World Journal of Stem Cells

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.4252/wjsc.v6.i1.11

World J Stem Cells 2014 January 26; 6(1): 11-23
ISSN 1948-0210 (online)

TOPIC HIGHLIGHT

WJSC 6th Anniversary Special Issues (3): Embryonic stem cells

Neural differentiation from pluripotent stem cells: The role of natural and synthetic extracellular matrix

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Supported by FSU start up fund and FSU Research Foundation GAP award; partial support from National Science Foundation, No.1342192
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Received: October 1, 2013 Revised: October 23, 2013
Accepted: November 2, 2013
Published online: January 26, 2014

Abstract

Neural cells differentiated from pluripotent stem cells (PSCs), including both embryonic stem cells and induced pluripotent stem cells, provide a powerful tool for drug screening, disease modeling and regenerative medicine. High-purity oligodendrocyte progenitor cells (OPCs) and neural progenitor cells (NPCs) have been derived from PSCs recently due to the advancements in understanding the developmental signaling pathways. Extracellular matrices (ECM) have been shown to play important roles in regulating the survival, proliferation, and differentiation of neural cells. To improve the function and maturation of the derived neural cells from PSCs, understanding the effects of ECM over the course of neural differentiation of PSCs is critical. During neural differentiation of PSCs, the cells are sensitive to the properties of natural or synthetic ECMs, including biochemical composition, biomechanical properties, and structural/topographical features. This review summarizes recent advances in neural differentiation of human PSCs into OPCs and NPCs, focusing on the role of ECM in modulating the composition and function of the differentiated cells. Especially, the importance of using three-dimensional ECM scaffolds to simulate the *in vivo* microenvironment for neural differentiation of PSCs is highlighted. Future perspectives including the immediate applications of PSC-derived neural cells in drug screening and disease modeling are also discussed.

Key words: Pluripotent stem cells; Neural differentiation; Extracellular matrix; Three-dimensional; Drug screening

Core tip: Neural cells derived from human pluripotent stem cells (hPSCs), including oligodendrocyte progenitor cells and neural progenitor cells, emerge as an unlimited and physiologically relevant cell source for drug screening, disease modeling, and regenerative medicine. Natural and synthetic extracellular matrices play an important role in regulating neural differentiation, cell migration, and the derived neural cell maturation. Recent advances in neural differentiation of hPSCs on extracellular matrices in 2-D and 3-D systems are reviewed in this paper. The immediate applications of the derived neural cells in drug screening and disease modeling are also discussed.

Li Y, Liu M, Yan Y, Yang ST. Neural differentiation from pluripotent stem cells: The role of natural and synthetic extracellular matrix. *World J Stem Cells* 2014; 6(1): 11-23 Available from: URL: http://www.wjgnet.com/1948-0210/full/v6/i1/11.htm DOI: http://dx.doi.org/10.4252/wjsc.v6.i1.11

INTRODUCTION

Human pluripotent stem cells (hPSCs), including human

embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), have extensive proliferation potential and the unique ability to produce any type of somatic cells[1,2]. Due to their self-renewal ability, hPSCs potentially can provide unlimited numbers of neural cells for cell therapy and drug discovery[3,4]. For example, oligodendrocyte progenitor cells (OPCs) derived from hESCs have been tested in Geron's Phase I clinical trial approved by Food and Drug Administration in 2010 to treat spinal cord injury (SCI)[5,6]. OPCs derived from hiPSCs have also been shown to myelinate and rescue a mouse model of congenital hypomyelination[7]. Compared to other animal cells and tissues, hPSCs can provide physiologically relevant cells to deliver more efficacious medicines and to provide accurate models for drug screening[8,9]. For example, *in vitro* model of amyotrophic lateral sclerosis (ALS) can be established from the motor neurons differentiated from hPSCs, which are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation[10]. Compared to hESCs, hiPSCs can be derived from individual patients, providing "personalized" medicine and the *in vitro* models to study pathological neural development and disease progression[11]. For neurological diseases where somatic neural cells are limited in number, hPSCs emerge as a powerful tool for drug screening, disease modeling, and regenerative medicine.

The ability to obtain high-purity and functionally mature neural cells is the pre-requisite to fulfill the potential of hPSCs in neurological disease treatments. Differentiating hPSCs into OPCs or neural progenitor cells (NPCs) with a high purity has been demonstrated, but their function and maturation are still under investigation[12-14]. Extracellular matrix (ECM) plays an important role in neural differentiation of hPSCs and the maturation of the derived neural cells[15]. ECM proteins, through the interaction with integrins expressed on the neural cells, modulate cell survival, migration, proliferation, and the differentiated cell function[16]. Besides ECM composition, the mechanical property of ECMs is also found to regulate neural lineage commitment of hPSCs recently. To better understand the *in vivo* development and the "niches", *i.e.*, microenvironment, of neural tissue development[15], three-dimensional (3-D) ECMs, both natural and synthetic, have been investigated for efficient neural differentiation of hPSCs. 3-D ECM scaffolds provide not only physical support for cell adhesion, but also the structural and biomechanical cues that can be transduced into biochemical signals, affecting cellular composition during neural differentiation[17,18]. By regulating biochemical composition, biomechanical properties, and physical structure of 3-D ECMs, neural differentiation of hPSCs can be effectively controlled.

