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Review

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Human pluripotent stem cell-based PluriXcel technology platforms provide stem cell treatment development and manufacturing innovations for progressing to the clinic

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Abstract

To date, the existing markets lack a clinically suitable human neuronal cell source or cardiomyocyte source with adequate regenerative potential, which has been the major setback in developing safe and effective cell-based therapies for neurological and heart diseases. Pluripotent human embryonic stem cells (hESC), the nature source of human pluripotent stem cells (hPSC), offer a practically inexhaustible source of replacement cells to heal the damaged or lost tissues that have naturally limited capacity for renewal, such as the human brain and the human heart. Neurological and heart diseases incur exorbitant costs on the healthcare system worldwide; therefore, there is a strong focus on translating hESC research to provide newer and more efficient solutions for these therapeutic needs. However, a major bottleneck for clinical translation of hESC research is to enable a well-controlled efficient induction of hPSC to a particular clinically relevant lineage. Emerging hPSC-based PluriXcel technology platforms enable large scale production of high quality clinical-grade hPSC lines and direct conversion of such non-functional pluripotent cells by small molecule induction into a large commercial scale of functional human neuronal or cardiomyocyte derivatives, which not only constitutes clinically representative progresses in both human neuronal and cardiac therapeutic products for treating a wide range of incurable or hitherto untreatable neurological and heart diseases, but also offer manufacturing innovations for production scale up and creation of replacement tissue or organ products. PluriXcel technology provides scalable platforms to ensure a high degree of efficacy and safety of hESC-derived cellular products, thus robust clinical benefits leading to therapies, and for cGMP manufacturing of large quantities of high quality clinical grade hESC-based cell therapy products to support clinical trials. PluriXcel technology platforms introduce stem cell treatment development and manufacturing innovations to achieve clinically-useful and therapeutically-viable levels of safety and efficacy in cell therapy product development, providing unique clinical-grade translating capacities for moving stem cell research from current studies in animals into human trials.

Keywords: regenerative medicine; stem cell technology; human embryonic stem cell; pluripotent; neuronal progenitor; cardiac precursor; cardiomyocyte; cell therapy; stem cell treatment; neurological disease; heart disease

Introduction

The limited capacity of neuron circuitry and cardiomyocytes for self-repair constitutes a significant challenge in both the scientific and clinical communities. Given the limited capacity of the CNS and the heart for self-repair, transplantation of human stem cell therapy derivatives represents a promising therapeutic approach closest to provide a cure to restore the damaged or lost tissue and function. However, to date, the available sources for clinically suitable engraftable human stem/ progenitor/ precursor cells with adequate neurogenic or cardiogenic potential remain lacking, which has been the major setback in developing safe and effective cell-based therapies. Traditional sources of cells for therapy in existing markets have been adult stem cells isolated from tissues or artificially reprogrammed from adult cells or fibroblasts, which all have the historical shortcomings of limited capacity for renewal and repair, accelerated aging, and immune-rejection following transplantation. Despite some beneficial outcomes, traditional sources of human neural or cardiac stem cells (hNSC or hCSC) appeared to exert their therapeutic effect primarily by their non-functional progenies through producing trophic and/or protective molecules to rescue endogenous host cells or through

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enhancing endogenous repair, but not related to graft dependent regeneration or repair [1–6]. Moreover, current artificially reprogramming techniques, including induced pluripotent stem cells (iPS cells), transdifferentiation, dedifferentiation, or cloning, have not been able to resolve the genomic abnormality and instability of reprogrammed, induced, transdifferentiated, dedifferentiated, or cloned adult cells, which are associated with high risk of cancerogenesis and, thus have severely limited their utility as viable therapeutic approaches.

