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Editorial

LEVERAGING INTEGRATIVE TECHNOLOGIES TO TRANSLATE STEM CELL AND CELL REPROGRAMMING POTENTIAL FOR NEURODEGENERATIVE DISEASES

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Abstract

Keywords:

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Stem cell technology has a rich history spanning several decades and has evolved significantly since its inception in the mid-20th century. A pivotal moment occurred in 2006 when Shinya Yamanaka's laboratory at Kyoto University successfully developed induced pluripotent stem cells (iPSCs), marking a paradigm shift in cell biology (Takahashi and Yamanaka, 2006). This breakthrough specifically addresses the ethical concerns associated with using embryonic stem cells (ESCs) (Moradi *et al*., 2019) and paves the way for developing treatments for various diseases where effective therapies are currently lacking. In 2018, Japan launched its first clinical trial utilising iPSC-derived neurons to treat Parkinson's disease (PD). This initiative is part of broader research efforts focused on neurodegenerative disorders, including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (Takahashi, 2020). Clinical trials involving iPSC-based therapies are currently being conducted for various diseases in countries such as Australia, China, and the USA. However, none of these trials have received regulatory approval, mainly due to concerns regarding safety and efficacy. Safety issues, particularly tumorigenicity, arise from residual stemness and genetic abnormalities encountered during iPSC generation. Additionally, the complex nature of neurodegenerative diseases, the microenvironmental factors that influence cell fate post-transplantation, and the inherent variability in the purity of iPSC-derived neurons presents significant challenges to treatment efficacy. These obstacles underscore the need for further optimisation and innovative strategies to enhance treatment outcomes.

Organoids represent a significant breakthrough in cell biology, serving as three-dimensional (3D) organ mimetics derived from stem cells grown *in vitro*. They open new avenues for research and therapeutic solutions. In 2008, Hans Clevers' laboratory developed intestinal organoids from mouse intestinal stem cells (Sato *et al*., 2009). This was followed by significant advancements in developing cerebral organoid cultures, which effectively mimic brain tissue *in vitro* (Lancaster *et al*., 2013). Subsequent studies have successfully generated organoid models for neurodegenerative diseases (Zhao *et al*., 2020; D'Sa *et al*., 2023). However, these models have common limitations, including a lack of vascularisation, size constraints, and variability between batches. They cannot fully replicate the complexity of the human brain due to missing crucial cell types, such as immune cells, and their inability to reproduce the microenvironmental cues present in mature or diseased brain states. The introduction of assembloids, which combine different organoids and cell types, marks a significant advancement in replicating the complex architecture and functionality of native tissues. Nevertheless, this increased complexity can lead to variability in outcomes, and the technical requirements for culture and maintenance remain challenging. Regarding the rejuvenation phenomenon during reprogramming, current iPSC-derived brain organoid models primarily reflect the early stages of human brain development (Pitrez *et al*., 2024). As a result, they may not accurately capture the pathophysiological characteristics of neurodegenerative diseases, such as AD, in older individuals. Despite these limitations, organoids and assembloids hold promise for personalised medicine applications, including

