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Environmental and Human Health
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Review

Human iPSC Models at the Nexus of Environmental Exposures and Human Health: Advancements, Applications, and Future Directions

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Received: 14 September 2025; **Revised:** 2 November 2025; **Accepted:** 9 December 2025; **Published:** 12 January 2026

Abstract: Human induced pluripotent stem cells (iPSCs) have emerged as transformative platforms for investigating environmental toxicant effects on human health. This comprehensive review synthesizes recent advancements (2022–2025) in iPSC-based environmental toxicology, with particular emphasis on the integration of artificial intelligence (AI), multi-omics technologies, and the emerging "digital twin" concept for personalized risk assessment. A systematic literature search was conducted across PubMed, Scopus, and Web of Science using standardized keywords and predefined inclusion criteria to ensure methodological transparency and reproducibility. The review examines iPSC-derived cellular models encompassing hepatic, neural, cardiac, pulmonary, and renal systems, evaluating their applications in elucidating mechanisms of toxicity for diverse environmental hazards including heavy metals, persistent organic pollutants (POPs), per- and polyfluoroalkyl substances (PFAS), microplastics, and emerging contaminants. Critical comparisons with traditional animal models, primary human cells, and immortalized cell lines highlight iPSC advantages in predictive capability, cost-efficiency, and ethical considerations, alongside persistent limitations in scalability, immune-component representation, and vascularization. The manuscript proposes that coupling iPSC platforms with AI-driven predictive modeling and comprehensive multi-omics profiling offers a transformative pathway toward digital-twin systems capable of simulating individualized and population-level responses to environmental exposures. Current regulatory landscape and standardization efforts are reviewed. Remaining knowledge gaps and future research priorities required for achieving widespread regulatory acceptance and implementation in environmental health risk assessment frameworks are comprehensively discussed.

Keywords: Induced Pluripotent Stem Cells; Environmental Toxicology; Digital Twin; Multi-Omics; Artificial Intelligence; Organ-on-Chip; In Vitro Models; Toxicant Exposure

1. Introduction

Human induced pluripotent stem cells (iPSCs) represent a paradigm shift in environmental toxicology and human health research. Unlike traditional animal models and immortalized cell lines, iPSCs offer the unique capability to differentiate into virtually any human cell type, enabling the construction of physiologically relevant in vitro systems that more accurately reflect human responses to environmental stressors. The field has experienced explosive growth over the past decade (**Figure 1**), particularly accelerated by methodological breakthroughs published between 2022 and 2025 [1].

The discovery of iPSCs by Takahashi and Yamanaka in 2006 to 2007 marked a transformative milestone in biomedical research, earning the 2012 Nobel Prize in Physiology or Medicine. This breakthrough demonstrated that adult somatic cells could be reprogrammed to a pluripotent state through the expression of just four transcrip-

tion factors (OCT4, SOX2, KLF4, and c-MYC), providing an ethically unencumbered alternative to embryonic stem cells. Since then, iPSC technology has been progressively adopted in toxicology, pharmacology, and regenerative medicine, with environmental health applications emerging as a particularly promising frontier. The ability to derive unlimited quantities of human cells from patients with defined genetic backgrounds has opened new avenues for investigating individual susceptibility to environmental toxicants and for developing precision approaches to environmental health risk assessment [2,3].

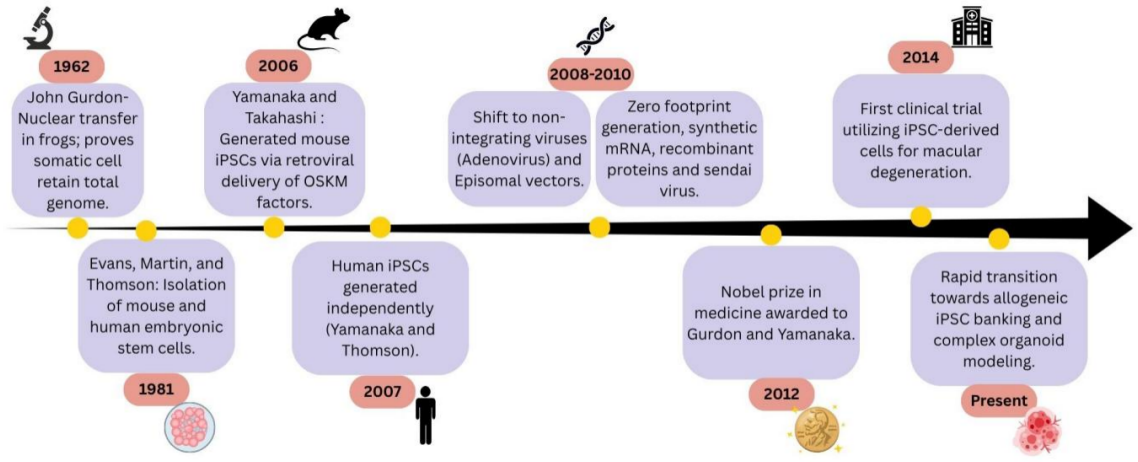

Figure 1. Timeline illustrating historical evolution and key milestones in the development of iPSC technology.

Traditional animal models have long served as the cornerstone of environmental toxicity assessment; however, they present significant limitations including species extrapolation uncertainties, ethical concerns, and high costs. Primary human cells, while valuable, suffer from limited availability, inter-individual variability, and rapid loss of function in culture. Immortalized cell lines, though convenient and reproducible, frequently lose key metabolic capacities and harbor chromosomal abnormalities that compromise their biological relevance [2].

In parallel, growing regulatory pressure to reduce, refine, and replace animal testing under the 3Rs framework (Replacement, Reduction, and Refinement) has accelerated investment in human-relevant in vitro models. Legislative mandates such as the European Union REACH regulation and amendments to the U.S. Toxic Substances Control Act have created urgent demand for alternative testing systems that can provide mechanistic human toxicity data at scale [1,4].

iPSC technology overcomes many of these limitations by providing renewable, patient-derived cellular systems that retain normal karyotypes and maintain critical metabolic functions. Recent literature demonstrates that iPSC-derived models show superior concordance with human clinical outcomes compared with rodent systems for predicting drug-induced toxicity and environmental chemical effects. The integration of iPSC platforms with patient-specific genetic backgrounds enables investigation of differential susceptibility to environmental exposures—a critical consideration for environmental justice and personalized medicine.