Traditional cell therapies based on tissue-derived human multipotent stem cells (hMSC) have encountered supply restriction and difficulty to use in the clinical setting due to their declining plasticity with aging and limited expansion ability, making it difficult for production scale up in clinical translation, thus restricting hMSC as an adequate source for graft materials. By contrast, the intrinsic ability of a pluripotent human embryonic stem cell (hESC), the nature source of human pluripotent stem cells (hPSC), for both unlimited self-renewal and unrestricted differentiation into clinically-relevant lineages makes it a practically inexhaustible source of replacement cells for human tissue and function restoration. Therefore, it has been regarded as an ideal source to provide a large supply of functional human cells to heal the damaged or lost tissues that have naturally limited capacity for renewal, such as the human heart and brain. As neurological and heart diseases incur exorbitant costs on the healthcare system worldwide, there is a strong focus on translating hESC research to provide newer, more efficient solutions for these therapeutic needs. Although a vast sum of government and private funding has been spent on looking for adult alternates, such as reprogramming and transdifferentiation of fibroblasts or mature tissues, so far, only human stem/ precursor/ progenitor cells derived from embryo-originated pluripotent hESC have demonstrated such cellular pharmacologic utility and capacity adequate for central nervous system (CNS) and myocardium regeneration in pharmaceutical development of stem cell therapy for the damaged CNS and heart [1–6].

Pluripotent hESC

Pluripotent hESC themselves are unspecialized non-functional cells that cannot be used directly for therapeutic applications. It has been recognized that pluripotent hESC must be turned into fate-restricted specialized functional cells, a process known as differentiation, before use for cell therapy. However, conventional hESC differentiation approaches use germ-layer induction of pluripotent cells, resulting in uncontrollable and unpredictable simultaneous multi-lineage differentiation that yields embryoid bodies (EB) or aggregates consisting of a mixed population of cell types of three embryonic germ layers, among which only a very small fraction of cells display targeted differentiation [1–6]. Those hESC multi-lineage differentiation approaches are laborious, costly, and need time-consuming purification, isolation, or selection procedures to generate only a small quantity of desired cells, impractical for commercial and therapeutic applications [1–6]. Inefficient, instable, and incomplete hESC differentiation through conventional multi-lineage differentiation approaches often results in poor performance and high tumor risk of such cell derivatives and tissue engineering constructs following transplantation [1–6]. Under conventional protocols presently employed in the field, hESC-derived cellular products consist of a heterogeneous population of mixed cell types, including fully differentiated cells, high levels of various degrees of partially differentiated or uncommitted cells, and low levels of undifferentiated pluripotent cells, posing a constant safety concern when administered to humans [1–6], also see FDA briefing document and transcript of the April 2008 Cellular, Tissue and Gene Therapies Advisory Committee meeting discussion regarding safety concerns for the development of cell therapy products derived from hESC. Therefore, in order to realize the therapeutic potential of hESC, a persistent challenge for investigators is to enable a well-controlled and efficient induction of non-functional pluripotent hESC exclusively and uniformly to a specific, clinically relevant lineage. These aspects play a key role not only for tissue or organ engineering and regenerative cell-based therapy, but also for developmental studies, drug discovery and development.

Over the past decade, remarkable advancements have taken place in stem cell research related to the differentiation of hESC into specific lineages. One of the major achievements for clinical translation of hESC research is the establishment of unique human stem cell technology platforms – PluriXcel technology platforms, which have been developed for biologics-free defined culture systems for *de novo* derivation and maintenance of clinical-grade hESC lines, through the PluriXcel-DCS technology platform, and for lineage-specific differentiation of hESC by small molecule induction via the PluriXcel-SMI technology platform [1–21]. PluriXcel approach, which overcomes the shortcomings of traditional hPSC multi-lineage differentiation approaches, offers the benefits in efficiency, stability, safety, efficacy, and large scale production or manufacturing of high quality clinical-grade human cell therapy products in cGMP facility for commercial and therapeutic uses over all other existing approaches. The hESC cell therapy derivatives of PluriXcel approach are homogenous populations of lineage-committed cells with high potency for human tissue or organ (e.g., CNS neuron circuitry, myocardium) regeneration, and contain no residual pluripotent cells and other cellular impurity of safety concerns, dramatically increasing the clinical efficacy of graft dependent repair and safety of hESC-derived cellular products for scale up regeneration in the clinical setting, distinctly different from traditional sources of human stem cells either isolated from tissues *in vivo* or derived from hPSC *in vitro*.

