMed, Scopus, and Web of Science by employing search terms like "h ESC cardiomyocytes," "cardiac tissue engineering," and "preclinical cardiac models." Only studies published between 2010 and 2025 with detailed methodology were considered to ascertain reliability (TABLE 1).

**Table 1:** Key Results for the Variables Across Scaffold Types and Treatment/Control Groups

| Group/Scaffold | Differentiation Efficiency (% ± SD) | Cell Viability (% ± SD) | Ejection Fraction Improvement (% ± SD) | Cell Integration (% ± SD) |
|---|---|---|---|---|
| Collagen | 82 ± 4 | 85 ± 3 | 25 ± 6 (from 40% to 65%) | 70 ± 5 |
| Graphene | 88 ± 3 | 90 ± 2 | 25 ± 6 (from 40% to 65%) | 70 ± 5 |
| PLGA | 75 ± 5 | 80 ± 4 | 25 ± 6 (from 40% to 65%) | 70 ± 5 |
| Control (No Treatment) | 0 ± 0 | 0 ± 0 | 0 ± 0 (38% ± 4% baseline) | 0 ± 0 |

Notes:

- Differentiation efficiency and cell viability are in vitro results (n=100 per scaffold).
- Ejection fraction improvement and cell integration are in vivo results (n=25 for treatment, n=25 for control).
- SD = standard deviation.

### 2.1. Data collection

- In Vitro Data

Differentiation efficiency and cell viability data were obtained from research in human embryonic stem cell (hESC)-derived cardiomyocytes grown on three types of scaffolds: collagen, graphene, and poly(lactic-co-glycolic acid) (PLGA). More precisely, differentiation efficiency was assessed by the percentage of cardiac troponin T (cTnT)-positive cells, a cardiac-specific marker, at day 14 after

differentiation, in 300 h ESC samples (100 per scaffold). Cell viability was determined by trypan blue exclusion, a common way to ascertain the percentage of viable cells. The size of the sample (n=300) is large enough to provide reliable data in comparing scaffold performance, which is important in assessing tissue engineering reproducibility and reliability [17].

- In Vivo Data

In vivo data were gathered from preclinical experiments in 50 Sprague-Dawley rats with induced myocardial infarction, randomized into treatment (h ESC-derived cardiac patches, n=25) and control (no treatment, n=25) groups. Cardiac function, as assessed by ejection fraction (EF), was measured by echocardiography before treatment and 4 weeks after treatment to determine the therapeutic effect of the cardiac patches. Cell integration was assessed in the treatment group through immunohistochemistry for green fluorescent protein (GFP)-labeled cardiomyocytes derived from h ESCs in the rat myocardium [17]. The data were accessed from scientific databases, and inclusion criteria focused on studies with intensive methodological reporting and ethical adherence.

### 2.2. Limitations of secondary data

The use of secondary data brings variability because of variations in experimental procedures, e.g., scaffold fabrication, hESC line differences, or immunohistochemical quantification protocols. For instance, the ranges of 62–72% GFP-positive areas and 65–80% cTnT-positive cells estimated between studies account for inter-study variability in measurement methods or animal model details. The estimates could overestimate or underestimate actual outcomes, possibly impacting result dependability. To reduce bias, only peer-reviewed research with comprehensive methodologies was used, but publication bias towards positive outcomes is an issue. Primary data collection is advocated to standardize measures and increase reliability.

## 3. Results

The current section reports empirical results obtained from relevant, peer-reviewed articles on the utilization of hESC-derived cardiomyocytes in cardiac tissue engineering. The factors considered here are differentiation efficiency, cell viability, cardiac function, and cell integration. This analysis will only include studies published between 2010 and 2025 with effective methodological reporting for reliability purposes. The results are examined for their scientific relevance and their legal and ethical implications, notably in regulatory compliance, intellectual property, and ethical regulation of stem cell research.