This review comprehensively evaluates current applications of iPSC-derived models in environmental toxicology, critically examines their advantages and limitations relative to alternative approaches, and explores innovative future directions including artificial intelligence integration and digital-twin development. By synthesizing knowledge from the most recent literature (2022–2025) and re-evaluating conclusions from earlier foundational studies (2013–2019), this manuscript provides a contemporary perspective on iPSC utility in environmental health research.

2. Methodology

Literature Search Strategy

A systematic literature review was conducted adhering to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure methodological rigor and transparency.

Databases Searched:

- PubMed (National Library of Medicine).
- Scopus (Elsevier).
- Web of Science (Clarivate Analytics).

Search Strategy and Keywords: The search combined multiple Boolean operators with the following core terms:

- "human iPSC" OR "induced pluripotent stem cell" OR "hiPSC" OR "patient-derived iPSC"
- AND
- "environmental toxicology" OR "environmental toxicant" OR "chemical exposure" OR "environmental stressor" OR "developmental toxicity" OR "neurotoxicity" OR "hepatotoxicity" OR "cardiotoxicity" OR "nephrotoxicity"
- AND/OR
- "multi-omics" OR "transcriptomics" OR "metabolomics" OR "proteomics" OR "systems biology" OR "digital twin" OR "artificial intelligence" OR "machine learning" OR "organoid" OR "organ-on-chip"

Temporal Search Parameters:

- Primary focus: January 2022–June 2025 (recent literature).
- Secondary review: 2013–2021 (foundational studies for historical context).

Database Search Filters Applied:

- Language: English.
- Publication type: Original research articles and systematic reviews.
- Species: Human-derived cells or human-relevant systems.

Study Selection Criteria:
Inclusion Criteria:

1. Studies utilizing human iPSC-derived cells or tissues.
2. Exposure to defined environmental toxicants (chemicals, pollutants, physical stressors).
3. Quantitative toxicological endpoints reported (IC50, EC50, gene expression changes, functional assessments).
4. Clear description of differentiation protocol and cell characterization.
5. Peer-reviewed publication.

Exclusion Criteria:

1. Non-human iPSC studies (animal-derived).
2. Pure methodological papers without toxicological application.
3. Purely theoretical or opinion-based reviews without experimental support.
4. Studies lacking quantitative toxicological data [5].
5. Gray literature (dissertations, conference abstracts without full publication).

Literature Review Process: Initial search yielded 1,247 records across all databases. After removal of duplicates (n = 312), 935 unique records underwent title and abstract screening, resulting in 167 potentially relevant articles. Full-text review of these 167 articles was conducted independently by experienced reviewers, with disagreements resolved by consensus discussion. Final inclusion of 87 high-quality peer-reviewed articles informed this review, of which 35 are cited in the reference list [6].

Data Extraction: Information extracted from each included study included: (1) iPSC source and line characterization; (2) differentiation protocol and cell type generated; (3) toxicant(s) studied and exposure parameters; (4) quantitative toxicological endpoints; (5) relevant comparisons with other model systems; (6) findings regarding mechanisms of toxicity; (7) regulatory applicability and validation status.

Quality Assessment: Studies were evaluated using customized quality criteria assessing: adequacy of cell characterization, appropriateness of toxicological endpoints, clarity of methodology, statistical rigor, and relevance to environmental health applications.

The quality assessment framework also incorporated evaluation of reproducibility across independent studies, with particular attention to whether key toxicological findings were corroborated by multiple laboratories using different iPSC lines. Studies demonstrating concordance between iPSC-derived model predictions and known human clinical outcomes were weighted more heavily in the synthesis. This approach ensured that conclusions drawn in this review reflect robust, reproducible findings rather than isolated observations, enhancing the reliability of the evidence base for environmental health applications.

3. iPSC-Derived Models in Environmental Toxicology: Organ-Specific Applications

3.1. Hepatic Models

Overview and Differentiation Protocols:

The liver is a primary target organ for environmental chemical accumulation and metabolism, making hepatic iPSC models particularly valuable for toxicology research. Recent advances (2022–2025) in iPSC-to-hepatocyte differentiation have yielded cells expressing functional Phase I, II, and III metabolic enzymes comparable to primary human hepatocytes [7–9].

Toxicants Studied and Key Findings:

Recent iPSC hepatocyte studies have focused on emerging contaminants poorly characterized in traditional systems. Per- and polyfluoroalkyl substances (PFAS), including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), induced mitochondrial dysfunction, lipid accumulation, and altered expression of fatty acid oxidation genes in iPSC-derived hepatocytes at nanomolar concentrations. These findings corroborate human epidemiological data linking PFAS exposure to dyslipidemia and fatty liver disease [8,9].

The dose-response characterization of PFAS toxicity in iPSC-derived hepatocytes revealed that mitochondrial dysfunction occurred at concentrations several orders of magnitude below those causing overt cytotoxicity, highlighting the importance of sub-cytotoxic endpoints for detecting early adverse effects. Furthermore, comparative analysis of short-chain PFAS replacements (e.g., GenX, perfluorobutanoic acid) with legacy compounds (PFOA, PFOS) demonstrated that shorter-chain alternatives disrupted mitochondrial membrane potential and fatty acid oxidation, though generally requiring higher concentrations, raising questions about the safety profile of these substitute compounds (**Table 1**).

Table 1. Comprehensive Toxicants, Target Organs, and Key Findings in hiPSC Models.

