Introduction

Human pluripotent stem cells (embryonic or induced pluripotent cells) have the intrinsic property to self renew indefinitely and the capacity to differentiate into almost all mature cell types, making them attractive candidates for cell therapy based regenerative medicine [1]. The advent of human iPSC technology in 2007 further revolutionized this field [2, 3]. iPSCs are derived from patient somatic cells and reprogrammed into the pluripotent stage state using viral transfection [4] or footprint free reprogramming using Sendai virus [5]. They are typically derived from fibroblasts obtained from biopsy punch [6], while alternate less invasive cell source includes peripheral blood mononuclear cells [7], renal epithelial cells from urine [8], dental pulp [9]. The advantage of iPSC over embryonic stem cell lies in the avoidance of ethical concerns associated with hESCs. Furthermore, it allows the use of patient specific cells, paving the way towards personalized medicine.

Hence, In addition to regenerative therapy, hPSCs have multiple critical *in-vitro* applications (Fig 1 schematic), including *in-vitro* disease modeling, providing a human specific platform to perform efficacy and toxicity screens for novel chemical entities [10, 11]. The advent of CRISPR/ Cas9 technology has further facilitated the generation of genetically defined human iPSCs, initiating the generation of human 'disease in a dish'. Until now, the study of rare disease have been restricted by the limited availability of cell models adequately representing the disease pathophysiology [12]. Immortalized cancer cell lines typically served as the primary cell source, which is restrictive since its long term culture often results in accumulated genetic defects or chromosomal aberrations. In contrast, the iPSCs offer an unrestrictive cell source, they are genetically stable and are amenable to genetic manipulation either to reproduce a disease phenotype or correcting a genetic defect.

In this review we provide a comprehensive discussion of the advances in the field of hPSCs, particularly focusing on its application in regenerative medicine and in disease modeling with subsequent application for drug discovery purposes. Future of iPSC based technology will be largely determined by the phenotype of the differentiated tissue/ organ, and its functional maturation. The advent of organoid technology has shown promise in reproducing the phenotype and function of the native organ to a certain extent. We have discussed the current advances in the organoid technology and offered our perspective on current challenges and future outlook on hPSC based technology.

iPSCs in Regenerative Medicine Applications

The potential of hPSCs to generate functional tissue, renders their impact in treatment of degenerative diseases, where the primary treatment option is replacement of damaged tissue with healthy donor tissue. While limited supply of donor tissue along with associated immune complications restricts this treatment option, hPSC derived tissues promises to overcome such restrictions by generating near functional tissue from isogenic or HLA matched cell source. The feasibility of replacing damaged tissue by iPSC derived tissue/ organ has been demonstrated in multiple studies, some of which have successfully progressed towards clinical trials. The first clinical trial was initiated in 2014, using human iPSC derived retinal pigmented epithelial (RPE) cells to treat macular degeneration [13-15]. To date, 11 approved clinical trials involving hPSC-based therapies have been registered at the National Institutes of Health clinical trials website (listed in **Table 1).** However, the cell dose required to treat patients in a commercial scale is not yet achievable with the current culture practices. In addition, current hPSC culture procedure lacks stringent quality control, and do not meet good manufacturing practices (GMP) standards [16]. These

shortcomings have inspired the development of techniques for controlled, scalable culture of hPSCs [17, 18] under GMP conditions [19].