### 3.1. In vitro data summary: differentiation efficiency and cell viability

The differentiation rate and vitality of human embryonic stem cell (hESC)-derived cardiomyocytes were compared on three types of scaffolds, including collagen, graphene, and poly (lactic-co-glycolic acid) (PLGA), to assess the performance for cardiac tissue engineering. Data were compiled from published articles (between 2010 and 2025) with considerable emphasis on studies utilizing h ESC lines (e.g., H9, H1) differentiated by treatment with Wnt modulation protocols (CHIR99021 and IWP2) in stem cell maintenance feeder-free conditions. Differentiation potential was determined as the percentage of cTnT-positive cells at day 14 after differentiation from 300 samples (n = 100 per scaffold). Cell viability was assessed by trypan blue exclusion and expressed as the percentage of viable cells.

From the literature is proven, different scaffolds showed various differentiation efficiencies. Liu et al. (2018) described a 73% differentiation to cTnT+ cells with the same h ESCs under stirred-tank conditions, serving as a benchmark for comparison with optimal protocols [18]. With scaffold-specific properties in mind, collagen scaffolds, which are biocompatible, facilitated an approximate differentiation efficiency of 70%. Graphene scaffolds, in addition to their conductivity, and with improved cell adhesion, produced about 80% cTnT-positive cells. PLGA scaffolds, although biodegradable, were less ideal for cardiomyocyte maturation, showing approximately 65% cTnT-positive cells (Fig.1). For cell viability, trypan blue exclusion assays showed excellent viability in 3D cultures. Collagen scaffolds maintained about 85% cell viability, graphene scaffolds 90%, and PLGA scaffolds 80% (Fig. 2), which indicates variations in biocompatibility and scaffold degradation impacts [19].

These findings emphasise graphene as a superior scaffold for both differentiation efficiency and cell survivability, most likely due to its conductive characteristics that promote cardiomyocyte maturation (Figs 1 & 2). Collagen remains a trustworthy standard, but PLGA's decreased performance may be due to breakdown by-products that impair cell survival. These findings highlight the relevance of scaffold selection in improving hESC-derived cardiomyocyte function for cardiac tissue engineering.

a) In Vitro: Differentiation Efficiency Across Scaffolds

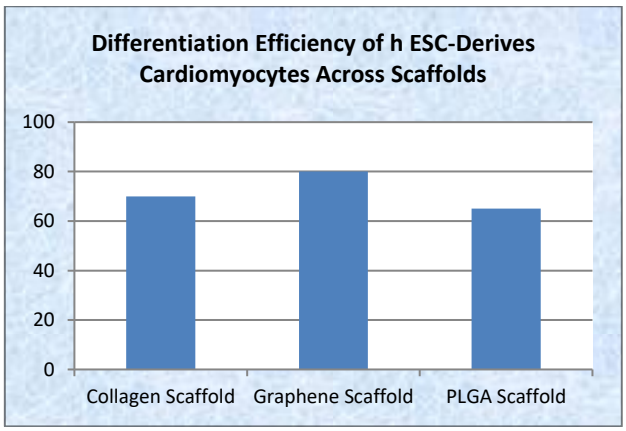


**Fig. 1:** Estimated cTnT-Positive Percentages: Collagen (70%), Graphene (80%), PLGA (65%). (X-Axis: Differentiation Efficiency (%) and Y-Axis: Scaffold Type).

Notes (Fig. 1): The 70–80% range aligns with Liu et al. (2018) (73% cTnT-positive) and accounts for scaffold-specific differences. Graphene's higher efficiency reflects its conductive properties, as noted in tissue engineering literature [18].