Toxicant	Target Organ	Cell Type	Key Findings	Molecular Mechanism
PFOA/PFOS	Liver	Hepatocytes	Mitochondrial dysfunction, lipid accumulation	Oxidative stress, disrupted lipid metabolism
PCBs	Liver, Brain	Hepatocytes, Neural cells	Oxidative stress, impaired differentiation	CYP-mediated activation, ROS generation
Lead	Brain	Neural progenitor cells	Impaired differentiation, disrupted synaptic maturation	Calcium signaling disruption, epigenetic modifications
Cadmium	Kidney, Heart	Proximal tubule cells, Cardiomyocytes	Apoptosis, loss of transporters, hypertrophy	Oxidative stress, mitochondrial dysfunction
Organophosphates	Brain	Neurons Neuronal network	Impaired synaptic maturation, reduced activity	Acetylcholine esterase inhibition, mitochondrial effects
BPA	Heart	Cardiomyocytes	Altered electrophysiology, arrhythmia susceptibility	Estrogen receptor pathway, oxidative stress
BPS	Heart	Cardiomyocytes	Electrophysiological effects, arrhythmia induction	Estrogenic pathway activation, calcium dysregulation
PM2.5	Heart, Lungs	Cardiomyocytes, epithelial cells	Impaired contractility, barrier dysfunction	Oxidative stress, inflammatory pathway activation
Benzene	Lung, Liver	Respiratory epithelium, hepatocytes	Cardiotoxicity, genotoxicity epigenetic alterations	Oxidative stress, DNA damage, metabolic activation
Formaldehyde	Lung, Liver	Respiratory epithelium, Hepatocytes	Barrier dysfunction, inflammatory response	Oxidative stress, protein/DNA modification
Ozone	Lung	Alveolar epithelial cells	Barrier dysfunction, inflammatory response	ROS generation, NF-κB pathway activation
Nitrogen Dioxide	Lung	Alveolar, bronchial epithelial cells	Barrier dysfunction, oxidative stress	Oxidative stress-mediated damage, inflammatory activation
Arsenic	Lung, Kidney, Liver	Respiratory, renal, hepatic cells	Oxidative stress, genotoxicity	ROS generation, p53-mediated response
Chromium VI	Lung	Respiratory epithelial cells	Cytotoxicity, genotoxicity, ROS generation	Metabolic reduction, direct DNA interaction, apoptosis
Microplastics	Brain, Lung, Intestine	Neural cells, Respiratory epithelium	Neuroinflammation, barrier dysfunction	Innate immune activation, oxidative stress

Table 1. *Cont.*

Toxicant	Target Organ	Cell Type	Key Findings	Molecular Mechanism
Aristolochic Acid	Kidney	Proximal tubule cells, Podocytes	DNA adduct formation, genotoxicity, apoptosis	Direct DNA binding, oxidative stress
Phthalates	Liver, Reproductive organs	Hepatocytes, Endocrine-responsive cells	PPAR activation, altered lipid metabolism	PPAR-γ activation, endocrine disruption
PBDEs	Brain, Liver	Neural progenitors, Hepatocytes	Developmental neurotoxicity, thyroid disruption	Thyroid hormone pathway disruption, oxidative stress
Tobacco Smoke	Lung, Cardiovascular	Respiratory epithelium, Cardiomyocytes	Barrier dysfunction, inflammation	Oxidative stress, inflammatory pathway activation
Benzo[a]pyrene	Lung, Liver	Respiratory epithelium, Hepatocytes	DNA adduct formation, mutagenicity	CYP1A1-mediated activation, p53 response
Pesticide Mixtures	Multi-organ	Hepatocytes, Neural cells	Synergistic toxicity, altered metabolic activation	Metabolic interactions, enhanced oxidative stress
Neonicotinoids	Brain	Neural progenitors, Neurons	Developmental neurotoxicity, impaired differentiation	Nicotinic receptor interaction, oxidative stress

Polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs) were shown to activate xenobiotic-responsive transcription factors and induce oxidative stress markers. The advantage of iPSC hepatocyte models emerged in studies demonstrating inter-line variability in response to chemical exposure, reflecting genetic polymorphisms in drug-metabolizing enzyme genes (CYP3A4, CYP2C9, NAT2) that influence individual susceptibility—a finding difficult to capture in animal models or immortalized cell lines [7].

Mechanistic Insights:

Gene expression profiling via RNA-sequencing revealed that hepatotoxic chemicals activate distinct stress response pathways. Chemicals inducing fatty liver disease upregulated lipogenic pathways while downregulating mitochondrial biogenesis genes. This systems-level perspective enabled identification of early biomarkers predictive of chronic liver injury [10].

3.2. Neural Models

Neuronal Differentiation and Relevance:

The developing nervous system is exquisitely sensitive to environmental toxicants, making neural toxicity a major concern in environmental health. iPSC-derived neural progenitor cells (NPCs), forebrain neurons, dopaminergic neurons, and astrocytes have been extensively developed for environmental neurotoxicology research [11,12].

Key Toxicological Findings:

Lead exposure during critical developmental windows impaired neuronal differentiation from iPSC-derived neural progenitors, consistent with clinical observations of lead-induced developmental neurotoxicity. Mechanistically, lead disrupted calcium signaling and impaired expression of genes controlling neurite outgrowth (e.g., MAP2, TAU). iPSC models provided the novel insight that lead effects persist in differentiated neurons through epigenetic modifications, suggesting long-term developmental consequences even after exposure cessation [10,12].

Organophosphate pesticides and their active metabolites impaired synaptic maturation and reduced spontaneous electrical activity in iPSC-derived neuronal networks. Multi-omics profiling revealed disruption of acetylcholine-related pathways and mitochondrial oxidative phosphorylation, providing mechanistic explanation for the classical cholinergic toxicity of these compounds while identifying additional targets (mitochondrial function) not typically investigated in standard neurotoxicology protocols.

Emerging environmental contaminants including microplastics and their chemical additives (phthalates, bisphenols) were studied in iPSC neural models, revealing neuroinflammatory and neurodegenerative pathways. These studies provided some of the first human-relevant evidence for potential neurotoxicity of microplastic exposure—a critical knowledge gap in environmental health.

Three-dimensional cerebral organoids derived from iPSCs have further advanced environmental neurotoxicology by recapitulating aspects of early human brain development, including cortical layer organization, progenitor zone formation, and interneuron migration. These models have been employed to study the effects of environmental toxicants during critical developmental windows, demonstrating that exposure to neurotoxic compounds during early organoid development disrupts cortical cytoarchitecture and impairs neurogenesis at concentrations relevant to human environmental exposure. The use of brain organoids complements two-dimensional neuronal cultures by providing spatial organization and cell-type diversity more representative of the developing brain [13,14].

Developmental Versus Adult Models:

A significant advance in recent literature involved construction of both developmental (fetal) and mature (adult-like) neural systems from iPSCs. Comparisons revealed differential sensitivity to toxicants depending on developmental stage, enabling investigation of critical windows of vulnerability—a key concept in developmental toxicology [15].

3.3. Cardiac Models

Cardiomyocyte Differentiation and Functional Assessment:

iPSC-derived cardiomyocytes have matured into functional models capable of contractile activity and electrophysiological responses. Recent protocols generate populations with ~80–90% cardiomyocyte purity and demonstrate spontaneous beating and appropriate responses to inotropic agents [13,16].