	Study Title	Registration Number	Status	Sponsor/Collaborator	Location	Estimated Enrollment	Estimated Study Completion
Ophthalmic Diseases	Safety and efficacy study of OpRegen for treatment of advanced dry-form age-related macular degeneration	NCT02286089	Phase I/II study; recruiting	BioTime Inc./ CellCure Neurosciences Ltd.	United States: California; Israel	24	Sep 2019
	Safety and tolerability of sub-retinal transplantation of hESC-derived RPE (MA09-hRPE) cells in patients with advanced dry age-related macular degeneration (Dry AMD)	NCT01344993	Phase I/II study; completed	Astellas Institute for Regenerative Medicine	United States: California, Florida, Massachusetts, Pennsylvania	13	Aug 2015
	Sub-retinal transplantation of hESC-derived RPE (MA09-hRPE) cells in patients with SMD	NCT01345006	Phase I/II study; completed	Astellas Institute for Regenerative Medicine	United States: California, Florida, Pennsylvania	13	Aug 2015
	Long term follow up of sub-retinal transplantation of hESC-derived RPE cells in SMD patients	NCT02445612	Observational study; long-term follow-up study to NCT01345006; active, not recruiting	Astellas Institute for Regenerative Medicine	United States: California, Florida, Pennsylvania	13	Dec 2019
	Long term follow up of sub-retinal transplantation of hESC-derived RPE cells in patients with AMD	NCT02463344	Observational study; follow-up study to NCT01344993; active, not recruiting	Astellas Institute for Regenerative Medicine	United States: California, Florida, Massachusetts, Pennsylvania	11	Dec 2019
	Study of subretinal implantation of human ESC-derived RPE cells in advanced dry AMD	NCT02590692	Phase I/II study; active, not recruiting	Regenerative Patch Technologies, LLC	United States: Arizona, California	16	Sep 2022
	A Safety surveillance study in subjects with macular degenerative disease treated with human ESC-derived retinal pigment epithelial cell therapy	NCT03167203	Phase I/II study; enrollment by invitation	Astellas Institute for Regenerative Medicine	United States: Missouri; United Kingdom	36	Dec 2029
Neurological Disorders	A Phase 1 Safety Study of GRNOPC1 in Patients With Neurologically Complete, Subacute, Spinal Cord Injury	NCT01217008	Phase I study; completed	Asterias Biotherapeutics, Inc.	United States: Alabama, California, Georgia, Illinois, Maryland, Pennsylvania, Wisconsin	5	July 2013
	Dose escalation study of AST-OPC1 in spinal cord injury	NCT02302157	Phase I/II study; completed	Asterias Biotherapeutics, Inc.	United States: California	25	Dec 2018
Diabetes	A safety, tolerability, and efficacy study of VC-01™ combination product in subjects with type I diabetes mellitus	NCT02239354	Phase I/II study; active, not recruiting	ViaCyte/ California Institute for Regenerative Medicine (CIRM)	United States: California, Georgia, Illinois, Indiana, Missouri, Pennsylvania, Wisconsin	69	Jan 2021
	Three year follow-up safety study in subjects previously implanted with VC-01™	NCT02939118	Observational study; enrollment by invitation	ViaCyte	not provided	200	Nov 2021
	A safety, tolerability, and efficacy study of VC-02™ combination product in subjects with type 1 diabetes mellitus and hypoglycemia unawareness	NCT03163511	Phase I/II study; recruiting	ViaCyte/ California Institute for Regenerative Medicine (CIRM)	United States: California, Maryland, Minnesota, Ohio; Canada: Alberta, British Columbia	55	Dec 2020
Heart Disease	The Transendocardial Autologous Cells (hMSC or hBMC) in Ischemic Heart Failure Trial (TAC-HFT) (TAC-HFT)	NCT00768066	Phase I/II study; completed	University of Miami/ The EMMES Corporation	United States: Florida	65	Dec 2015
	PercutaneOus StEm Cell Injection Delivery Effects On Neomyogenesis in Dilated CardioMyopathy (The POSEIDON-DCM Study) (PoseidonDCM)	NCT01392625	Phase I/II study; completed	Joshua M Hare/ National Heart, Lung, and Blood Institute (NHLBI)	United States: Florida	37	Feb 2018

Table 1: Clinical trials in the United States for human embryonic stem cell-based therapies

Disease Modeling using iPSCs

In parallel to regenerative medicine, iPSCs are emerging as an attractive platform for disease modeling and drug discovery applications. Conventionally, human diseases are studied in well established animal models, which offers an in vivo setting to investigate pathological mechanisms. Such animal models have also served a critical role in discovering therapeutic strategies to debilitating diseases. However, substantial interspecies differences between human and commonly used rodent models often restricts the adequate recapitulation of human disease pathophysiology [20, 21]. Thus therapeutic strategies developed on animal models have often failed in the clinic, thereby necessitating the development of human specific disease models to complement current animal models.

Human iPSCs are particularly useful in modeling human diseases with defined genetic causes. Disease modeling with iPSCs involve derivation of iPSCs with the disease causing mutation, followed by differentiation of the iPSCs into disease relevant cells. These iPSCs could potentially be derived directly from patients, to provide disease relevant cells, along with paving the way for personalized disease modeling, the central theme for precision medicine.