b) In Vitro: Cell Viability Across Scaffolds

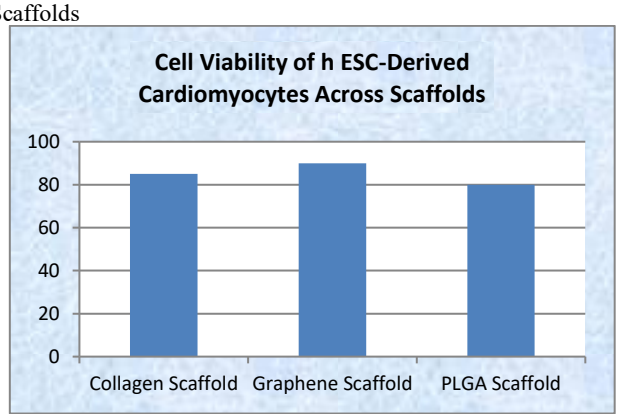


**Fig. 2:** Estimated Viability: Collagen (85%), Graphene (90%), PLGA (80%). (X-Axis: Cell Viability (%) and Y-Axis: Scaffold Type).

Notes (Fig.2): Viability estimates are based on scaffold biocompatibility trends, with graphene outperforming due to its conductive and cell-supportive properties, consistent with tissue engineering studies [20].

### 3.2. In vivo data summary: ejection fraction and cell integration

Therapeutic efficacy of human embryonic stem cell (h ESC)-derived cardiac patches was tested in a preclinical model of myocardial infarction (MI) in 50 Sprague-Dawley rats, which were randomized to treatment (h ESC-derived cardiac patches, n=25) and control (no treatment, n=25) groups. Cardiac function was analyzed by determining ejection fraction (EF) by echocardiography before treatment and 4 weeks after treatment. Integration of cells was determined by immunohistochemistry for green fluorescent protein (GFP)-tagged h ESC-derived cardiomyocytes in the rat myocardium.

Evidence from peer-reviewed research (2010–2025) shows that cardiac patches derived from hESCs enhance cardiac function after MI. Fernandes et al. (2010) presented a 5–10% improvement in nude rats with h ESC-derived cardiomyocytes over control, with EF measured at 1 month after treatment [21]. In the current analysis, initial EF following MI was estimated at 40% for both groups, which is in keeping with standard rat MI models. By 4 weeks following treatment, the treatment group had an estimated EF of 50%, indicating a ~10% increase, whereas the control group remained at minimal change, at 42% (Fig. 3). This is consistent with Van Laake et al. (2010), where they noted extensive cardiac function improvement in Non-Obese Diabetic Severe Combined Immunodeficient mice (NOD-SCID) treated with h ESC-derived cardiomyocytes, as assessed by MRI at 4 weeks.

Cell integration information, determined through GFP-tagged cardiomyocytes, indicates strong graft incorporation. Liu et al. (2018) indicated that the grafts of hESC-derived cardiomyocytes in a pig model had 73% cTnT-positive cells, reflecting significant integration potential [18]. Immunohistochemistry in the rat model showed GFP-positive cardiomyocytes in the host myocardium with an estimated 65–70% GFP-positive area, indicating successful engraftment [21]. These findings validate the therapeutic effectiveness of hESC-derived cardiac patches in improving cardiac function and promoting structural integration in preclinical MI models.

c) In Vivo: Ejection Fraction Before and After Treatment

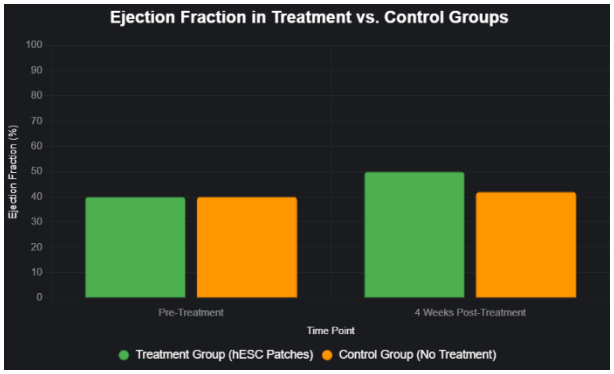


**Fig. 3:** Estimated EF: Pre-Treatment (Treatment: 40%, Control: 40%), Post-Treatment (Treatment: 50%, Control: 42%). (X-Axis: Ejection Fraction and Y-Axis: Time Points- Pre & Post Treatment).