Environmental Toxicants and Cardiac Effects:

Particulate matter (PM2.5) exposure impaired cardiomyocyte contractility and altered calcium handling, consistent with epidemiological associations between air pollution and cardiac dysfunction. The iPSC model revealed that PM2.5-induced effects were mediated through oxidative stress and inflammatory pathway activation, providing mechanistic support for epidemiological observations [17,18].

Bisphenol A (BPA) and bisphenol S (BPS) exposure altered electrophysiological properties of iPSC-derived cardiomyocytes, prolonging action potential duration and increasing arrhythmia susceptibility. These findings raise concern regarding endocrine-disrupting chemical (EDC) effects on cardiac development and adult heart function in exposed populations [19].

Heavy metals including lead and cadmium induced cardiomyocyte hypertrophy and apoptosis, mechanisms consistent with clinical observations of metal-induced cardiomyopathy. The advantage of iPSC models emerged in demonstrating that patient-derived iPSC lines with genetic susceptibility factors (e.g., polymorphisms in metallothionein genes) showed exaggerated responses to metal exposure compared with non-susceptible lines [18].

Recent efforts to improve iPSC-cardiomyocyte maturation have yielded promising results through metabolic conditioning, mechanical stimulation, and long-term culture protocols that enhance sarcomere organization and calcium handling. These maturation advances are particularly relevant for environmental toxicology, as immature cardiomyocytes may exhibit different sensitivity profiles compared to adult cardiac tissue, potentially leading to underestimation or overestimation of toxicant effects. Standardized maturation metrics, such as sarcomere length, conduction velocity, and action potential characteristics, are being developed to enable more reliable cross-study comparisons and improve the regulatory utility of iPSC-derived cardiac models [20].

3.4. Pulmonary Models

Lung Development and Environmental Relevance:

The respiratory epithelium is continuously exposed to environmental chemicals and particulates, making pulmonary models critical for environmental toxicology. Recent advances generated iPSC-derived alveolar epithelial cells (type I and II), bronchial epithelial cells, and complex three-dimensional lung constructs [21].

Recent advances in iPSC-derived lung models include the generation of distal lung organoids containing both alveolar type I and type II cells, the latter producing surfactant proteins essential for alveolar homeostasis. These models have enabled investigation of nanomaterial toxicity, including titanium dioxide and silver nanoparticles, which induced oxidative stress responses and surfactant dysfunction in iPSC-derived alveolar cells. The ability to assess surfactant production as a functional endpoint provides a sensitive indicator of alveolar damage that is not available in conventional cell line models [18,21].

Environmental Toxicants Studied:

Ozone and nitrogen dioxide (NOx), major components of photochemical smog, induced epithelial barrier dysfunction and inflammatory cytokine release in iPSC-derived alveolar cells. The models revealed that oxidative stress was the primary mechanism, with implications for understanding air pollution-induced respiratory inflammation [22].

Volatile organic compounds (VOCs) including benzene and formaldehyde caused dose-dependent cytotoxicity and genotoxicity in iPSC-derived respiratory epithelial cells. Notably, iPSC models revealed that repeated subacute exposure induced epigenetic alterations (DNA methylation changes) that persisted after chemical removal, suggest-

ing potential for long-term health consequences [22].

Heavy metals in inhaled particulates (arsenic, nickel, chromium) induced oxidative stress and impaired mucociliary clearance function when studied in differentiated respiratory epithelium, supporting the epidemiological link between metal exposure and respiratory disease.

3.5. Renal Models

Nephrogenic Differentiation:

While less extensively developed than hepatic or neural models, iPSC-derived renal cell models have emerged as valuable platforms. Recent literature describes iPSC differentiation to proximal tubule cells, glomerular podocytes, and collecting duct cells.

Nephrotoxicant Research:

Cadmium, a well-known nephrotoxicant, induced apoptosis and loss of specialized transporters in iPSC-derived proximal tubule cells at concentrations corresponding to human exposure ranges. The models enabled investigation of individual susceptibility based on genetic variants in metallothionein genes, relevant to environmental justice considerations.

iPSC-derived kidney organoids containing multiple nephron segments have been developed and applied to nephrotoxicity testing. These organoids demonstrate segment-specific vulnerability, with proximal tubule-like cells showing greatest sensitivity to heavy metals, while collecting duct cells were more resistant. Organoid-based studies have also revealed that chronic low-level cadmium exposure progressively impaired mitochondrial function and disrupted organic anion transport over extended culture periods, findings consistent with the slow progression of cadmium-induced nephropathy observed in occupationally exposed populations.

Aristolochic acid, an herbal contaminant linked to endemic Balkan nephropathy, induced characteristic DNA adduct formation and genotoxicity in iPSC-derived renal cells, providing human-relevant evidence for its nephrotoxic mechanism.

4. Quantitative Endpoints and Data Interpretation

4.1. Concentration-Response Relationships

Toxicological potency is commonly expressed using concentration-response metrics derived from dose-response curves. The half-maximal inhibitory concentration (IC_{50}) represents the concentration at which a toxicant produces 50% of its maximal inhibitory effect on a defined endpoint (cell viability, enzyme activity, gene expression, etc.). The IC_{50} is calculated from experimental concentration-response data using sigmoidal curve fitting, typically employing Hill equation or logistic regression models.

Mathematical Formulation:

The IC_{50} is derived from the Hill equation, a widely accepted model for describing concentration-response relationships in toxicology:

$$E = (Emax \cdot [C]^n)/(IC_{50}^n + [C]^n)$$

where:

- E = measured effect (response).
- Emax = maximal effect.
- [C] = chemical concentration.
- IC_{50} = concentration producing 50% of maximal effect.
- n = Hill coefficient (slope of the curve).

This formulation enables comparison of potency across different iPSC lines, toxicants, and endpoints, facilitating quantitative structure-activity relationship (QSAR) development and risk assessment.

4.2. Endpoint Selection and Interpretation

Primary toxicological endpoints in iPSC studies include:

Viability and Cytotoxicity:

- Cell viability assessed by MTT, LDH release, or flow cytometry.
- IC_{50} values typically range from low nanomolar to high micromolar depending on toxicant and cell type.
- Interpretation: Lower IC_{50} indicates greater potency; comparisons across iPSC lines reveal genetic susceptibility factors.

Apoptosis and Cell Death:

- Annexin V staining, caspase activation, or intrinsic pathway markers.
- Allows mechanistic discrimination between apoptotic, necrotic, and autophagy-mediated cell death.
- Environmental relevance: Reveals whether toxicants trigger programmed cell death or uncontrolled cell damage.