This review summarizes recent advances and the development of protocols for *in vitro* differentiation of hPSCs to OPCs and NPCs with high purity and desired function. To provide the 3-D microenvironment that more resembles *in vivo* tissues than traditional 2-D cultures, 3-D neural differentiation systems based on various natural and synthetic ECMs have been extensively studied and are discussed in this review, with an emphasis on the effects of ECMs on neural lineage commitment of hPSCs. Current progress in the application of hPSC-derived neural cells for drug screening is also discussed and highlighted.

PLURIPOTENT STEM CELL-DERIVED OLIGODENDROCYTE PROGENITOR CELLS

Oligodendrocytes derived from OPCs can remyelinate axons upon maturation. However, transplanting OPCs instead of mature oligodendrocytes is a better strategy to restore neural function[19]. While OPCs from somatic tissues are limited in cell number, OPCs derived from hPSCs provide novel alternative autologous or allogeneic cell sources. There are two types of OPC differentiation protocols from hPSCs in general: epithelial growth factor (EGF)-dependent protocol and platelet-derived growth factor (PDGF)-dependent protocol (Table 1)[20]. OPCs were initially derived from hESCs through embryoid body (EB) formation in the presence of fibroblast growth factor (FGF)-2, retinoic acid (RA), and EGF for 4 wk followed by attaching the neural spheres onto Matrigel-coated surface for another 2 wk (6-wk protocol)[21]. A high-purity population of OPCs was achieved with the expression of more than 70% NG2, oligodendrocyte transcription factor 1 (OLIG1), OLIG2, and (sex determining region Y)-box 10 (SOX10) using this EGF-dependent protocol. When culturing the cells on human laminin in the absence of mitogen EGF, the derived OPCs displayed minimal neuronal and astrocyte markers, and could mature into oligodendrocytes, which expressed Gal C, O4, and myelin basic protein. Transplanting hESC-OPCs was shown to remyelinate axons and restore the locomotor function in a rat contusion model[19]. The transplanted OPCs phenotypically replaced lost oligodendrocytes, remyelinated axons, and also secreted neurotrophic factors to establish a repair environment in the lesion[22,23]. This EGF-dependent OPC differentiation protocol has been successfully used in a manufacturing process in a cGMP (*i.e.*, current Good Manufacturing Practices) facility to produce OPCs for treating SCI patients in Phase I clinical trials[6]. Although the preliminary safety data were obtained, additional trials are required to demonstrate the efficacy of the hESC-derived OPCs. Different protocols have been developed later using different induction factors, including sonic hedgehog (Shh) protein, PDGF, insulin-like growth factor (IGF)-1, bone morphogenetic protein antagonists such as noggin, neurotrophic factors such as neurotrophin (NT)-3 and ciliary neurotrophic factor, with or without EGF[24-28]. High-purity OPCs were obtained, and they also showed remyelination capacity in animal study[29]. The main drawback of these PDGF-dependent protocols is their lengthy (10-14 wk) and complicated procedures with multiple growth factors and multiple steps of suspension and adherent cultures, which are difficult and expensive to scale up for

Table 1 Protocols and *in vivo* studies for oligodendrocyte progenitor cells differentiation from human pluripotent stem cells

Growth factors	Cell source	Cell characteristics	Ref.
EGF-dependent protocol			
RA/EGF (short FGF-2 exposure)	hESC lines: H1 and H7	Olig1 (80%-90%), Sox10 (76%-84%), NG2 (95%); remyelinated in a rat thoracic contusion model	Nistor *et al*[21], 2005; Keirstead *et al*[19], 2006; Li *et al*[13], 2013
RA/EGF (short FGF-2 exposure)	hiPSC lines: Royan hiPSC1, hiPSC8	> 90% Olig2, Sox 10, > 80% NG2 and PDGFRα; tested in a rat model of optic chiasm demyelination	Pouya *et al*[33], 2011
RA/EGF (short FGF-2 exposure)	hESC H7 line	OPCs remyelinated in a rat cervical contusion model	Sharp *et al*[94], 2010
RA/EGF (short FGF-2 exposure)	hiPSC lines: 201B7, 253G1	$O4^+$ cells were observed in EGF-dependent protocol	Ogawa *et al*[20], 2011
PDGF-dependent protocol			
PDGF/FGF-2 (short EGF exposure)	SNUhES1 line	PDGFRα (81%), A2B5 (90.4%), NG2 (91.3%) and O1 (81%); myelinate axons in co-cultures with fetus hippocampal neurons.	Kang *et al*[24], 2007
RA/Shh/FGF-2/PDGF	hESC lines: H1, H9 H14	> 80% $PDGFR\alpha^+$, also co-express Olig2, Nkx2.2, Sox10 and NG2	Hu *et al*[26], 2009
RA/Shh/FGF-2/PDGF	hiPSC lines: K04, C14, and C27	70%-90% $Olig2^+/Nkx2.2^+$, OPCs myelinated the brains of myelin-deficient shiverer mice	Wang *et al*[7], 2013
FGF-2/EGF/PDGF/CNTF plus laminin; Shh, PDGF, IGF-1, EGF, FGF-2 and CNTF plus RA and laminin	hESC lines: HS360 and HS362; Regea 06/040 and Regea 08/023	> 90% $NG2^+$, > 80% $PDGFR\alpha^+$; multilayered myelin sheet formation around axons was detected in co-culture with neuronal cells	Sundberg *et al*[28], 2010 Sundberg *et al*[27], 2011
Noggin/FGF-2/FGF-4/PDGF/EGF	hESC H1 line	> 95% cells expressing Sox10, A2B5, PDGFRα, NG2, O4, O1; increased neurological response in a rat contusion model	Kerr *et al*[29], 2010

RA: Retinoic acid; EGF: Epidermal growth factor; FGF-2: Fibroblast growth factor 2; hESC: Human embryonic stem cell; hiPSC: Human induced pluripotent stem cell; PDGFR: Platelet-derived growth factor receptor; PDGF: Platelet-derived growth factor; Shh: Sonic hedgehog; CNTF: Ciliary neurotrophic factor; IGF-1: Insulin-like growth factor-1.

generating cells needed for clinical studies.