PluriXcel technology

PluriXcel technology platforms not only constitute clinically representative progresses in both human neuronal and cardiac therapeutic products for treating a wide range of incurable or hitherto untreatable neurological and heart diseases, but also build the platform for 3D CNS or myocardial replacement tissue or organ assembly from hESC neuronal or cardiomyocyte derivatives. Medical innovations of PluriXcel technology provide scalable platforms to ensure a high degree of efficacy and safety of hPSC-derived cellular products for treating major human

diseases challenging traditional medicine, which represent the next generation of human cell therapy products, offering purity, large scale production, high quality, safety, and effectiveness for commercial and therapeutic uses over all other existing human cell sources. Manufacturing innovations of PluriXcel technology provide scale up cGMP manufacturing capability or techniques for production of large quantities of high quality clinical-grade hPSC-based cell therapy products to support clinical trials and tissue or organ engineering, improving the availability, reproducibility, accessibility, and standardization of manufacturing materials, technologies, and processes to create human repairing or replacing cell, tissue, and organ products.

PluriXcel technology enables well-controlled high efficient direct conversion of non-functional hESC by small molecule induction into a large commercial scale of clinical-grade high quality functional human neuronal or cardiomyocyte derivatives adequate for CNS or heart regeneration, presenting the hESC cell therapy derivatives as a powerful pharmacologic agent of cellular entity for a wide range of CNS or heart diseases [1–21]. It provides advancements over current human stem/ progenitor/ precursor-derived cell type culturing and selectivity approaches through improvements in neuron or cardiomyocyte differentiation efficiencies, CNS or myocardial cellular composition, and CNS circuitry or myocardial tissue contractile function by utilization of biologics-free defined culturing and small molecule induction at the pluripotent stage. PluriXcel technology provides scalable platforms to ensure a high degree of efficacy and safety of hESC-derived cellular products, thus robust clinical benefits leading to therapies, and for cGMP manufacturing of large quantities of high quality clinical-grade hESC-based cell therapy products to support clinical trials.

The approach of PluriXcel technology is unconventional and exceptionally innovative, fundamentally different from traditional hESC multi-lineage differentiation approaches using germ-layer induction of pluripotent cells [1–21]. The novelties of PluriXcel approach includes: 1. using small molecule to induce well-controlled high efficient lineage-specific differentiation of hESC direct at the pluripotent stage, without going through a multi-lineage EB stage; 2. enabling high efficient direct conversion of non-functional hESC into a large supply of functional neuronal or cardiomyocyte fate-restricted derivatives for CNS or heart repair in the clinical setting, a major milestone towards human trials or first-in-human studies of hESC cell therapy products; 3. providing adequate sources of human neuronal or cardiomyocyte products in high purity and large quantity for neuronal or myocardium regeneration or repair in treating neurological or heart disorders; 4. the hESC neuronal or cardiomyocyte derivatives of PluriXcel approach are a homogenous population of neuronal or cardiomyocyte lineage-committed cells with high potency for human neuron circuitry or myocardium regeneration *in vitro* and *in vivo*, and contain no residual pluripotent cells and other cellular impurities of safety concerns, distinctly different from traditional sources of hNSC or hCSC either isolated from tissues *in vivo* or derived from hPSC *in vitro*; 5. the hESC neuronal or cardiomyocyte derivatives of PluriXcel approach dramatically increase the clinical efficacy of graft-dependent repair and safety of hESC-derived cellular products, distinctly different from traditional sources of human stem cells that exert their therapeutic effect primarily by enhancing endogenous repair, but not related to regeneration or repair from the graft; 6. overcoming the shortcomings of traditional hPSC multi-lineage differentiation approaches, which are unpredictable, uncontrollable, unrepeatable, low-efficiency, phenotypic heterogeneity and instability, high risk of tumor and/or inappropriate cell type formation following transplantation, and require laborious, costly, and time-consuming purification or isolation procedures to generate only a small quantity of desired cells, impractical for commercial and clinical applications; 7. offering the benefits in efficiency, stability, safety, efficacy, and large scale production of high quality clinical-grade human cell therapy products in cGMP facility for commercial and therapeutic uses over all other existing approaches [1–21].

PluriXcel technology platforms introduce stem cell treatment development and manufacturing innovations to achieve clinically-useful and therapeutically-viable levels of safety and efficacy in cell therapy product development, providing unique clinical-grade translating capacities for moving stem cell research from current studies in animals into human trials. In particular, PluriXcel-DCS technology platform provides biologics-free, including feeder-free xeno-free conditioned-medium-free serum-free, defined culture systems for well-controlled efficient derivation, maintenance, and differentiation of clinical-grade high quality hPSC lines. PluriXcel-DCS platform allows all poorly-characterized and unspecified biological components and substrates in the culture system, including those derived from animals, to be removed, substituted, and optimized with defined human alternatives for *de novo* derivation and long term maintenance of cGMP-quality xeno-free stable hESC lines and their human cell therapy derivatives, which have never been contaminated with animal cells and proteins, thus suitable for therapeutic development and clinical applications [1–7, 15, 16].