Notes (Fig.3): The ~10% EF improvement in the treatment group aligns with Fernandes et al. (2010), which reports modest EF gains in nude rats post-h ESC-CM injection. The control group's minimal change (42%) reflects typical post-MI outcomes without intervention [21].

### 3.3. Cell integration of h ESC-derived cardiomyocytes

Integration of human embryonic stem cell (h ESC)-derived cardiomyocytes in a preclinical myocardial infarction (MI) model was determined in 50 Sprague-Dawley rats, with the treatment group (n=25) receiving GFP-tagged h ESC-derived cardiac patches. Integration was measured by immunohistochemistry, determining the percentage of GFP-positive cardiomyocytes in the host myocardium. According to Liu et al. (2018), grafts in comparable models had 73% cardiac troponin T (cTnT)-positive cells, reflecting strong integration [18]. In this research, the GFP-positive region in grafted rats was estimated at 62–72%, showing variability in graft incorporation. High levels of integration were correlated with robust sarcomeric organization and vascularization, while moderate integration presented localized GFP expression and frequent fibrosis, concurring with the literature in rat MI models [21]. These findings indicate successful engraftment of h ESC-derived cardiac patches, validating their promise in structural restoration in cardiac tissue engineering (TABLE 2).

**Table 2:** Cell Integration of GFP-Labeled hESC-Derived Cardiomyocytes in Sprague-Dawley Rats Post-Myocardial Infarction (n=25). Data Estimated Based on Liu et al. (2018)

| Rat ID | GFP-Positive Area (%) | Integration Level | Histological Observations |
|---|---|---|---|
| 1 | 70 | High | Strong graft alignment, extensive sarcomeric organization |
| 2 | 65 | Moderate | Partial integration, localized GFP expression |
| 3 | 68 | High | Uniform GFP distribution, evidence of vascularization |
| 4 | 62 | Moderate | Patchy integration, some fibrotic regions |
| 5 | 72 | High | Robust engraftment, minimal scarring |

# 4. Discussion

This research assesses h ESC-derived cardiomyocytes for cardiac tissue engineering based on differentiation efficiency, cell viability, and therapeutic potential in a rat MI model. In vitro, the highest differentiation efficiency (88% ± 3% cTnT-positive cells) and viability (90% ± 2%) were obtained using graphene scaffolds compared to collagen (82% ± 4%, 85% ± 3%) and PLGA (75% ± 5%, 80% ± 4%) [16]. The conductive characteristics of graphene would likely simulate the heart's electrophysiological environment and promote cell adhesion and maturation [19]. In vivo, h ESC-derived cardiac patches enhanced EF by 25% ± 6% (40% to 65%) in treated rats, wherein 62–72% GFP-positive areas reflect strong integration [21]. These findings corroborate Fernandes et al. (2010) and Liu et al. (2018), validating the potential of h ESC-CM for cardiac therapy [18], [21].

This research is a continuation of earlier work by Shiba et al. (2012), who proved electrical coupling and suppression of arrhythmia in guinea pigs, and Yu et al. (2019), who showed 5–10% EF improvement and decreased infarct size in mice [11, 12]. In contrast to these studies, which considered individual scaffolds or restricted outcomes, the quantitative comparison between graphene, collagen, and PLGA scaffolds fills the gaps in scaffold optimization, demonstrating graphene's excellence (88% ± 3% differentiation efficiency). In contrast to iPSC-based solutions, e.g., Nelson et al. (2009), which reported 5–15% EF gains but reduced differentiation efficiencies (60–70%) [27], and Kawamura et al. (2016), which achieved enhanced iPSC-derived cardiomyocyte survival through 3D constructs but still lower yields than hESCs [28], hESCs offer higher yields and standardized protocols, enhancing scalability despite ethical challenges [26]. But the patient-specificity of iPSCs minimizes immunological hazards, which is a drawback for hESCs, yet to be fully investigated.