The differentiation of iPSCs to oligodendrocytes was initially performed with mouse iPSCs for the possible application in SCI[30]. A lower percentage of $O4^+$ cells was obtained compared to the differentiation from mouse ESCs[31]. However, the variability of iPSC lines due to different tissues of origin and reprogramming methods may account for the difference[32]. The differentiation of hiPSCs to oligodendrocytes was performed using two types of hESC-OPC protocols based on PDGF- or EGF-induced differentiation[20]. The $O4^+$ oligodendrocytes were only observed in the EGF-dependent protocol with a low induction efficiency (< 0.01%). Later, the protocol developed by Nistor *et al*[21] was tested for hiPSC differentiation, and more than 90% of the differentiated cells expressed OPC markers (OLIG2, NG2, and O4), similar to that obtained with hESC-OPCs. The derived OPCs were transplanted into a demyelinated rat model and showed maturation into oligodendrocytes and the ability of remyelination[33]. An *OLIG* gene targeting protocol was also developed for hiPSCs, providing the possibility of genetic correction of patient-specific hiPSCs for cell therapy[34]. High-purity (70%-90%) $Olig2^+/Nkx2.2^+$ OPCs were obtained from hiPSCs treated with RA, Shh, FGF-2 and PDGF, and these OPCs were shown to myelinate the brains of myelin-deficient shiverer mice[7]. Given the progress made for OPC differentiation from hiPSCs, there is an urgent need for a clinical relevant system to generate a large amount of hiPSC-OPCs for drug screening and autologous transplantation. ECM is an important component during OPC differentiation, affecting both the differentiation efficiency and the derived cell function (Table 2). Thus, understanding the cell-ECM interactions and development of defined ECM substrates are critical steps for future clinical applications[13].

EFFECTS OF ECM ON OPC DIFFERENTIATION FROM PSCS

For various types of OPC differentiation protocols, replating the neural progenitors on ECM-coated surface is always part of the procedure[21,26]. The most common ECMs that have been used for OPC differentiation include laminin, fibronectin, alone or with poly-D-lysine, and Matrigel, which comprises mostly of laminin (Table 2). Oligodendrocytes were reported to express the laminin receptor α6β1 integrin[25]. Laminin is thus a potent promoter of oligodendrocyte survival and myelination. Direct comparison of various ECM proteins including fibronectin, laminin, and Matrigel was performed on OPCs isolated from embryonic day 15 rat spinal cords. All three ECMs were found to promote OPC survival, proliferation, migration, and maturation as compared to poly-D-lysine[35]. Recently, another ECM protein, vitronectin, was shown to promote oligodendrocyte differentiation from hESCs by synergistically interacting with Shh protein[36]. Besides α6β1, vitronectin receptors αvβ1, αvβ3, and αvβ5 are also differentially expressed at different OPC developmental stages and play an important role in modulating OPC migration, proliferation, and differentiation[37]. Especially, vitronectin-derived synthetic peptide acrylate surface (VN-PAS), which contains the active binding site of vitronectin, has been shown to support high-purity OPC derivation from hPSCs (Figure 1)[13]. Compared to Matrigel-coated surface, VN-PAS supported higher NG2 expression with similar expressions of nestin and