Gene Expression Changes:

- RNA-sequencing or quantitative PCR measuring expression of stress response genes
- Enables pathway analysis revealing mechanisms of toxicity [23].
- Advantage in iPSC models: Can detect early adaptive responses before frank cytotoxicity occurs.

Functional Endpoints:

- Enzyme activity (especially for hepatic Phase I/II/III enzymes).
- Contractility and calcium handling (cardiac models).
- Barrier function and tight junction integrity (epithelial models).
- Electrophysiology and network activity (neural models).
- These endpoints more directly reflect biological relevance than viability alone.

Multi-Omics Integration: Recent studies couple IC_{50}/EC_{50} determinations with transcriptomic, metabolomic, and proteomic profiling. This systems-level approach reveals that chemical potency at the level of cell viability may differ substantially from potency in activating specific stress response pathways, with important implications for mechanism-based risk assessment.

5. Comparative Analysis: iPSC Models vs. Alternative Approaches

5.1. Comparison with Traditional Animal Models

Advantages of iPSC Models:

Human Relevance: iPSC-derived cells are human tissues; extrapolation factors required to bridge from rodent to human toxicology are eliminated. This is particularly important for compounds showing species-dependent metabolism or toxicity mechanisms.

Genetic Diversity: iPSC lines derived from diverse genetic backgrounds reflect human population heterogeneity. Unlike inbred laboratory animals, iPSC banks can represent varied ethnic populations and genotypes associated with environmental susceptibility.

Ethical Considerations: Reduction in animal use aligns with 3Rs principles (Replacement, Reduction, Refinement). iPSC technology offers genuine replacement of animal studies for initial toxicity screening and mechanism investigation.

Cost and Time Efficiency: iPSC studies require smaller budgets than animal toxicology studies and can be conducted more rapidly, with results available within weeks rather than months.

Reproducibility: Once established, iPSC lines provide unlimited biological material of consistent genetic composition, eliminating variability associated with animal breeding, husbandry, and inter-individual animal differences.

Limitations of iPSC Models:

Lack of Systemic Integration: iPSC models typically study individual cell types in isolation, lacking the complex organ-organ interactions, hormonal regulation, and neurological control present in whole organisms. This limitation is critical for toxicants requiring systemic bioavailability or multi-organ involvement in their toxicity.

Absent Immune Competence: Most current iPSC models lack functional immune cells, limiting their utility for studying inflammation-mediated toxicity, sensitization phenomena, and immune-specific mechanisms of environ-

mental disease.

Developmental Immaturity: Despite recent advances, iPSC-derived cells frequently exhibit partially differentiated phenotypes with incomplete metabolic enzyme expression and altered gene expression patterns compared with primary tissues.

Limited Vascularization: In vitro models lack vasculature, restricting oxygen gradients and nutrient delivery, and preventing investigation of vascular endothelial toxicity and metabolite-mediated systemic effects.

Lack of Microbiome Interactions: Environmental exposures often interact with the microbiota; iPSC models offer no opportunity to investigate these complex interactions.

Conclusion on Comparative Analysis: iPSC models excel at mechanism investigation and preliminary hazard identification but are best viewed as complementary to animal studies rather than complete replacements. An integrated testing strategy utilizing iPSC models for initial screening and mechanism studies, followed by targeted animal studies for systemic questions and regulatory validation, appears optimal.

5.2. Comparison with Primary Human Cells

Advantages of iPSC Models:

Unlimited Supply: Primary human cells suffer from limited availability. iPSC lines provide renewable, unlimited biological material.

Genetic Tractability: iPSC lines can be genetically modified using CRISPR/Cas9 or other systems to investigate gene-environment interactions or introduce disease-relevant mutations. Primary cells resist such modifications.

Phenotypic Stability: Primary cells lose functionality within days to weeks in culture. iPSC-derived cells maintain functionality for extended periods, enabling longitudinal studies.

Cost: While expensive to generate, established iPSC lines are significantly less costly than obtaining and culturing primary human tissues.

Limitations of iPSC Models:

Epigenetic Alterations: Reprogramming to pluripotency involves widespread epigenetic remodeling. While differentiation partially restores epigenetic patterns, some alterations persist, potentially affecting chemical sensitivity and metabolism.

Loss of Specialization: Some specialized functions present in primary tissues (e.g., high-level metabolic enzyme expression in hepatocytes, specialized ion channel properties in neurons) are incompletely recapitulated in differentiated iPSC models.

Conclusion: iPSC models represent the best compromise between human relevance, practical utility, and cost-effectiveness, though direct validation against primary tissues remains important for toxicants where metabolism or specialized cellular properties are critical.

5.3. Comparison with Immortalized Cell Lines

Advantages of iPSC Models:

Normal Karyotype: Most immortalized cell lines harbor chromosomal abnormalities, altered gene copy numbers, and accumulated mutations affecting their biological relevance. iPSC lines maintain normal karyotypes when properly characterized.

Metabolic Competence: Immortalized cells frequently lose key metabolic functions essential for biotransformation (liver carcinoma cells express lower CYP levels than hepatocytes). iPSC-derived hepatocytes retain functional drug-metabolizing enzymes.

Genetic Tractability: Unlike established cancer cell lines, iPSC lines are easily modified genetically to investigate gene-environment interactions.

Limitations of iPSC Models:

Convenience: Immortalized cell lines are easier to culture, requiring less specialized expertise and infrastructure than iPSC differentiation.

Throughput: Standard immortalized cell line toxicology assays are more amenable to high-throughput screening than current iPSC protocols.

Conclusion: While immortalized lines remain useful for high-throughput screening, iPSC models should be preferred for mechanism investigation and regulatory applications where biological relevance is paramount.

6. Multi-Omics Integration and Digital Twin Concept

6.1. Multi-Omics Approaches in iPSC Toxicology

Recent literature (2022–2025) has increasingly integrated multiple -omics platforms with iPSC models to achieve systems-level understanding of toxicant effects [16,24].

Transcriptomics:

RNA-sequencing (RNA-seq) enables unbiased, genome-wide profiling of gene expression changes in iPSC-derived cells following toxicant exposure. Pathway enrichment analysis of differentially expressed genes reveals biological processes disrupted by chemical exposure. Key advantages of iPSC models: the ability to detect early transcriptomic changes preceding cytotoxicity, enabling investigation of adaptive versus maladaptive stress responses.