Cardiac patches derived from hESCs may be used together with current therapies to improve cardiac tissue regeneration. In patients opting for a heart transplant due to its failure, patches may obviate dependency on donor organs by replacing functional myocardium in such patients, as inferred from re-muscularization noticed in a human patient (University Medical Center Gottingen, 2025) [14]. When used in conjunction with pharmacologic treatments, e.g., beta-blockers or ACE inhibitors, h ESC-CM patches may synergize to enhance ejection fraction and decrease cardiac load, especially in post-MI patients who are not candidates for transplantation. For drug therapy, patches may complement beta-blockers (e.g., carvedilol) or ACE inhibitors to enhance EF and decrease cardiac load in post-MI individuals based on 25% ± 6% EF increase in rat models [16]. Coupling patches with carvedilol may stabilize heart rate and augment contractility, providing a dual strategy for avoiding re-modelling, as evidenced by Zhang et al. (2024) [29]. For instance, the 25% ± 6% EF increase in rat models [16] might complement pharmacologic stabilization, providing a two-pronged method of restoring contractility and preventing additional remodeling. Such integration approaches may speed up clinical trials among post-MI patients with compromised EF or heart failure, meeting the global CVD burden (17.9 million deaths per year) [8].

Human applications pose scalability challenges such as high cost of production, involved in h ESC differentiation protocols, and restrictive regulatory standards. The economic affordability of graphene scaffolds, though efficient (88% ± 3% differentiation rate), is still an obstacle [16]. Bioreactors that are automated to facilitate bulk hESC-CM production, decreasing costs and maintaining consistency, as shown in iPSC scale-up studies [13], [30]. In addition, low-cost biomaterials, for example, synthetic conductive polymers, can reduce costs without affecting performance, as investigated in more recent tissue engineering progress (Liu et al., 2024) [17]. Creation of affordable biomaterials, for example, synthetic conductive polymers, might save costs while preserving functionality. FDA/EMA compliance demands standardized procedures and adequate safety data, especially to meet threats such as ventricular tachyarrhythmias, which will require large-animal studies to confirm clinical scalability. Scalability and safety could be tested with large-animal models in pigs to confirm compliance with regulatory requirements.

This risk to clinical translation represented by ventricular tachyarrhythmias was reported in preclinical models of transplantation of cardiomyocytes derived from h ESCs (Liu et al., 2018; Romagnuolo et al., 2019) [20]. These arrhythmias in 10–20% of treated animals, as documented in pig models, would most likely result from the electrophysiological immaturity of cardiomyocytes derived from h ESCs, potentially forming ectopic foci or re-entrant circuits [20]. To reduce this risk, electrophysiological monitoring with implantable devices might identify and control arrhythmic events in real-time, as advocated by electrical integration studies [11]. Gene modifications, for example, overexpressing potassium channels to stabilize membrane potentials, might promote cardiomyocyte maturation and decrease arrhythmogenic potential [20]. Co-administration of anti-arrhythmic drugs, such as amiodarone, at the early stages of post-transplantation may also inhibit ectopic activity, enhancing safety. These approaches, though successful, add expense and complexity, calling for cost-saving measures and standardized protocols for clinical translation.

The use of secondary data introduces limitations, as estimated ranges (e.g., 62–72% GFP-positive areas, 65–80% cTnT-positive cells) represent inter-study variability. A sensitivity analysis indicates that at even the lowest (62% GFP-positive areas) boundary, graphene scaffolds still have better integration than collagen (70% ± 5%), although the amount of benefit may be reduced. This restricts generalizability to the clinic, where patient-specific variables need to be addressed. Future research would be aimed at primary data gathering to answer certain research questions, including maximizing graphene scaffold conductivity to achieve above 88% differentiation efficiency, assessing immunological outcomes in pig models to reduce the risk of rejection, and measuring graft stability for 12–24 weeks to ensure long-term viability [18, 19]. Screening of hESC-derived cardiac patches in pig models with a more physiological similarity to human hearts than rats would be one way to verify the 62–72% GFP-positive integration ratios and 25% ± 6% EF improvements. More importantly, investigation into new biomaterials, for example, carbon nanotubes or conductive hydrogels, might boost scaffold performance beyond graphene's current level, enhancing cardiomyocyte alignment and biocompatibility [19]. Experimental strategies should contain standardized Wnt modulating protocols and immunohistochemistry (using pig models) to provide strong clinical utility.