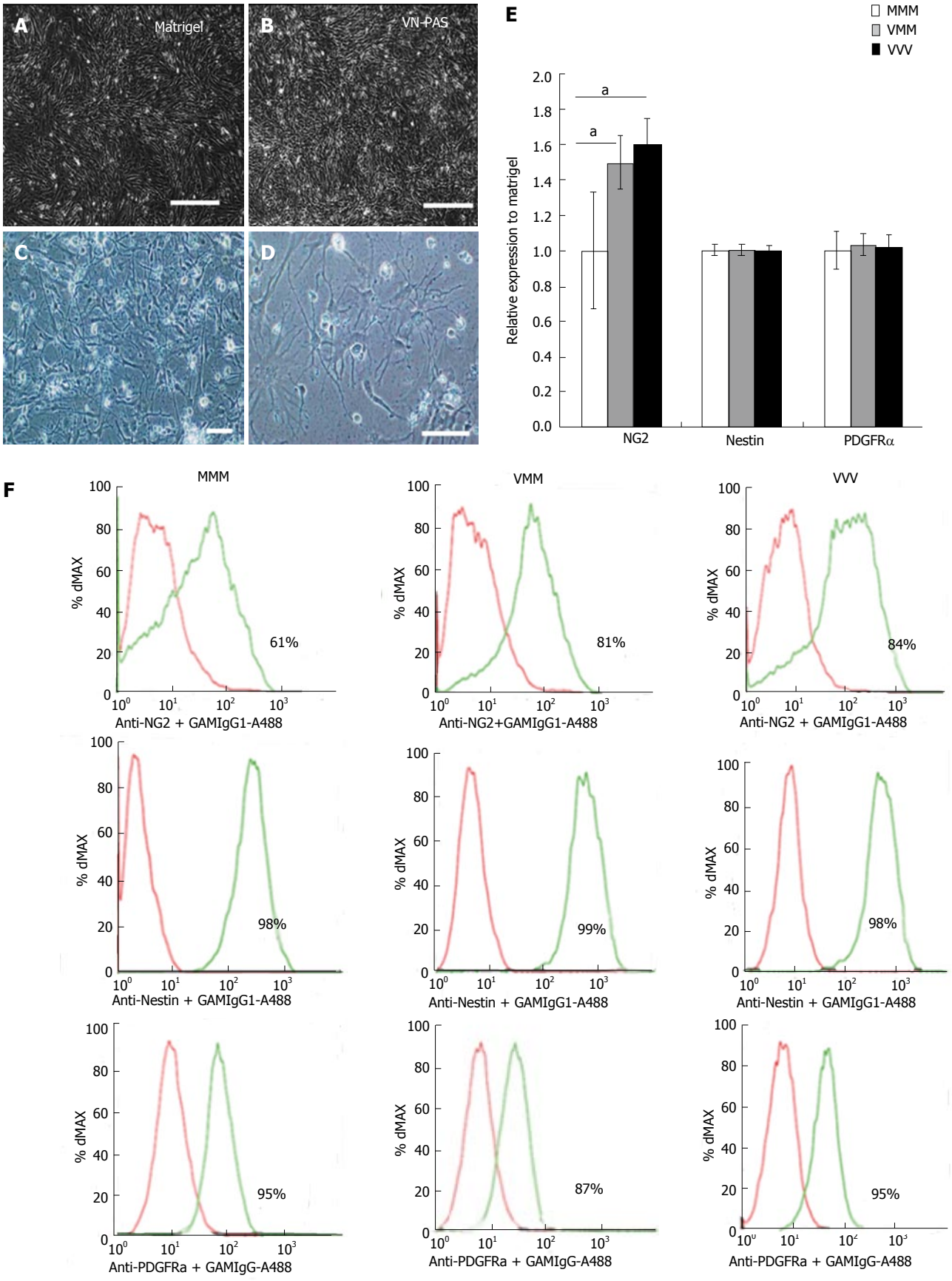

Figure 1 Oligodendrocyte progenitor cells derived from human pluripotent stem cells. A: Morphology of day 41 Oligodendrocyte progenitor cells (OPCs) derived from cells grown on Matrigel; B: Morphology of day 41 OPCs derived from cells grown on vitronectin-derived synthetic peptide acrylate surface (VN-PAS); scale bar: 200 μm; C and D: Oligodendroglial morphology after OPC maturation; C: Low magnification; D: High magnification, scale bar: 100 μm; E: OPC marker expression; MMM: all the steps of human pluripotent stem cell (hPSC) expansion and differentiation were performed on Matrigel; MVV: hPSC expansion on Matrigel and differentiation on VN-PAS; VVV: All the steps of hPSC expansion and differentiation were performed on VN-PAS. $^{a}P < 0.05$ *vs* MMM. F: Flow cytometry histograms of OPC markers; PDGFRα: Platelet-derived growth factor receptor alpha. This figure is adapted from Li *et al*[13].

Table 2 Effects of extracellular matrices proteins on neural differentiation of pluripotent stem cells

ECM protein	Integrins	Cell source	Neural differentiation	Ref.
Laminin Matrigel (rich in laminin)	α6β1 α3β1	HESC lines: TE03 and TE06	Neuronal generation and neurite outgrowth were significantly greater on laminin and laminin-rich Matrigel than fibronectin, poly-D-lysine, and collagen Ⅰ	Ma *et al*[16], 2008
Fibronectin	α5β1	Oligodendrocyte progenitor cells from rats	Promoted OPC survival, proliferation, migration, process extension, and OPC purity	Hu *et al*[35], 2009
Vitronectin	αvβ1, αvβ3, αvβ5	hESC lines: Miz-hES4, Miz-hES6	Promoted oligodendrocyte differentiation in the presence of RA/Shh/Noggin	Gil *et al*[36], 2009
Collagen Ⅳ	α1β1, α10β1	Rabbit neural progenitor cells	Conducive for both neuronal and glial cell differentiation	Raghavan *et al*[61], 2013
Collagen Ⅰ	α2β1	Rabbit neural progenitor cells	Conducive for both neuronal and glial cell differentiation	Raghavan *et al*[61], 2013
Vitronectin-derived synthetic peptide acrylate surface	αvβ5	hESC line: H1	Promoted oligodendrocyte progenitor differentiation; higher NG2 expression compared to Matrigel	Li *et al*[13], 2013
Heparan sulfate	Binding heparin	Rabbit neural progenitor cells	Addition of heparan sulfate to collagen mixtures improved neuronal differentiation	Raghavan *et al*[61], 2013

ECM: Extracellular matrix; hESC: Human embryonic stem cells; RA: Retinoic acid; Shh: Sonic hedgehog; OPC: Oligodendrocyte progenitor cells.

platelet-derived growth factor receptor alpha, demonstrating the active role of ECM-integrin interactions in OPC differentiation. In addition to the single ECM protein, decellularized ECM derived from bone marrow mesenchymal stem cells (MSCs) has also been evaluated for its ability to support neural cell growth[38]. Compared to poly-D-lysine, MSC-ECM enhanced the differentiation into astrocytes and oligodendrocytes beside neurons, prolonged survival, and better protected the cells from nutrient and growth factor deprivation.