Till date there has been multiple reports in developing disease models with iPSCs. Most prominently, Alzheimer disease [22-24] and Parkinson disease [25, 26] has been modeled using neurons derived from iPSCs. The pathology of amyotrophic lateral sclerosis (ALS) was modeled using a co culture of astrocyte with neuron [20] The Next Generation Genetic Association Studies consortium aims to generate iPSCs from patients with genetic variants correlated with cardiovascular disease [27]. Till date this initiative has produced a bank of iPSC lines from patients with various disease conditions, including insulin resistance, lipid disorders, myocardial infarction, pulmonary hypertension, coronary artery disease, platelet aggregation deficiency, QT intervals and ECG cardiac traits, left ventricular hepertrophy and sickle cell anemia (http://www.wicell.org/home/stem-cell-lines/collections/collections.cmsx).
Modeling of infectious diseases have also been demonstrated, by introducing microbes into the disease models [28], including Helicobacter pylori (H. pylori) [29], Zika virus (ZIKV) and Norovius (NoV) [30, 31]. These models are expected to be instrumental in drug discovery efforts for future pathogenic organisms.

iPSCs for drug discovery applications

iPSC based disease models are currently being utilized for drug screening application, including both phenotypic screening and target based screening [20, 32]. Such screening tests have demonstrated the feasibility of therapeutic compound identification [33-35] using iPSC models of multiple diseases including Alzheimer's disease [36], Familial dysautonomia [37], long QT syndrome [38, 39], Rett Syndrome [40], schizophrenia [41], spinal Muscular Atropy [42, 43] and Timothy syndrome [44]. Importantly, in 2012 a high throughput screening of a large chemical library was conducted on iPSC model [37]. Compounds identified from this screening have proceeded to clinical trials [45] as a landmark iPSC utilization testbed.

An important study was the report by Ogawa, in studying cystic fibrosis transmembrane regulator (CFTR) using patient derived cholangiocyte organoids. In this seminal study the authors demonstrated derivation of functional cholangiocyte from iPSCs using 3D culture models. They also demonstrated development of disease model from patient derived iPSCs, resulting in protein misfolding and translocation to cell membrane, and reversal of the diseased phenotype using inhibitors to reduce misfolding and stabilize protein [46].

Organoid models derived from iPSC

Success of both regenerative medicine and disease modeling/ drug discovery applications detailed earlier rely heavily on successful derivation of mature tissue/ organ from hPSCs. Over the last decade, hPSCs have been cultured, propagated and differentiated predominantly under adherent cultured configuration, which renders them a two dimensional (2D) monolayer geometric configuration [20, 21]. Derivation of tissue specific cells from iPSCs cultured under such 2D configurations have generally resulted in an immature phenotype, resembling a fetal state [47]. In the native tissue environment, the cells are exposed to a complex, heterotypic 3 dimensional (3D) environment supported by multiple other cells types and extracellular matrix (ECM) [48]. Such dynamic, reciprocal interactions are beginning to be realized as essential to reproduce the adult mature function of the hPSC derived tissue [49]. Thus recent efforts are focusing on generating tissue/ organ specific 'organoid' models (meaning 'resembling and organ' [32]) with the goal of reproducing the in vivo, tissue specific niche environment [48, 50].

The development of intestinal organoids marked a major development in the stem cell field, when endogenous stem cell components were demonstrated to have long term and stable culture of near physiological epithelia [32, 51, 52]. In subsequent work, intestinal organoids were generated from iPSC derived cells [53, 54]. Over the last decade substantial efforts have been directed to derive organoids for various organs, using both adult stem cells as well as iPSCs (**Table 2**). Prominent examples include the derivation of stomach and gastric organoids from murine ESC [55], human ESCs and iPSCs [56]. Takebe et al generated liver organoids, referred as 'liver bud', using iPSC derived hepatocytes along with human endothelial cells and mesenchymal stem cells [57]. Upon implantation the liver organoids developed into vascularized, functional, liver tissue. Recently, cholangiocyte organoids have also been reported from hPSCs [46, 58]. Lung organoids have been derived from human ESCs, expressing both proximal and distal lung markers reminiscent of branching morphogenesis [59, 60]. Our group have reported the generation of pancreatic islet organoids by controlled aggregation of hPSC derived pancreatic progenitor cells using a novel hydrogel substrate [61]. This substrate allows inclusion of multiple cell population, towards generation of an islet mimetic with stromal and endothelial support along with the endocrine cells. Our group along with others have demonstrated that reproducing the native 3D islet configuration in hPSC derived organoids significantly enhanced the insulin secretion and glucose responsiveness of the derived islets [61-63].