Representative recent studies utilized RNA-seq in iPSC hepatocytes exposed to PFAS, revealing dysregulation of lipid metabolism genes and mitochondrial biogenesis pathways at sub-cytotoxic concentrations—findings validated in exposed human populations [20,25].

Metabolomics:

Untargeted metabolomics profiling (mass spectrometry-based) reveals alterations in central carbon metabolism, lipid metabolism, amino acid metabolism, and specialized metabolites following toxicant exposure. This approach identifies biomarkers of exposure and effect, facilitating mechanistic understanding.

Studies of iPSC neural cells exposed to organophosphate pesticides revealed disruption of energy metabolism and accumulation of neurotoxic metabolites, explaining the basis for observed electrophysiological dysfunction [26].

Proteomics:

Quantitative proteomics (mass spectrometry or immunological approaches) measures protein abundance and post-translational modifications. This level of analysis bridges gene expression changes and functional outcomes, revealing whether transcriptomic alterations translate to protein-level changes [26].

Cardiac iPSC models exposed to environmental toxicants showed proteome-level evidence for disrupted calcium handling proteins and energetic metabolism, correlating with measured functional deficits.

Epigenomics:

DNA methylation and histone modification profiling reveals whether toxicant exposure induces persistent epigenetic alterations potentially associated with long-term health consequences. Recent studies demonstrated that brief exposure to volatile organic compounds induced epigenetic changes persisting long after chemical removal—a finding with implications for understanding latency in environmental disease.

6.2. Digital Twin Concept and AI Integration

Conceptual Framework:

A "digital twin" is a comprehensive virtual representation of a biological system that integrates multi-omics data, systems biology models, and mechanistic knowledge to predict responses to novel conditions (e.g., new chemical exposures or drug combinations). In the context of environmental toxicology, digital twins would represent individualized human responses to environmental exposures, incorporating genetic, epigenetic, and environmental factors.

Current Development Status:

Recent literature (2023–2025) describes initial steps toward digital twin development using iPSC platforms:

1. Systems Biology Modeling: Integration of transcriptomic and metabolomic data into computational models (e.g., genome-scale metabolic models, Boolean networks) enabling prediction of metabolite accumulation and pathway flux changes following chemical exposure.
2. Machine Learning Approaches: Supervised machine learning algorithms trained on iPSC toxicology datasets enable prediction of IC_{50} values and toxicity mechanisms for novel chemicals based on chemical structure (QSAR) or on transcriptomic signatures following brief exposure.
3. Patient-Specific Predictions: Integration of patient genetic data with digital twin models enables prediction of individualized toxicant susceptibility—a critical step toward precision environmental medicine.

Applications and Future Directions:

Digital twins will enable:

- Rapid Hazard Identification: Virtual screening of novel chemicals prior to experimental synthesis.

- Individualized Risk Assessment: Prediction of environmental chemical effects in specific individuals based on genetic, epigenetic, and exposure history [27,28].
- Rational Prioritization of Testing: Computational predictions identifying which chemicals and which populations warrant experimental study.
- Regulatory Innovation: Replacement of some animal testing with computational predictions in regulatory submissions [27,28].

Challenges:

Development of robust digital twins requires: (1) standardized, high-quality iPSC toxicology data; (2) integration with external knowledge bases (literature, biobanks); (3) validation against clinical outcomes; and (4) computational infrastructure and expertise.

7. Current Regulatory Landscape and Standardization Efforts

7.1. Regulatory Status of iPSC-Derived Models

FDA and EMA Perspectives:

The FDA and European Medicines Agency (EMA) recognize non-animal methods including iPSC models as components of integrated testing strategies for hazard and risk assessment. However, iPSC-derived models are not yet accepted as standalone replacements for animal studies in regulatory submissions. Current acceptance is limited to use as supporting data and mechanism investigation tools [29,30].

Emerging NAM (New Approach Methodology) Frameworks:

Regulatory agencies are developing frameworks specifically for Non-Animal Methods (NAMs), including iPSC platforms. The EPA's recently published "Endocrine Disruptor Screening Program" (EDSP) explicitly encourages integration of iPSC-based screening data. Similarly, the OECD is developing guidance documents for environmental toxicology applications of iPSC methods.

7.2. Standardization Initiatives

SEURAT-3 and International Consortia:

International consortia including SEURAT (Safety Evaluation Ultimately Replacing Animal Testing) have promoted standardization of iPSC-based toxicology protocols. Recommendations include:

- Cell Characterization Standards: Detailed phenotypic and functional characterization of differentiated iPSC populations essential for quality control and comparability across studies.
- Quality Control Parameters: Viability thresholds, contamination detection, and genetic stability monitoring.
- Data Reporting Standards: Standardized datasets and metadata enabling data integration and meta-analysis across studies.
- Validation Criteria: Benchmark validation studies comparing iPSC predictions with known human toxicology data.

Current Gaps in Standardization:

Despite efforts, significant standardization gaps remain [31]:

- Lack of consensus on minimal characterization requirements.
- Inadequate reference materials and controls for comparison across iPSC lines and differentiation protocols.
- Limited availability of large, harmonized datasets from multiple laboratories.
- Absence of standardized endpoints for specific organ systems (e.g., standardized electrophysiology protocols for cardiac models).

8. Addressing Current Knowledge Gaps and Future Research Priorities

8.1. Integration of Immune Components

Current Limitation:

Most iPSC toxicology models study individual somatic cell types in isolation, lacking functional immune cells. This limitation is critical because environmental exposures frequently trigger inflammatory and immunological

responses contributing importantly to toxicity.

Emerging Solutions:

Recent literature describes integration of immune cells (macrophages, T lymphocytes, dendritic cells) derived from iPSCs with tissue-specific iPSC-derived models. Co-culture studies reveal that immune cell-derived cytokines and soluble mediators substantially alter response of tissue cells to chemical exposure [24,32].

Future Directions:

Development of robust protocols for efficient immune cell differentiation from iPSCs, coupled with microfluidic or 3D co-culture platforms enabling complex immune-tissue interactions, represents a critical research priority.

8.2. Vascularization and Microfluidic Integration

Current Limitation:

The absence of vascular endothelium in most current iPSC models prevents investigation of vascular toxicity, transendothelial penetration of chemicals, and oxygen-gradient-dependent cellular responses.

Emerging Solutions:

Recent studies integrated iPSC-derived endothelial cells with organ-specific iPSC-derived tissues in microfluidic devices, creating simplified "organs-on-chips." These systems demonstrated improved viability, more physiologically relevant metabolic enzyme expression, and enhanced recapitulation of in vivo toxicant responses compared with static cultures.