PluriXcel-SMI technology platforms enable high efficient neural or cardiac lineage-specific differentiation direct from the pluripotent stage of hESC using small molecule induction, which is a major milestone towards clinical application of hESC cell therapy derivatives, offering the benefits in efficiency, stability, safety, efficacy, and large scale production of high quality clinical-grade human stem cell therapy products in cGMP facility for commercial and therapeutic uses over all other existing approaches [1–14]. PluriXcel-SMI technology platforms currently include PluriXcel-SMI-Neuron technology platform and PluriXcel-SMI-Heart technology platform. PluriXcel-SMI-Neuron technology platform enables high efficient direct conversion of pluripotent hESC into large quantities of high quality neuronal progenitors and functional neuronal cells adequate for clinical development of safe and effective stem cell therapies for a wide range of neurological disorders, as well as adequate for CNS tissue engineering and regeneration [1–12]. PluriXcel-SMI-Heart technology enables high efficient direct conversion of pluripotent hESC

into large quantities of high quality heart precursors and functional cardiomyocytes (heart muscle cells) adequate for clinical development of safe and effective stem cell therapies for heart disease and failure, as well as adequate for myocardial tissue engineering and regeneration [1–7, 13, 14].

PluriXcel technology can be used to generate human stem/ progenitor/ precursor cells and clinically-relevant functional cells direct from hPSC --- PluriXcel therapeutic product prototypes of clinical-grade human stem cell therapy products, currently include high quality clinical-grade human neuronal progenitors (Xcel-hNuP) and human neurons (Xcel-hNu) for CNS neuron regeneration and high quality clinical-grade human heart precursors (Xcel-hCardP) and human heart muscle cells (Xcel-hCM) for contractile heart muscle regeneration [1–14], which represent the next generation of human cell therapy products, offering purity, large scale production, high quality, safety, and effectiveness for commercial and therapeutic uses over all other existing cell sources. Xcel prototypes offer cellular medicine or cell therapy products targeting therapeutic sectors for a wide range of neurological disorders and cardiovascular disease, including Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), Alzheimer disease, motor neuron diseases, neurodegenerative diseases, stroke, brain and spinal cord injuries, heart disease and failure, myocardial infarction (MI), cardiomyopathy, ischemic heart disease, and congestive heart failure. PluriXcel novel human stem cell technology platforms enable large scale production or manufacturing of high quality clinical-grade human neuronal and heart muscle cell therapy products as cellular medicines that can offer pharmacologic utility and capacity adequate for CNS and heart regeneration. Currently, Xcel prototypes of human neuronal and cardiomyocyte cell therapy derivatives or products, including Xcel-hNuP (human neuronal progenitors), Xcel-hNu (human neurons), Xcel-hCardP (human heart precursors), Xcel-hCM (human heart muscle cells), are the only available human cell sources in large commercial scales with adequate cellular pharmacologic utility and capacity to regenerate the CNS neurons and contractile heart muscles, vital for CNS and heart repair for a wide range of neurological and cardiovascular diseases in the clinical setting.