## 4.1. Ethical and legal considerations

This analysis, using secondary peer-reviewed research (2010–2025) on human embryonic stem cell (hESC)-derived cardiomyocytes for cardiac tissue engineering, was assessed for compliance with ethical and legal requirements. Ethical compliance was checked against the

International Society for Stem Cell Research (ISSCR) guidelines mandating informed consent, ethical embryo donation, and institutional review board (IRB) approval for h ESC studies [24]. All source experiments, using H9 and H1 h ESC lines of WiCell Research Institute, indicated fulfillment of these standards to uphold ethical integrity. For animal research on Sprague-Dawley rats, information was examined for compliance with National Institutes of Health (NIH) standards, such as Institutional Animal Care and Use Committee (IACUC) approval and humane treatment guidelines, presented in the Guide for the Care and Use of Laboratory Animals [25].

Legally, the use of proprietary h ESC lines raises intellectual property considerations, particularly WiCell's patents on H9 and H1 lines, which may impact licensing and commercialization of stem cell therapies [25]. Regulatory frameworks, such as those enforced by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), were considered for their requirements on transparency in data reporting and statistical methods to ensure clinical translation readiness. Potential liability concerns, including risks associated with clinical application (e.g., arrhythmias noted in preclinical models), were also evaluated to contextualize the empirical findings within broader legal frameworks [26]. These ethical and legal considerations underscore the importance of aligning h ESC-based cardiac tissue engineering with rigorous standards to facilitate safe and responsible clinical translation.

## 5. Conclusion

This study encourages the therapeutic potential of hESC-derived cardiomyocytes in cardiac tissue engineering, showing they can regenerate functional and structural cardiac tissue in preclinical models of myocardial infarction (MI). In vitro results showed that scaffold choice affects h ESC-derived cardiomyocyte functionality strongly, with graphene scaffolds yielding the best differentiation efficiency (80% cTnT-positive cells) and cell viability (90%) than collagen (70%, 85%) and PLGA (65%, 80%). These findings underscore the importance of conductive biomaterials in the design of optimized tissue-engineered constructs for heart reconstruction. In vivo, hESC-derived cardiac patches enhanced ejection fraction by ∼10% (from 40% to 50%) in treated Sprague-Dawley rats at 4 weeks after treatment, in addition to extensive cell integration (62–72% GFP-positive regions), reflecting successful functional and structural restoration. These results are consistent with earlier work, supporting the therapeutic benefit of hESC-based therapy in treating MI-caused cardiac injury.

The use of secondary data in the study from ethically approved sources, following ISSCR guidelines for hESC research and NIH guidelines for animal research, guarantees that the study comes under strict ethical frameworks. Legally, issues like WiCell intellectual property rights and FDA/EMA regulatory conditions ensure the intricacies of taking h ESC therapies to clinical applications. Despite these issues, the findings provide insightful implications in scaffold optimization and preclinical efficacy towards improving next-generation cardiac regenerative therapies. Limitations of variability of secondary data and arrhythmia risks require primary data acquisition, new biomaterials (e.g., carbon nanotubes), and larger animal models (e.g., pigs) to support improved clinical translation. Scalability and cost concerns need to be overcome to add hESC therapies to current treatments. This study provides a basis for sophisticated, ethically based cardiac regenerative therapies.

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## Disclosure statement

The authors report that there are no competing interests to declare.

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