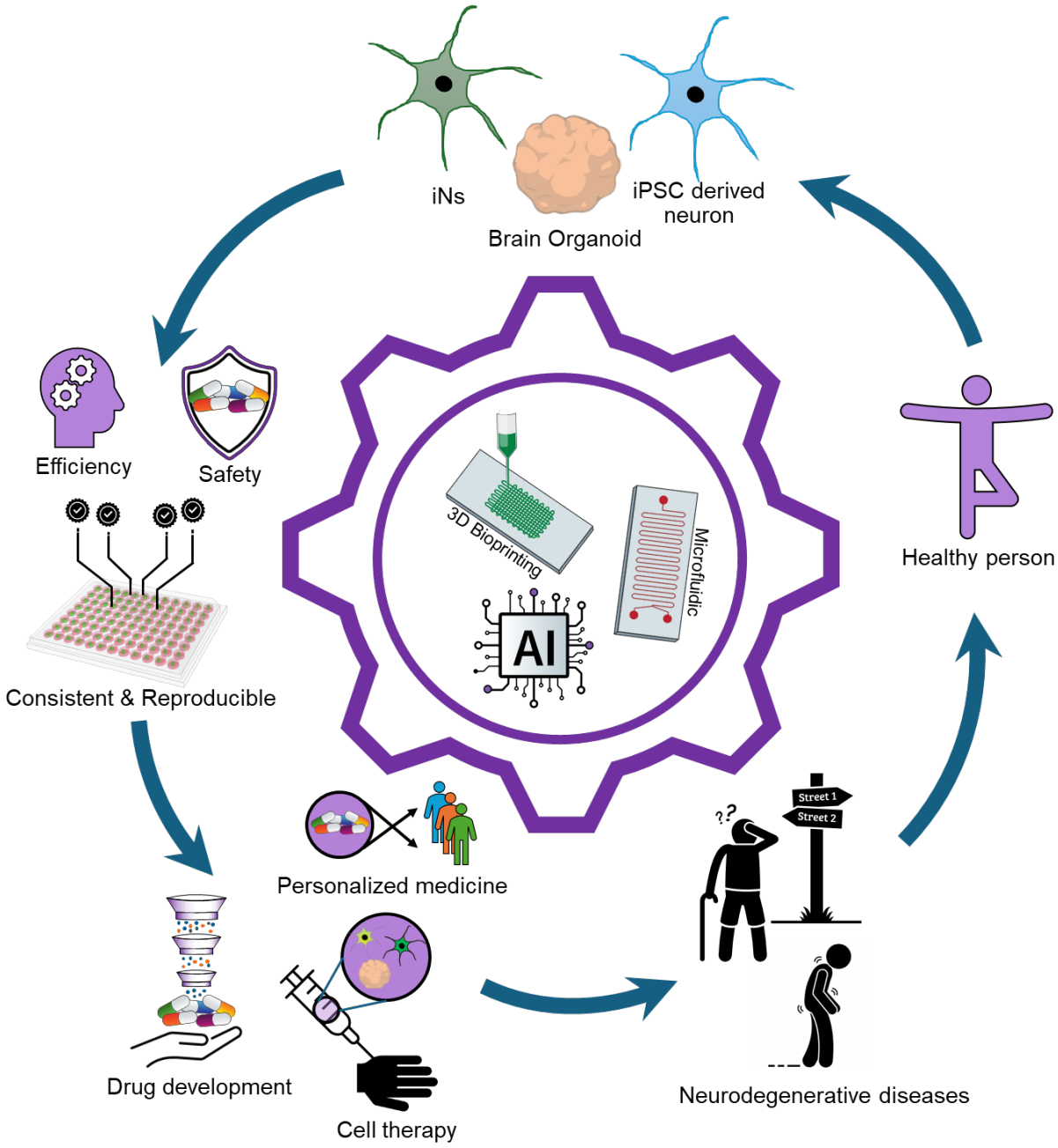

Fig. 1. Integrative technologies enhance the efficiency, safety, and reproducibility of stem cell research and cell reprogramming to treat neurodegenerative diseases. Microfluidics enables precise control of the microenvironment. Three-dimensional (3D) bioprinting facilitates the creation and manipulation of complex structures. Artificial intelligence (AI) and machine learning (ML) enable managing and analysing large datasets and facilitate automated manufacturing processes. iNs, induced neurons; iPSC, induced pluripotent stem cell.

diseases like cystic fibrosis and neurodegenerative conditions.

Additionally, organoids can serve as transplantation materials, offering an advanced alternative for cell therapy by closely mimicking the structure and function of real organs. This approach may reduce the risk of tumour formation and enhance cell viability compared to traditional therapies (Cao *et al*., 2023). Furthermore, organoid transplantation can facilitate more accurate modelling of the human brain, improving our understanding of neurodegenerative disorders and increasing the likelihood of successful drug development. However, ethical concerns arise regarding the creation of chimeric entities that may possess consciousness (Revah *et al*., 2022). Concurrently, advances in stem cell technology have led to direct neuronal reprogramming (dNR) techniques, representing a transformative approach in neuroscience. By modulating specific neural lineage transcription factors, these methods enable the conver-

sion of somatic cells, such as fibroblasts or astrocytes, into induced neurons (iNs) (Harati *et al*., 2023). Unlike iPSCs, iNs bypass a stem cell intermediate, reducing tumorigenesis risk. Additionally, dNR can be performed *in vivo*, allowing the conversion of local astrocytes or glial cells into functional neurons within the brain (Zhou *et al*., 2020). This technique can potentially restore neuronal function directly at the site of injury or degeneration. Despite challenges related to dNR conversion efficiency and the functional maturity of iNs for therapeutic applications, these cells exhibit characteristics of the aged state, making them valuable for modelling age-related neurodegenerative diseases (Sun *et al*., 2024).