Besides natural ECM proteins, synthetic ECMs have also been developed to better control biochemical and biomechanical microenvironments. Synthetic ECMs such as chitosan and poly (lactic) acid have been used to promote myelination by providing suitable environment to activate Schwann cell function post SCI[39]. OPCs have been shown to be mechanosensitive; the survival, proliferation, and migration of OPCs in polyacrylamide gels were optimal on intermediate stiffness (0.7-1 kPa) while differentiation efficiency increased with the substrate stiffness[40]. Chitoson was tested as the substrate for oligodendrocyte differentiation from neural stem cells (NSCs), where stiff surface (> 7 kPa) promoted NSC differentiation into oligodendrocyte while soft surface (< 1 kPa) promoted oligodendrocyte maturation and myelination[41]. Hybrid-scaffolds combining synthetic ECMs with cell-derived ECMs would be a better strategy as they could provide both biomechanical stability and the large amount of neurotropic factors in treating spinal cord[42]. ECMs not only modulate the late-stage OPC differentiation, but also provide a cell delivery strategy to enhance the *in vivo* remyelination and tissue regeneration[42]. However, the effects of ECMs during differentiation of hiPSC into OPCs and using ECM in cell delivery of hiPSC-derived OPCs have not been well studied.

PLURIPOTENT STEM CELL-DERIVED NEURAL PROGENITOR CELLS

NPCs and NSCs are able to differentiate into neurons, astrocytes, and oligodendrocytes, with neuronal lineage as the dominant population in most cases. Robust neural differentiation has been observed from various hESC and hiPSC lines, although variations among cell lines exist (Table 3)[12,43,44]. The differentiation of hPSCs into NPCs has been performed either by monolayer induction or by the formation of EBs in suspension, with inducing factors including RA, FGF-2, EGF and Shh, *etc*[45-49]. Recently, the synergistic induction using two inhibitors of SMAD signaling, noggin and SB431642, resulted in efficient neural differentiation for various hPSC lines[12,50]. SMADs are intracellular proteins that transduce extracellular signals from TGF-β ligands to the nucleus where they activate downstream gene transcription. The derived neural progenitors demonstrated the ability to further differentiate into dopaminergic neurons, when treated with Shh and FGF8, and motor neurons, when treated with brain-derived neurotrophic factor, ascorbic acid, Shh and RA[12]. Both monolayer induction and EB formation methods produced high-purity (> 80%) NSCs or NPCs. However, the populations obtained in different studies had different potential to differentiate into mature neuronal types. For example, FGF-2/EGF expanded hiPSC-derived NSCs showed a high tendency to differentiate into γ-aminobutyric acid neurons while RA/FGF-2 induced hESC-derived NPCs differentiated easily into motor neurons[46,47].

Specific neuronal cell types are required for treating particular neurological diseases. For example, protocols of motor neuron differentiation have been developed by several groups due to their potentials to treat SCI, ALS, and muscular atrophy, *etc*[11,51]. For the application in treating Alzheimer's disease, the hiPSC-derived neuronal cells were shown to express amyloid precursor protein and capable of secreting Aβ protein[52]. To treat stroke-damaged brain, early-stage neural progenitors expressing nestin, Pax6, and Musashi have been used in several studies[45,53,54]. Human ESC-derived NPCs were transplanted into the cortex rats after permanent distal middle cerebral artery occlusion. Some improvements in sensorimotor functions were observed but more complicated functions were not restored[45]. HiPSC-derived NPCs have

Table 3 Protocols and *in vivo* studies for neural progenitor cells differentiation from human pluripotent stem cells

Growth factors	Cell source	Cell characteristics	Ref.
EB-based protocol			
FGF-2/RA/ascorbic acid, db-cAMP, HAg	16 hiPSC lines	13 of the cell lines produced functional motor neurons. Treat with BDNF, GDNF, CNTF to produce motor neurons (4%-15% ISL$^+$ neurons)	Boulting *et al*[43], 2011
RA/FGF-2	hESC lines: H7, hCSC14, hCSC14-CL1	Produce neuronal progenitors (> 95% nestin and Musashi-1), can become cholinergic neurons, GABA neurons, *etc*	Nistor *et al*[47], 2011
Isolated Rosette expanded with EGF/FGF-2	hiPSC lines	Generate long-term expandable neuro-epithelial like stem cells (Lt-NES); in stoke model, the cells improved recovery of fine forelimb movements	Oki *et al*[53], 2012
RA/Shh	hESC lines H1 and H9	To generate motor neurons (50% HB9$^+$ motor neurons)	Hu *et al*[51], 2009
Monolayer-based protocol			
Noggin and SB431542	hESC line: H9 hiPSC lines: iPS-14, iPS-27	> 80% PAX6$^+$ cells; Shh/FGF8 for midbrain dopamine neurons; BDNF, Shh, RA, ascorbic acid for motor neurons	Chambers *et al*[12], 2009
Noggin and SB431542	hESC line: H9 hiPSC line: 253G4	Treat BDNF, GDNF, NT-3 for forebrain neurons which secrete Aβ for drug screening.	Yahata *et al*[52], 2011
Noggin only; FGF-2 may be added at later stage	hESC line: H1, H7, H9	> 90% nestin, musashi, and PSA-NCAM; For TH neurons, add Shh/FGF8/ascorbic acid; followed by BDNF, GDNF, ascorbic acid and laminin	Gerrard *et al*[48], 2005
FGF-2	hESC line: SA002 and AS034	> 90% nestin, NCAM; For neuronal lineage, add Shh/FGF8; after differentiation TH$^+$ cells, MAPab$^+$ cells and astrocytes existed	Axell *et al*[49], 2009