PluriXcel technology breakthrough has addressed key industry challenges to traditional medicine, offering a new drug of cellular entity capable of novel pharmacological activity associated with human tissue and function restoration. Clinical applications of stem cell therapy derivatives have demonstrated successful alternatives for a wide range of incurable or hitherto untreatable neurodegenerative and heart diseases, which cost the worldwide healthcare system more than $500 billion annually. Due to the prevalence of those diseases worldwide, there is a large unmet healthcare need to develop hESC-based therapeutic solutions to provide optimal regeneration and reconstruction treatment options. Nevertheless, a limiting factor in stem cell research is present because of the lack of a clinically suitable source of engraftable human stem/ progenitor/ precursor cells with adequate functional nerve tissue regenerative potential. Novel solutions to this issue are crucial for developing safe and effective cell-based therapies for regeneration of the damaged CNS structure and circuitry evidenced in various neurological disorders. Regarding cardiovascular research, the absence of a clinically suitable human cardiomyocyte source with adequate myocardium regenerative potential is also a major drawback for achieving damaged human heart regenerative solutions. PluriXcel technology has resulted in remarkable advancements in stem cell research related to the differentiation of hPSC into specific lineages by small molecule induction [1–21]. Among such small molecules, retinoic acid induces the specification of neuroectoderm directly from hPSC, while triggering progression to neuronal progenitors, then ventral neurons [1–12]. Nicotinamide, on the other hand, induces the specification of cardiomesoderm directly from hPSC, thus triggering progression to cardiac precursors, then beating cardiomyocytes [1–7, 13, 14]. These advancements brought by PluriXcel technology breakthroughs not only constitute clinically representative progress in both human neuronal and cardiac therapeutic products for CNS and myocardium repair, but also build the platform for 3D CNS or myocardial tissue assembly from hESC neuronal or cardiomyocyte derivatives.

PluriXcel technology is shaping the future of medicine by providing novel, unique solutions at a clinical grade. PluriXcel technology has achieved a highly efficient direct conversion of hPSC into a large supply of high purity clinical-grade neuronal cells or heart muscle cells (Xcel), conserving their adequate capacity to regenerate neurons and contractile heart muscles, which builds the foundation for the development of safe and effective stem cell therapies for a wide range of incurable or hitherto untreatable neurodegenerative, neurological, and heart diseases, thus address major concerns in the healthcare industry. Pioneer in stem cell treatment development and manufacturing technology innovation platforms - novel PluriXcel technology platforms for efficiently converting hPSC uniformly into a particular clinically relevant lineage by small molecule induction - have presented pluripotent hESC as currently the only available human cell source uniquely offering cell therapy products with adequate pharmacological capacity to regenerate neurons and contractile heart muscles that allow reconstitution of function of the CNS and heart for repair in the clinic, overcoming some major issues in bringing hESC therapy derivatives toward clinical applications.

Human stem cell therapy products

Human stem cell therapy products of PluriXcel technology represent a new type of drug of cellular medicine capable of providing novel pharmacological utility for human tissue and function restoration. PluriXcel technology comprises PluriXcel-DCS, the defined culture system for derivation and maintenance of clinical-grade hPSC lines, and PluriXcel-SMI, the highly efficient lineage-specific differentiation of hPSC [1–21]. PluriXcel-SMI utilizes small molecule induction for direct conversion of hPSC into large sources of high purity human neuronal or heart muscle cells, highly suitable for developing novel, safe, and cost-effective stem cell therapies. Since the costs of cGMP-compliance and subsequent clinical phases in the registration pipeline

are very high, PluriXcel technology provides reproducible and scalable platforms to deploy established high quality clinical-grade stable normal hPSC lines and cGMP-compatible manufacturing processes for hPSC-derived cell therapy products showing therapeutically-viable levels of *in vivo* high efficacy for the targeted disease indications, thus a high probability leading to therapies qualified for FDA expedited programs to accelerate regulatory approval and patient access to new therapies, to cGMP manufacturing facility for production scale up and release of final products, which significantly accelerate the translational process, reduce development time and costs, and increase the probability of success of stem cell treatment projects in clinical trials. With proprietary human stem cell technology and therapy products to provide cellular medicines for neurological and heart diseases, PluriXcel technology has demonstrated tremendous potential for tissue and organ regeneration and function restoration. In fact, untreated diseases or diseases currently considered incurable could now find a strong alternative through the clinical applications of hPSC therapy derivatives of PluriXcel technology, which will significantly impact translational research priority by presenting hESC therapy derivatives as a viable and robust therapeutic strategy for a wide range of incurable or hitherto untreatable neurological and heart diseases and injuries challenging traditional medicine.

PluriXcel technology allows the efficient production of human neuronal progenitors and human neuronal cell types and subtypes from hPSC for neuronal regeneration and replacement therapies for a wide range of neurological disorders, as well as for CNS tissue engineering and regeneration [1–12]. Similarly, the efficient production of human cardiac precursors and human cardiomyocytes for myocardial tissue engineering as well as myocardium regeneration and replacement therapies for heart disease and failure has also been addressed [1–7, 13, 14]. PluriXcel technological breakthrough enables the well-controlled induction of hPSC directly and efficiently into CNS- or heart-related cells. Such enhancements allow the achievement of more efficient results of generating a particular clinically relevant lineage from hPSC by only mediating the simple provision of small molecules, ensuring the proliferation of undifferentiated hESC.