An interdisciplinary approach integrating advanced technologies is essential for overcoming the challenges of translating established knowledge in stem cells and cell reprogramming into effective treatments for neurodegenerative diseases.

Microfluidic systems enable precise control over the cellular microenvironment by regulating media flow dynamics, shear stress, and chemical gradients. This capability enhances reproducibility and facilitates the development of conditions that mimic *in vivo* environments. For instance, a microfluidic chamber device integrated with a decellularised human brain extracellular matrix (ECM) can create brain-like microenvironments. Coupled with the brain-specific biochemical and mechanical cues from the ECM, the gravity-driven flow system optimises conditions, significantly promoting the structural and functional maturation of human brain organoids (Cho *et al*., 2021). Moreover, the simultaneous culture of multiple cell types within a microfluidic system enhances the study of their interactions and better simulate *in vivo* microenvironments. This is particularly beneficial in drug development, as demonstrated by a microfluidic co-culture system mimicking ALS disease through the interaction of neurons and muscle cells (Stoklund Dittlau *et al*., 2021). Additionally, microfluidic systems can be integrated into manufacturing processes, as evidenced by the controlled fusion of brain organoids into assembloids, meeting requirements for uniformity and scalability (Zhu *et al*., 2023).

3D bioprinting offers precise control over the spatial arrangement of cells and biomaterials. Tailoring the composition and architecture of printed constructs enhances their functionality and reproducibility across various manufacturing processes. A notable example is the Orthogonally Induced Differentiation (OID) method, which has successfully generated vascularised and patterned cortical organoids. This approach employs 3D bioprinting with differentiation-programmed stem cells to establish specific neural regions spatially, improving the reproducibility of complex multicellular tissue-like structures (Skylar-Scott *et al*., 2022). The potential of 3D bioprinting is further explored in efforts to replicate the intricate and functional complexity of the brain, focusing on the spatial printing of cells and organoids to construct functionally active and structurally analogous brain-like tissues (Roth *et al*., 2023; Yan *et al*., 2024).

Artificial intelligence (AI) and machine learning (ML) technologies, while still in their early stages, show significant promise in managing large, complex biological datasets such as multi-omics and high-content imaging data. For example, applying machine learning to immunostaining images of neurons derived from patient-specific iPSCs could help predict mechanistic subtypes of PD (D'Sa *et al*., 2023). These advancements promote personalised and precision medicine by enabling more accurate and rapid treatment adjustments based on individual disease mechanisms. AI and ML enhance the efficiency and effectiveness of drug discovery, particularly through AI-driven high-throughput screening (HTS) processes. A notable example is the development of a deep learning-based neural organoid platform, which effectively addresses the challenges of HTS and the complexity of high-dimensional organoid image data, demonstrating its potential for drug screening in HD models (Metzger *et al*., 2022). Furthermore, AI and ML integration for cellular characterisation during manufacturing has been demonstrated in various studies, such as identifying and selecting human iPSC colonies for clonal expansion through image analysis combined with robotic systems (Powell *et al*., 2023). These automated manufacturing systems improve reproducibility and reduce safety risks associated with manual processes.

The application of stem cell and cell reprogramming technologies has transformed our understanding of cell fate mechanisms and opened new avenues for treating neurodegenerative diseases. However, several challenges remain in translating these scientific advances into effective clinical applications. Integrating advanced technologies—such as biotechnology, microfluidics, 3D bioprinting, and AI/ML—present multiple potential solutions to address these challenges, including safety, reproducibility, and efficiency (Fig. 1).

List of Abbreviations

iPSCs, induced pluripotent stem cells; PD, Parkinson's disease; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; HD, Huntington's disease; dNR, direct neuronal reprogramming; OID, Orthogonally Induced Differentiation; 3D, three-dimensional; AI, artificial intelligence; ML, machine learning; HTS, high-throughput screening; ESCs, embryonic stem cells; iNs, induced neurons; ECM, extracellular matrix.

Availability of Data and Materials

Not applicable.

Author Contributions

JH and PYW conceived the idea. JH wrote the initial draft. JH and PYW revised the final version. All authors read and approved the final manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. PYW is serving the Editorial Board members of this journal. We declare that PYW had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to YL.

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