EB: Embryoid body; FGF-2: Fibroblast growth factor-2; RA: Retinoic acid; db-cAMP: Dibutyryl-cAMP; HAg: A small molecule agonist of the sonic hedgehog pathway; hESC: Human embryonic stem cell; hiPSC: Human induced pluripotent stem cell; BDNF: Brain-derived neurotrophic factor; GDNF: Glial cell-derived neurotrophic factor; CNTF: Ciliary neurotrophic factor; GABA: Gamma-Aminobutyric acid; Shh: Sonic hedgehog; NT-3: Neurotrophin-3; PSA-NCAM: Polysialylated-neural cell adhesion molecule; TH: Tyrosine hydroxylase; NCAM: Neural cell adhesion molecule.

also been shown to engraft with little neuroblasts or morphologically mature neurons in a rat model[55,56]. Recently, transplantation of hiPSC-derived NSCs exhibited functional recovery and electrophysiological properties of mature neurons, and was proved to be a safe approach for neuron replacement in stroke-damaged brain[53]. However, the cell engraftment and *in vivo* maturation are yet to be improved. Transplantations of NPCs derived from hiPSCs for treating other neurological diseases such as ALS and muscular atrophy have also been demonstrated in proof-of-principle studies[47,57]. The neural progenitors survived and engrafted *in vivo*, and the nestin-positive cells differentiated into neuronal phenotype and motoneuron-like structure in both wild-type rats and the ALS rats harboring a mutated human *SOD1* (*G93A*) gene[57]. To eliminate the risk of tumorigenicity of the residual undifferentiated hPSCs, intermediate NPC and NSC lines were established from hPSCs, which can be maintained for more than 100 passages[46]. There are growing interests in functional NPC differentiation from hPSCs to generate neural cells with clinically relevant quality and quantity for preclinical and potential clinical studies[58]. Current challenges include the functional maturation of NSCs and NPCs both *in vitro* and *in vivo*[58]. Large-scale generation of a specific neural subtype also remains a major challenge for neuronal differentiation of hPSCs. Recreating the stem cell niches enriched with ECMs is being pursued to address these challenges[15].

EFFECTS OF ECM ON NPC DIFFERENTIATION FROM PSCS

ECM proteins have been shown to regulate the survival, proliferation, and neurite outgrowth of hESC-derived NPCs in a dose-dependent manner through integrin-ECM signaling (Table 2)[16]. Similar to OPCs, NPCs also express integrin α6β1 and its ligand laminin is a major ECM protein that regulates NPC differentiation. Neuronal generation and neurite outgrowth were significantly greater on laminin and laminin-rich Matrigel substrates than other substrates including fibronectin, poly-D-lysine, and collagen I [16]. Delivering NPCs in laminin- or fibronectin-based constructs into injured brain showed the improved survival, migration, and behavioral recovery at 8 wk post-transplant[59]. Endogenous ECMs derived from the RA-treated EBs also accelerated neural differentiation, demonstrating the signaling capacity of ECM environment associated with the lineage commitment[60]. The native ECMs derived from PSC aggregates had a high content of fibronectin, laminin, collagen Ⅳ and vitronectin (Figure 2), which after decellularization can be used as 3-D scaffolds to promote stem cell adhesion, proliferation and differentiation. Such ECM scaffolds contain the balanced composition with the sequestered biological factors which provide the unique signaling to mediate the coordinated cellular events of stem cells. The composition of ECM proteins consisting of laminin, collagen Ⅳ, and heparan sulfate was found to regulate the balance of neuronal and glial cell differentiation; the ECM containing a higher portion of laminin and heparan sulfate induced more neuronal differentiation[61]. Neural differentiation of PSCs is associated with the switch from E-cadherin expression to N-cadherin expression. Hence, recombinant ECM components based on E-cadherin and N-cadherin hybrid substratum were also shown to support neural differentiation of ESCs and iPSCs[62].