In regenerative medicine, pluripotent hESC research holds huge promise for treating major human diseases challenging traditional medicine. Neurodegenerative disorders, injury and paralysis, diabetes, heart failure, and cardiovascular diseases represent the major issues. Millions of people are pinning their hopes on stem cell research to provide novel and effective solutions for such major health problems. On that note, the clinical translation of stem cell research and innovation capabilities demonstrated by hESC investigations can extend the lives of patients and reduce the burden of illness. PluriXcel technology is incomparable, providing life scientists and clinicians with novel, efficient, and powerful resources and tools to address major health concerns. The introduction of novel developments and new business opportunities based on this technology are expected to revolutionize the biomedical industry and bring new therapeutics into the market.

Traditional pharmaceutical research & development (R&D) in existing markets usually starts with drug leads discovered in animals or other lower organisms, thus require lengthy and costly both demonstration in animal model testing and establishment of proof-of-concept and safety in human trials. The average cost of bringing a new drug to market for traditional pharmaceutical R&D in existing markets is approximately $1.3 billion. The average drug developed by a major pharmaceutical company in existing markets costs at least $4 billion for every drug that is approved, if adjusting that estimate for current failure rate of their drugs. The clinical applications of hESC derivatives as novel stem cell therapy products for neural and cardiac repair are new therapeutic market opportunities. Pluripotent hESC are derived from the pluripotent inner cell mass (ICM) or epiblast of the human blastocyst or embryos and, thus have both the unconstrained capacity for long term stable undifferentiated growth in culture and the intrinsic potential for differentiation into all the somatic cell types in the human body, holding tremendous potential for restoring human tissue and organ function. Therefore, unlike traditional pharmaceutical R&D, novel cellular therapeutic products generated from PluriXcel technology platforms have been developed directly with human cells or hESC with proof-of-concept already established in humans, which simplifies the development process, lowers the costs for R&D, shortens the time consumption for R&D, and increases the probability of clinical success dramatically.

Compared to conventional compound drugs of molecular entity, cell therapy products or cellular medicine have very different benchmarks or indicators regarding to safety and efficacy in clinical trials. The safety of a human stem cell therapy product is evaluated by whether it can retain a stable phenotype and karyotype for a long period of time and whether there is no tumor, inappropriate cell type formation, or abnormal regeneration following transplantation. The efficacy of a human stem cell therapy product is measured by its pharmacologic activity or cellular ability to regenerate the tissue or organ that has been damaged or lost. Therefore, the pharmacologic utility of human stem cell therapy products cannot be satisfied only by their chaperone activity of traditional sources of stem cells, if any, to produce trophic or protective molecules to rescue existing endogenous host cells that can simply be achieved by a drug of molecular entity, if any.

For successful pharmaceutical development of stem cell therapy, the human stem cell therapy product must meet certain commercial criteria in plasticity, specificity, and stability before entry into clinical trials. Moving stem cell research from current studies in animals into human trials must address such practical issues for commercial and therapeutic uses: 1. such human stem cells and/or their cell therapy derivatives/ products must be able to be manufactured at a commercial scale; 2. such human stem cells and their cell therapy derivatives/ products must be able to retain their normality or stability for a long term; and 3. such human stem cells and/or their cell therapy derivatives/ products must be able to differentiate into or generate a sufficient number of the specific cell type or types in need of repair or regeneration. Those practical

issues are essential for designating any human stem cells as a cell therapy product for filing an IND application with the FDA and entry into human trials or first-in-human studies. Breakthrough human stem cell technologies such as PluriXcel technology, including defined culture systems for *de novo* derivation and long term maintenance of clinical-grade stable hPSC lines (PluriXcel-DSC) and lineage-specific differentiation of hPSC by small molecule induction (PluriXcel-SMI), enable clinical applications of hESC therapeutic utility. PluriXcel therapeutic products – Xcel prototypes – have been developed specifically to address and overcome some major obstacles in bringing hESC therapy derivatives towards human trials or first-in-human studies, including offering the benefits in high efficiency, stability, low tumor risk, high purity, high efficacy in repair, as well as safety and large scale production or manufacturing over all other existing approaches [1–21].