Organoids of the ectoderm lineage are primarily derived from embryoid bodies, which are further induced towards the neural fate. Accordingly, retinal organoids have been generated from mouse ESCs in combination with defined ECM components [64, 65]. Inner ear organoids have been generated from mouse ESCs possessing mechanosensitive hair and prosensory cells [66, 67]. Eiraku pioneered the Serum Free Culture of Embryoid Body (SFEBq) method to generate brain organoids, resembling telencephalon [68]. Lancaster generated cerebral organoids using the spinning bioreactor, representing multiple regions of the brain with functional neurons [69].

Organoids are gaining popularity since they closely resemble the endogenous organ structure, including cellular organization. This will likely enable the study of disease pathology in a spatiotemporal context, and modeling of drug response at the level of an organ instead of individual cells.

Organoid	Type of Stem Cell	Aggregation Method	Cell Types/Structures	Applications	Refs
Intestine	Mouse ESCs Human iPSCs/ESCs	Differentiation Induced Aggregation; Matrigel Suspension Culture; Microinjection; Transwell Air-Liquid Interface; Microengineering Chip	Polarized, Columnar Epithelial Cells; Enterocyte Cells; Goblet Cells; Paneth Cells; Enteroendocrine Cells; Villus Region; Crypt Region; Mesenchymal Cells	Clostridium difficile Disease Model; Salmonella Disease Model; Developmental Model; Retroviral Infection Model; Transplantation; Small Intestine Model; In Vitro Matured Model	[52-54, 70-79]
Liver	Human iPSCs	Matrigel Surface Coat Induced Self-Assembly; Culture with Hepatic Cells on Ultralow Cluster Dish; Collagen I/Matrigel-treated Surface; Matrigel Suspension Culture	Liver Buds; Endothelial Cells (HUVEC); Primary Mesenchymal Stem Cells; Biliary Epithelial Cells (cholangiocytes)	In Vitro Functional Liver Model; Biliary Disease Model; Transplant	[46, 49, 57, 58, 80]
Lung	Human iPSCs/ ESCs	Matrigel Suspension Culture; Engineered/Decellularized Scaffold Aided Assembly	Mesenchymal Cells; Alveolar-like Epithelial Cells; Basal Cells; Goblet Cells; Clara Cells; Ciliated Cells; Type I and II Alveolar Epithelial Cells	Cystic Fibrosis Disease Model; In Vitro Lung Model; Hermansky-Pudlak Syndrome Type 2 In Vitro Model	[59, 60, 81-83]
Brain	Mouse ESCs Human iPSCs/ESCs	Spinning Bioreactor; Matrigel Suspension Culture; Ultralow Attachment Plate on an Orbital Shaker Suspension Culture;	Forebrain Region; Mid-brain Region; Hapothalamic Region; Cerebral Cortex Region; Outer Radial Glial Stem Cells;	Mid-brain Disease Model; Cerebral Model; Zika Virus Models; Developmental Model; Autism Spectrum Disorder Model	[30, 31, 68, 69, 84-91]
Kidney	Human iPSCs	Transwell Culture; Collagen I/ Matrigel Suspension Culture; Microwell Aggregation; Ultralow Attachment Plate Culture; V-bottom Plate Culture	Collecting Duct Network-associated Nephrons; Endothelial Cells; Podocytes; Proximal Tubules	Nephrogenesis Model	[92-96]
Pancreas	Human iPSCs	Matrigel Surface Coat Induced Self-Assembly; Amikagel; Matrigel Suspension	Exocrine Progenitor Cells; Endocrine Porgenitor Cells; Acinar-like Cells; Ductal-like Cells; Beta-like Cells; Endothelial Cells (HUVEC)	Islet Model; Adenocarcinoma Model; Acinar/ductal Model	[61, 97, 98]
Stomach	Mouse ESCs Human iPSCs/ESCs	Matrigel Suspension Culture; Gelatine-coated Dish Culture; Differentiation Induced Aggregation;	Antrum Domain; Corpus Domain (Fundic Epithelium); Gastric Gland-like Domain; Pit-like Domain; Gastric Endocrine Cells;	Gastric Disease Model	[29, 55, 56, 99]
Inner Ear	Mouse ESCs Human iPSCs/ESCs	Ultralow Adhesion U- or V-bottom Plate Culture	Sensory Epithelia; Sensory Neurons	In Vitro Model; Inner Ear Development Model	[66, 67, 100]
Retina	Mouse ESCs/iPSCs Human ESCs	Ultralow Adhesion Plate Culture; Rotating Wall Vessel Bioreactor; Lipidure-coated U-bottom Plate	Optic Cup; Pigment Epithelium; Cone and Rod Photoreceptors	Retinogenesis Model; Transplantation	[64, 101-106]
Thymus	Human iPSCs/ESCs derived HSC	Transwell Culture	T-cells; Artificial Antigen Presenting Cells; Human Embryonic Mesodermal Progenitors	T-cell Immunotherapy	[107]