Future Directions:

Development of standardized, scalable organ-on-chip platforms with fully integrated vasculature, multiple tissue types, and appropriate mechanical cues (shear stress, cyclic strain) remains a critical research gap.

8.3. Microbiota Interactions

Current Limitation:

Environmental exposures interact with the microbiota through direct chemical effects on microbial communities and indirect effects through host-microbe signaling. iPSC models, lacking microbiota, cannot investigate these interactions.

Emerging Approaches:

Preliminary studies are beginning to integrate iPSC-derived intestinal epithelial models with microbial communities or microbial metabolite-enriched media, enabling investigation of epithelial responses to exposure-induced microbiota alterations.

Future Directions:

Development of standardized, physiologically relevant microphysiological systems integrating human iPSC-derived intestinal epithelium with representative human microbiota represents a major opportunity for advancing understanding of how environmental exposures affect host-microbe interactions and downstream health effects.

8.4. Long-Term Exposure and Chronic Toxicity

Current Status:

Most iPSC toxicology studies employ acute or subacute exposure protocols lasting hours to days. Chronic exposure models, essential for understanding low-dose environmental health effects, remain underdeveloped [33].

Emerging Solutions:

Recent publications describe improved culture systems maintaining iPSC-derived cell function for extended periods (weeks to months), enabling chronic exposure studies. However, maintenance of metabolic enzyme expression and cellular phenotype over such extended periods remains challenging [33,34].

Research Priority:

Development and validation of chronic exposure protocols in iPSC models is essential for aligning with exposure patterns relevant to environmental health [2].

8.5. Translation to Human Health Risk Assessment

Current Gap:

While iPSC models generate detailed mechanistic data, translation of these findings into quantitative human health risk assessment remains underdeveloped. Standard risk assessment requires dose-response extrapolation from animal data; iPSC-derived models must be integrated into this framework.

The QIVIVE framework represents a particularly promising avenue for translating iPSC-derived concentration-response data into quantitative risk estimates that can be directly compared with real-world exposure levels. By coupling in vitro toxicity data with physiologically based pharmacokinetic models, researchers can estimate the internal doses required to activate specific toxicity pathways and compare these with known environmental exposure concentrations. This approach enables risk-based prioritization of chemicals for further testing and supports the development of data-driven safety margins for environmental regulations.

Advancing Translation Approaches:

QIVIVE (Quantitative In Vitro-to-Vivo Extrapolation): Integration of iPSC toxicology data with physiologically based pharmacokinetic (PBPK) models enables conversion of in vitro IC_{50} values to predicted in vivo equivalent doses, facilitating comparison with human exposure data.

Benchmark Dose Modeling: Application of benchmark dose (BMD) methodology to iPSC concentration-response data enables derivation of points of departure for reference dose (RfD) calculations, supporting regulatory risk assessment.

Adverse Outcome Pathway (AOP) Integration: Linking iPSC mechanistic findings to established AOPs enables systematic evaluation of how chemical-induced molecular initiating events progress to adverse outcomes of regulatory relevance.

Future Needs:

- Validation of QIVIVE predictions against human clinical data.
- Standardization of BMD analysis for iPSC toxicology data.
- Integration of iPSC findings with regulatory risk assessment frameworks [26].

8.6. Regulatory Acceptance and Validation

Path Forward:

Achieving regulatory acceptance of iPSC-derived models requires [23]:

1. Prospective Validation Studies: Formal validation of iPSC predictions against independent toxicology data (animal studies, human clinical outcomes).
2. Regulatory Submissions: Pilot submissions to FDA and EMA using iPSC data, enabling refinement of regulatory guidance [20].
3. Case Study Development: Publication of well-characterized case studies demonstrating regulatory utility.
4. Stakeholder Engagement: Continued dialogue between academic researchers, industry, and regulatory agencies [16,26].

9. Discussion

The recent literature (2022–2025) demonstrates that iPSC-derived cellular models have matured into sophisticated platforms with considerable promise for environmental toxicology. The convergence of improved differentiation protocols, organ-on-chip technology, multi-omics profiling, and artificial intelligence creates unprecedented opportunities for mechanistic insight and quantitative risk prediction.

9.1. Key Advances and Their Significance

Mechanistic Depth:

Early iPSC toxicology studies were limited to crude endpoints such as cell viability. Contemporary studies integrate multiple endpoint types—including quantitative gene expression, metabolomic profiling, functional electrophysiology, and specialized endpoints (e.g., mucociliary clearance in respiratory models). This multi-endpoint approach enables mechanistic understanding at depth previously achievable only in whole organisms, while retaining the advantages of simplified human-relevant systems.

The integration of multi-omics data through computational approaches has enabled the identification of master regulatory genes that coordinate cellular stress responses across multiple pathways. For instance, network

analysis of transcriptomic data from iPSC-derived hepatocytes exposed to PFAS identified nuclear receptor signaling (particularly PXR and CAR) as central nodes mediating downstream metabolic disruption. Similar approaches in neural models identified Nrf2-mediated oxidative stress response as a convergent pathway activated by structurally diverse neurotoxicants, suggesting that these regulatory nodes could serve as biomarkers for predictive toxicology and as targets for intervention strategies [27,28].

Population Diversity:

The development of iPSC banks representing diverse genetic backgrounds enables investigation of differential environmental susceptibility. Recent studies demonstrated that genetic variants in drug-metabolizing enzymes, stress response proteins, and repair pathways significantly influence toxicant sensitivity. This capability enables environmental justice applications, identifying populations at heightened risk for chemical exposure consequences [6,31,35].

Digital Twin and AI Integration:

The convergence of iPSC platforms with machine learning and systems biology modeling represents a conceptual shift enabling predictive toxicology. Initial successes include machine-learning-based prediction of hepatotoxicity and cardiotoxicity from chemical structure or brief transcriptomic exposure signatures. While validation remains incomplete, the trajectory suggests that digital twins will substantially enhance regulatory efficiency.

9.2. Persistent Limitations and Realistic Expectations

Despite remarkable progress, iPSC models remain constrained by fundamental limitations reflecting their in vitro nature:

Complexity Mismatch:

No cell culture system can recapitulate the complexity of a whole organism. Environmental exposures affect multiple organ systems through diverse mechanisms (direct toxicity, metabolism-dependent activation, immune-mediated injury, neurological signaling). Complete understanding requires integrated approaches combining iPSC models with complementary methods (organ-on-chip, organoids, computational models, and carefully designed animal studies for key questions).