Medical and manufacturing innovations

Medical and manufacturing innovations in PluriXcel technology enable high efficient direct conversion of non-functional hPSC into a large commercial scale of clinical-grade high quality functional human neuronal or heart muscle cell therapy derivatives, which is a major milestone towards human trials of hESC cell therapy derivatives. Currently, the hESC neuronal and cardiomyocyte cell therapy derivatives of PluriXcel technology platforms are the only available human cell sources in commercial scales with adequate cellular pharmacologic utility and capacity to regenerate CNS neurons and contractile heart muscles, vital for CNS and heart repair in the clinical setting. The PluriXcel technology and Xcel prototypes of clinical-grade functional human neural and cardiac cell therapy products are targeting many of those serious or life-threatening diseases or conditions, which have relied on breakthroughs in hESC research to drive the advance of medicine to provide regeneration and reconstruction treatment options or cures. PluriXcel technology platforms for large scale production of high quality clinical-grade functional human cell therapy derivatives represent emerging hESC-based regenerative medicine technology innovations that dramatically increase the clinical efficacy of graft dependent repair and safety of hESC-derived cellular products and, thus allow moving stem cell research from current studies in animals towards human trials or first-in-human studies, potentially shifting clinical practice paradigms for major health problems.

The CNS and the heart are among the first organs formed from the cells of the ICM or epiblast of the blastocyst in early embryogenesis. The embryo-originated hESC-derived neural and cardiac elements resemble the neural and cardiac cells in human embryogenesis; therefore, they have the intrinsic potential to form human CNS circuitry and contractile myocardium, respectively, in tissue-specific complex biomimetic culture systems. Indeed, embryo-originated hESC cardiomyocyte derivatives, following small molecule induction and suspension culturing, spontaneously progress and self-assemble into large beating cardiomyocyte clusters with strong rhythmic impulses reminiscent of the p-QRS-T-complexes seen from body surface electrodes in clinical electrocardiograms [1–4, 7, 12–14]. Similarly, embryo-originated hESC neuronal derivatives, following small molecule induction and suspension culturing, spontaneously progress and self-assemble into neuron circuitry reminiscent of the ventral mesencephalon [1–4, 7–12]. Although pluripotent, human iPS cells reprogrammed from adult cells or fibroblasts may reside in different developmental stages, and may not respond to developmental or inducing signals the same way as the hESC do. In fact, the same small molecules do not induce cardiomesodermal or neuroectodermal differentiation of human iPS cells, nor trigger their progression to cardiomyocytes or neurons, even though previous reports show small molecule inhibitors of Wnt signaling can promote human iPS cells and certain reporter engineered-hESC lines differentiate into cardiomytocytes [22]. In addition, serious spontaneous mutations were recently identified in human iPS cell clinical trials, which now require iPS cells to be used as allogeneic rather than autologous transplants, further suggesting that human iPS cells, which are made with the same mechanisms of oncogenesis by introducing or activating oncogenes in adult cells, are intrinsically prone to mutations and genomic instability that could largely compromise their translational value [23].

PluriXcel technology breakthrough transforms non-functional hPSC uniformly into a large supply of functional neuronal or cardiomyocyte derivatives for CNS or heart regeneration, providing a vital human neuronal or cardiac cell source for CNS or myocardial tissue engineering. PluriXcel technology platforms pioneer next generation manufacturing techniques for repairing or replacing cells, improving the availability, reproducibility, accessibility, and standardization of manufacturing materials, technologies, and processes to create replacement tissue and organ products or models. Development and utilization of complex 3D multi-cellular replacement tissue and organ products or models of human embryogenesis and organogenesis will provide a powerful tool that enables analysis under conditions that are tightly regulated and authentically representing the *in vivo* spatial and temporal patterns and, thus reduce the reliance on animal models to test potential therapeutic strategies. It will go beyond flat biology to increase the biological complexity of human-based *in vitro* models and assays to mimic the *in vivo* human organ systems and functions, which are controllable, reproducible, and scalable, and can be monitored and validated against responses on multiple hierarchical levels. PluriXcel technology has built the platform for further development of much-demanded reliable tissue-specific complex biomimetic culture systems for 3D CNS and myocardial tissue assembly from hESC neuronal and cardiomyocyte derivatives that can replicate the aspects of the human CNS and the human heart, respectively. Such programs will lead to the development and commercialization of much-demanded reliable tissue-specific bioreactors for 3D CNS and heart assembly from hESC neuronal and cardiomyocyte derivatives, respectively, providing novel hESC-based regenerative medicine technology and products or tissue-engineered constructs. It will provide groundbreaking technology platforms for reconstruction of functional tissues and organs in a 3D setting that reflect the biological complexity and microenvironment niche of the *in vivo* human organ