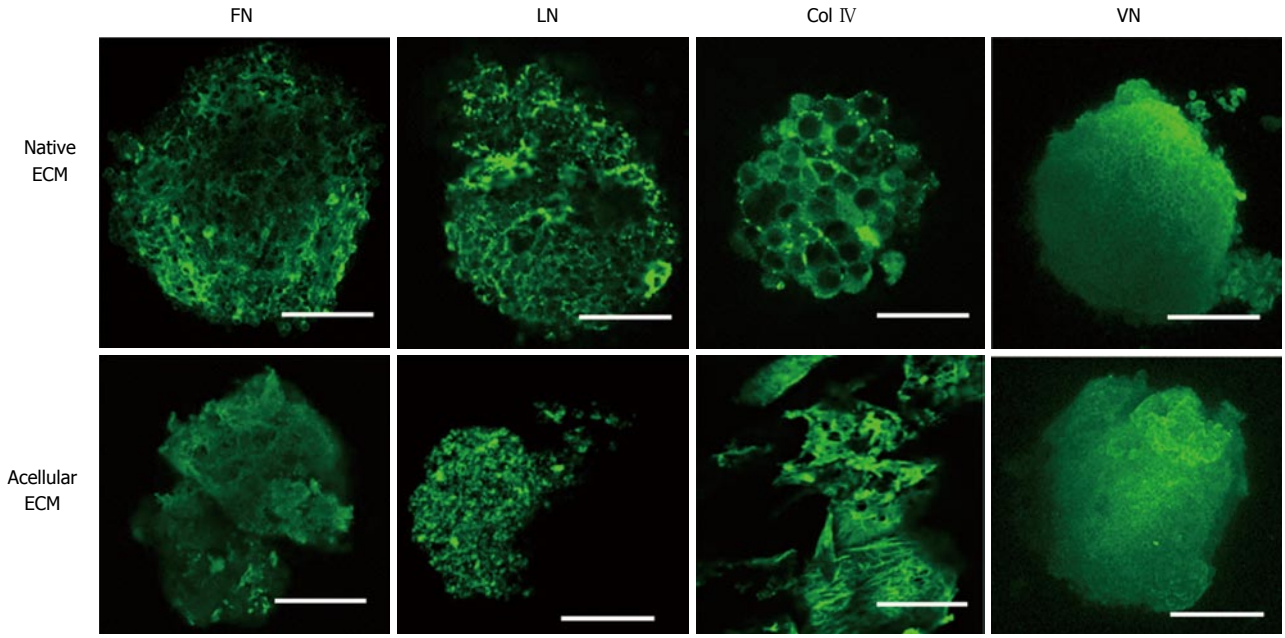

Figure 2 Three-dimensional extracellular matrix scaffolds derived from pluripotent stem cell aggregates. Confocal images of fibronectin (FN), laminin (LN), Collagen Ⅳ (Col Ⅳ), and vitronectin (VN) expression pre- and post-decellularization [acellular extracellular matrix (ECM) and native ECM, respectively]. Scale bar: 100 μm. For native Col Ⅳ, scale bar: 50 μm. The ECM scaffolds can be used for neural differentiation. Images are adapted from Sart *et al*[60].

Besides the ECM composition, the mechanical property of ECMs such as stiffness also affects neural differentiation. HPSCs are sensitive to biomechanical cues of the microenvironment[63-65] and respond quickly to stiffness change[65,66]. For hPSCs, a stiff surface was found to promote cell attachment and proliferation with dense F-actin expression while a soft surface led to cell detachment[67]. For neural lineage, soft hydrogels (100-500 Pa) promoted neuronal lineage while hard hydrogels (1-10 kPa) promoted glial differentiation[68-70]. Similarly, soft ECMs with a stiffness similar to that of the neural tissue (100-700 Pa) promoted the generation of early neural ectoderm from hPSCs, while this effect was less pronounced for hard ECMs (7.5 kPa)[71]. In studies simulating the biomechanical environment in each germ-layer, the scaffolds with high (1.5-6 MPa), intermediate (0.1-1 MPa), and low elastic moduli (< 0.1 MPa) were found to promote mesodermal, endodermal, and ectodermal differentiation of hPSCs, respectively[66,72]. ECMs may function as force sensors and transduce the biomechanical signals through the ECM-integrin-cytoskeleton pathway[73]. Therefore, the biomechanical elasticity of ECMs is a potent regulator for neural lineage commitment of hPSCs.

THREE-DIMENSIONAL NEURAL DIFFERENTIATION OF PSCS

Because cells *in vivo* are exposed to a 3-D ECM environment, 3-D neural differentiation in natural or synthetic ECM scaffolds has been studied to mimic the architecture and biological role of the ECM in modulating stem cell fate decision[17,74]. Different 3-D synthetic ECM scaffolds including hydrogels, microfibrous, and nanofibrous matrices have been used for neural differentiation from PSCs or PSC-derived neural precursors (Table 4)[75-78]. For example, using chitin-alginate 3-D microfibrous scaffolds together with RA and noggin, nestin-expressing neural progenitors were derived from three independent hiPSC and hESC lines[75]. Neuron growth factor-grafted poly(-caprolactone)-poly(-hydroxybutyrate) scaffolds were demonstrated to improve iPSC differentiation into neurons while inhibiting differentiation into other lineages[79]. In another example of a 3-D synthetic hydrogel-based system, PuraMatrix™, hESC-derived neuronal cells developed more branched neurite structures and formed more electrically active networks as compared to 2-D differentiation, better resembling the *in vivo* tissues[76]. Electrospun polyurethane fibrous scaffolds have been shown to preferably differentiate hESCs into the neuronal lineage over the glial lineage[80]. A 3-D system involving an air-liquid interface was shown to generate a self-organized three-dimensional neural tissue guided by endogenous developmental cues on hydrophilic polytetrafluoroethylene membrane[81]. Tissue-engineered fibrin scaffolds were developed to enhance PSC-derived NPC survival and direct differentiation into neurons[82]. All these studies demonstrated that 3-D scaffolds physically influenced neural lineage commitment from PSCs.