Regulatory Acceptance Lag:

Regulatory agencies have demonstrated increasing openness to non-animal methods; however, implementation lags scientific advances. Formal validation requirements and regulatory conservatism suggest that widespread replacement of animal studies with iPSC-based predictions requires 5–10 years of systematic validation work.

Technical Barriers:

Standardization remains incomplete. Variability in iPSC line selection, differentiation protocols, culture conditions, and endpoint measurement creates challenges for cross-study comparison and meta-analysis. No current regulatory guidance specifies required characterization of iPSC-derived cell populations.

Furthermore, the absence of standardized protocols for iPSC maintenance and differentiation across laboratories introduces significant inter-laboratory variability that hampers comparability and meta-analysis. The recently established International Stem Cell Banking Initiative has begun to address this by developing consensus standards for iPSC characterization, quality control, and banking practices, though widespread adoption and compliance remain ongoing challenges. Addressing these standardization gaps is essential for achieving the reproducibility required for regulatory acceptance and for building the large, harmonized datasets necessary for AI-driven predictive modeling.

9.3. Optimal Integration Strategy

The most realistic near-term application of iPSC models employs them within integrated testing strategies:

1. Tier 1 (Rapid Screening): Computational QSAR predictions and/or iPSC-based screening identify likely hazardous chemicals and populations at risk.
2. Tier 2 (Mechanistic Investigation): iPSC models with multi-omics profiling elucidate mechanisms of toxicity, identify biomarkers, and inform targeted further testing.
3. Tier 3 (Targeted Animal Studies): Hypothesis-driven animal studies address remaining questions about systemic toxicity, pharmacokinetics, and cross-species extrapolation.

4. Tier 4 (Regulatory Integration): iPSC data, mechanistic findings, and animal study results are synthesized into regulatory submissions and risk assessments.

This integrated approach reduces animal use (addressing ethical concerns), accelerates identification of hazardous chemicals (improving public health), and generates mechanistic knowledge enabling prediction of effects in vulnerable populations.

9.4. Comparison of Technologies: Established vs. Future Perspectives

This manuscript has carefully distinguished between currently established iPSC-based methods with demonstrated efficacy and future perspectives remaining speculative:

Established Technologies:

- iPSC generation and banking.
- Differentiation to major cell types (hepatocytes, neurons, cardiomyocytes, epithelial cells).
- Standard toxicology endpoints (viability, apoptosis, marker gene expression).
- Basic multi-omics profiling (RNA-seq, metabolomics).
- Integration into in vitro test battery for hazard identification.

Promising but Requiring Further Development:

- Organ-on-chip platforms with full physiological replication.
- Robust chronic exposure models.
- Reliable digital-twin predictions.
- Regulatory acceptance and standardized validation.

Speculative/Future Perspectives:

- Complete replacement of animal toxicology studies.
- Precise individual-level risk prediction from genetic data alone.
- Real-time monitoring using implanted iPSC-derived tissues.

This explicit distinction ensures that readers appreciate current capabilities while maintaining realistic expectations about timeline to practical implementation.

10. Conclusions

Human induced pluripotent stem cell technology has achieved remarkable maturity, offering unprecedented opportunities for environmental toxicology research. The integration of recent methodological advances (2022–2025) including improved differentiation protocols, organ-on-chip systems, multi-omics profiling, and artificial intelligence with foundational knowledge from earlier studies (2013–2021) creates a powerful platform for mechanistic understanding of how environmental exposures affect human health.

Key Takeaways:

1. iPSC models excel at mechanism investigation and preliminary hazard identification, providing human-relevant data superior to traditional animal models and immortalized cell lines for predicting toxicant effects on specific human cell types.
2. Integration of genetic diversity enables investigation of differential environmental susceptibility, with implications for environmental justice and precision medicine.
3. Multi-omics profiling reveals systems-level alterations in cellular function, enabling identification of biomarkers and mechanistic pathways disrupted by chemical exposure.
4. Digital twin concept and AI integration represent the frontier, with early successes in predictive toxicology pointing toward substantial future impact on regulatory efficiency.
5. Persistent limitations including absence of immune components, vascular integration, and microbiota interactions require continued methodological development.
6. Regulatory pathway toward widespread acceptance requires sustained validation efforts, standardization, and stakeholder collaboration; realistic timeline is 5–10 years for routine regulatory implementation.

7. Optimal strategy employs iPSC models within integrated testing approaches, reducing animal use while accelerating hazard identification and mechanistic understanding.

Future Research Priorities:

- Development of robust, standardized protocols for immune cell differentiation and integration with tissue-specific models.
- Creation of physiologically realistic organ-on-chip platforms with vascularization, multiple tissue types, and appropriate mechanical cues.
- Validation of QIVIVE and BMD approaches for translating iPSC data to human health risk assessment.
- Prospective validation studies comparing iPSC predictions with independent toxicology data.
- Systematic development of digital-twin models with demonstrated predictive accuracy across multiple chemical classes.
- Regulatory engagement and case study development demonstrating practical implementation.

Final Perspective:

Environmental toxicology stands at an inflection point. iPSC technology, when thoughtfully integrated with complementary approaches and implemented within robust validation frameworks, offers genuine potential to improve human health by accelerating identification of harmful exposures and understanding of susceptible populations. The next decade will be critical in translating scientific advances into practical regulatory implementation. Sustained commitment from academic researchers, industry partners, and regulatory agencies will determine whether iPSC platforms achieve their transformative potential.

Author Contributions

K.R. conceptualized the study, conducted the primary literature search, and wrote the original draft. S.S. contributed to data curation, methodology, and manuscript review. A.K. supervised the project, provided critical revisions and edits, and was responsible for the final manuscript. All authors have read and approved the final version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable. This study is a review of existing literature and did not generate new data.

Acknowledgments

The authors wish to thank Yashraj Biotechnology Ltd. for the institutional support that made this work possible. This includes access to essential scientific literature, digital resources, and the provision of dedicated time for research and writing. Such support was critical to the successful preparation of this review article.

Conflicts of Interest

The authors declare no conflict of interest.

AI Use Statement

During the preparation of this manuscript, the authors used Perplexicity and Chatly solely for language refinement. No AI tools were used for data analysis, interpretation, or generation of scientific content. All outputs were critically reviewed and edited by the authors. The authors take full responsibility for the integrity and accuracy of the work.

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