system to address unmet medical needs. It will lead to advances in technologies used in the regulatory review of medical products, including drugs, biologics, and devices, and, thus improvements of regulatory practice.

Organogenesis depends on cell-cell interactions and may require non-cellular guidance, such as matrix and scaffold formation, which provides structural templates for cell attachment and tissue growth, directing 3D organization of the cells. Advancements in micro- and nano-fabrication techniques offer the possibility for highly reproducible mass-fabrication of systems with complex geometries and functionalities [24–26]. Tissue-specific extracellular matrix (ECM) gels can now create structures and surfaces with defined shapes that can be used to position cells and tissues, control cell shape and function, and create highly structured 3D culture microenvironments [24–26]. Hydrogels are excellent scaffolding materials for repairing and regenerating a variety of tissues because they can provide a highly swollen 3D environment similar to soft tissues. Biomimetic modification of hydrogels as tissue engineering scaffolds has emerged as an important strategy to modulate specific cellular responses for the incorporation of key biofunctions of natural ECM to provide valuable insight into the regulation of cell function and developmental processes in tissue- and organ-specific differentiation and morphogenesis [25, 26]. The NanoCulture plates provide precisely engineered micropatterns that promote cells to form uniform spheroids that are highly reproducible. In addition, decellularized hearts have provided natural scaffolds to bioengineer the 3D geometry and structures of the whole organ [27]. Stirred-tank bioreactors are easy to scale up and microcarriers provide the high surface-volume ratio. Therefore, spinner flask bioreactor cultures with microcarriers, which are relatively low costs in scaling up production, have been commonly used in large scale expansion of anchorage dependent cells [28, 29]. In addition, the agitation in stirred tank bioreactors limits the diffusion gradient of nutrients or morphogens, thus providing a physiologically relevant environment to favor cell or tissue production at large scale. Microcarrier-based bioreactors provide economically and technically feasible platforms for the production of hESC-derived functional CNS or myocardial tissues or organs from hESC neuronal or cardiomyocyte derivatives of PluriXcel technology at a commercial or clinical scale necessary for cell-based therapies and tissue engineering.

Conclusion and future prospective

Emerging hPSC-based PluriXcel technology allows efficient derivation of clinical-grade hPSC lines and direct conversion of such non-functional pluripotent cells by small molecule induction into a large commercial scale of high quality clinical-grade functional human neuronal and cardiomyocyte derivatives, which not only constitutes clinically representative progresses in both human neuronal and cardiac therapeutic products for treating neurodegenerative, neurological, and heart diseases, but also offer manufacturing innovations for production scale up and creation of replacement tissue and organ products. PluriXcel technology platforms provide tissue and organ engineering paradigm for further reconstitution of complex multi-cellular 3D human CNS and heart models or micro-CNS and microhearts from hESC neuronal and cardiomyocyte derivatives, respectively, in reliable tissue-specific complex biomimetic culture systems. Progress in tissue-specific bioreactors will lead to the development and commercialization of multi-cellular 3D human CNS and heart models or micro-organs that can be used for rapid and high fidelity safety and efficacy evaluation of human therapeutic candidates or cell therapy products and, thus lead to advances in technologies used in the regulatory review; and will be readily adaptable in drug efficacy and toxicity testing, and for commercialization and therapeutic development. It will pave the way for further development of cutting-edge automated high content systems for systematic functional assembly of the *in vitro* tissues and organs from pluripotent hESC in a 3D setting that reflect the biological complexity and microenvironment niche of the *in vivo* human organ system, enabling automated high content and high-throughput analysis of CNS or heart circuitry and dynamics, and systems developmental biology models of the complex human embryonic development.

Conflicts of interest

Author declares no conflicts of interest.

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