Table 2: Overview of pluripotent stem cell derived organoid types and their applications

Outlook and Future Perspective

The great potential of iPSC technology is only beginning to be realized, with its applications in regenerative therapy along with human disease modeling for drug discovery applications. Significant progress has been made over the last decade in deriving various organ specific cell and tissue from hPSCs, several of which have proceeded to clinical trials. While highly promising, there are several challenges which needs to be overcome to realize the full potential of hPSCs.

Success of iPSC based disease modeling depends both on the derivation and generation of relevant cells source along with its differentiation into mature cells representing the disease phenotype. The gene editing efficiency of human ESC and iPSCs have significantly improved by the advent of technologies such as zinc finger nucleases (ZFN) [108, 109], transcription activator like effector nucleases (TALENs) [110, 111] and the CRISPR Cas9 system [112, 113] These site specific nucleases can induce DNA double stranded breaks at the site of gene modification, which enables researchers to correct mutations in patient iPSCs or to introduce disease causing mutations into wild type iPSCs, to create isogenic controls for iPSC based disease modeling.

Successful generation of mature functional organs is a critical factor component governing the success of this technology. In the current state while the iPSC derived cells are still representing an immature, fetal phenotype, they can still provide a relevant model to study disease with an early onset [20, 43, 114]. However the organoid models are proving to be valuable in enhancing the maturation and function of the hPSC derived organ models, by adequately reproducing the complexity of organ structure in 3D configuration and cellular composition [6].

While the 3D multicellular heterotypic environment renders functional benefit to the organoid system, it also offers some restrictions, which needs to be overcome in order for this technology to emerge as a viable therapy. The 3D organoid structures are typically subjected to diffusion limitation in the organoid core, compromising the organoid function and fate. This issue could be resolved by in vitro vascularization, currently an active area of research [33, 57]. The 3D structure also hinders dynamic, real time readouts and microscopy, thereby affecting the throughput of organoid based studies [6]. However the major restriction is in the lack of control on the formation of self organized organoids, which introduces variability in organoid phenotype. It is expected that novel biomaterial design will play a critical role to facilitate 3D organoid architecture in a controlled and reproducible manner. Accordingly various scaffolds have been investigated and incorporated into organoid engineering, including collagen, fibrin and matrigel [115]. We have reported the feasibility of generating uniform, size controlled heterotypic organoids using a novel substrate [61].

Rapid progress in iPSCs and associated technologies like genome editing and organoid modeling have resulted in a paradigm shift in biomedical research. iPSC based tissue/ organ models have paved the way to systematic modeling of human disease and its application to inform therapeutic development. Patient derived tissue/ organs and biobanking is expected to further enhance our understanding of heterogeneity between patients, paving the way to personalized therapies. Overall, stem cell based technologies are becoming invaluable tools for regenerative medicine, precision medicine, disease modeling, development of novel therapeutics and toxicity testing.

Funding

Work in the Banerjee lab was supported by grants from NSF (CBET 1547618, 1706674, 1803781) and NIH (UG3DK119973, AI126335).

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