The contact guidance and topography effects of 3-D scaffolds on neural differentiation were revealed in several studies recently[77,83]. The 3-D microfibrous poly(ethylene terephthalate) (PET) scaffolds have been shown to support neural differentiation of PSCs induced in an astrocyte-conditioned medium[84,85]. Compared to 2-D differentiation, 3-D differentiation in microfibrous matri-

Table 4 Three-dimensional natural and synthetic extracellular matrices scaffolds for neural differentiation of pluripotent stem cells

Scaffolds	Cell source	Neural differentiation	Ref.
Poly(lactic-co-glycolic acid) and poly(L-lactic acid) scaffolds	hESC	Enhanced numbers of neural structures and staining of nestin and β-tubulin Ⅲ were observed	Levenberg *et al*[74], 2005
Synthetic hydrogel matrix PuraMatrix	hESC-derived neuronal cells	HESC-derived neurons, astrocytes, and oligodendrocytes grew, matured and migrated in hydrogel; neuronal cells had electrically active connections.	Ylä-Outinen *et al*[76], 2012
Poly(epsilon-caprolactone)-poly(beta-hydroxybutyrate) scaffolds	Mouse iPSCs	Improved iPSCs to differentiate into neurons and inhibited other differentiations.	Kuo *et al*[79], 2012
Polycaprolactone nanofiber matrices	HESC-derived neural precursors	Aligned fibrous matrices showed higher rate of neuronal differentiation compared to random micro- and nano-fibers (62%-86% *vs* 27%-32%).	Mahairaki *et al*[77], 2011
Polyurethane nanofibrous scaffolds	hESC line SA002	Neuronal differentiation was preferred over astrocyte differentiation.	Carlberg *et al*[80], 2009
Tissue-engineered fibrin scaffolds	Mouse ESC-derived NPCs	Enhanced NPC survival and directed differentiation into neurons.	Johnson *et al*[82], 2010
PET microfibrous scaffolds	Mouse ESC D3 line	Enhanced neuronal differentiation indicated by nestin, Nurr1, and tyrosine hydroxylase compared to 2-D culture.	Liu *et al*[84,85], 2013, 2013
Multiwalled carbon nanotube modified PET microfibrous scaffolds	Mouse ESC D3 line	Enhanced neuronal differentiation compared to unmodified scaffolds	Zang *et al*[83], 2013
Chitin-alginate 3-D microfibrous scaffolds	hESC line: HUES 7 hiPSC lines: PD-iPS5 and hFib2-iPS4	Efficient neuronal differentiation: > 95% nestin$^+$; able to mature into neurons (> 90% β-tubulin Ⅲ$^+$)	Lu *et al*[75], 2012
3-D ECM scaffolds derived from ESC aggregates	Mouse ESC D3 line	ECM scaffolds derived from RA-treated EBs enhanced nestin and β-tubulin Ⅲ expressions	Sart *et al*[60], 2013
Inverted colloidal crystal (ICC) scaffolds containing alginate, poly(gamma-glutamic acid), and surface peptide; or chitin-chitosan-gelatin ICC scaffolds	Mouse iPSCs	Accelerated neuronal differentiation (β-tubulin Ⅲ expression) of iPSCs.	Kuo *et al*[86,87], 2013, 2013

hESCs: Human embryonic stem cells; iPSCs: Induced pluripotent stem cells; NPC: Neural progenitor cells; PET: Poly(ethylene terephthalate); ECM: Extracellular matrix; RA: Retinoic acid; EB: Embryoid body.

ces resulted in a higher percentage of nestin-positive cells (68% *vs* 54%) and upregulated the expressions of nestin, Nurr1, and tyrosine hydroxylase. Multiwalled carbon nanotubes (MWCNTs) were used to coat and provide nano-features on the surface of 3-D PET fibers, which significantly enhanced neuronal differentiation of ESCs compared to the surface without MWCNTs (Figure 3)[83]. Without MWCNTs, cells were flatly spread out on the PET membrane with few neurites formed. In contrast, with MWCNT, more neurons were observed across the surfaces of carbon nanotubes, forming a neural network with extensive neurite bridges between adjacent cells both on 2-D PET membrane and 3-D PET matrices. The 3-D differentiation in PET scaffolds was also demonstrated in stirred bioreactors for potential scale up[85]. The effects of fiber diameter and fiber orientation of polycaprolactone fiber matrices were evaluated for hESC-derived neural precursors[77]. The NPCs adhered on the aligned fibers showed a higher rate of neuronal differentiation as compared to cells cultured on random micro- and nano-fibers (62%-86% *vs* 27%-32%). The alginate, poly(-glutamic acid), and surface peptide based inverted colloidal crystal (ICC) scaffolds were shown to provide hexagonal crystals of polystyrene microspheres with interconnected pores, in which topography together with the surface peptide improved the differentiation of iPSCs into neuron cells[86]. Chitin-chitosan-gelatin scaffolds with ICC geometry were also found to accelerate neuronal differentiation of iPSCs compared to free-form constructs[87]. The topography with different surface gratings can increase the rate of neural differentiation of hPSCs, although the mechanisms that transduce the topographical signals into cell phenotype remain unknown[88]. By ingenious design of novel 3-D scaffolds, the neural differentiation from PSCs or the derived NPCs can be promoted.

DRUG SCREENING BASED ON HPSC-DERIVED NEURAL CELLS

Current drug screening methods using immortalized human lines or rodent models cannot accurately represent how various drugs would initiate the response in humans due to the physiological differences between animal and human as well as the lack of native metabolic and biological functions[89]. Although the sensitivity of human primary cells (*e.g.*, human cardiomyocytes) may give better response, these somatic cells are often limited by the available cell numbers. Estimates indicate that every 1% increase in predictability of toxicity in human would save up to $100 million in the pharmaceutical industry[90]. A human cell-based drug screening platform is thus desirable for drug discovery and mechanistic studies of various neurological diseases. Human PSCs, especially iPSCs, provide a great platform to generate allogeneic or patient-specific neural cells that are physiologically relevant for drug screening and disease modeling[8]. For example, Aβ-

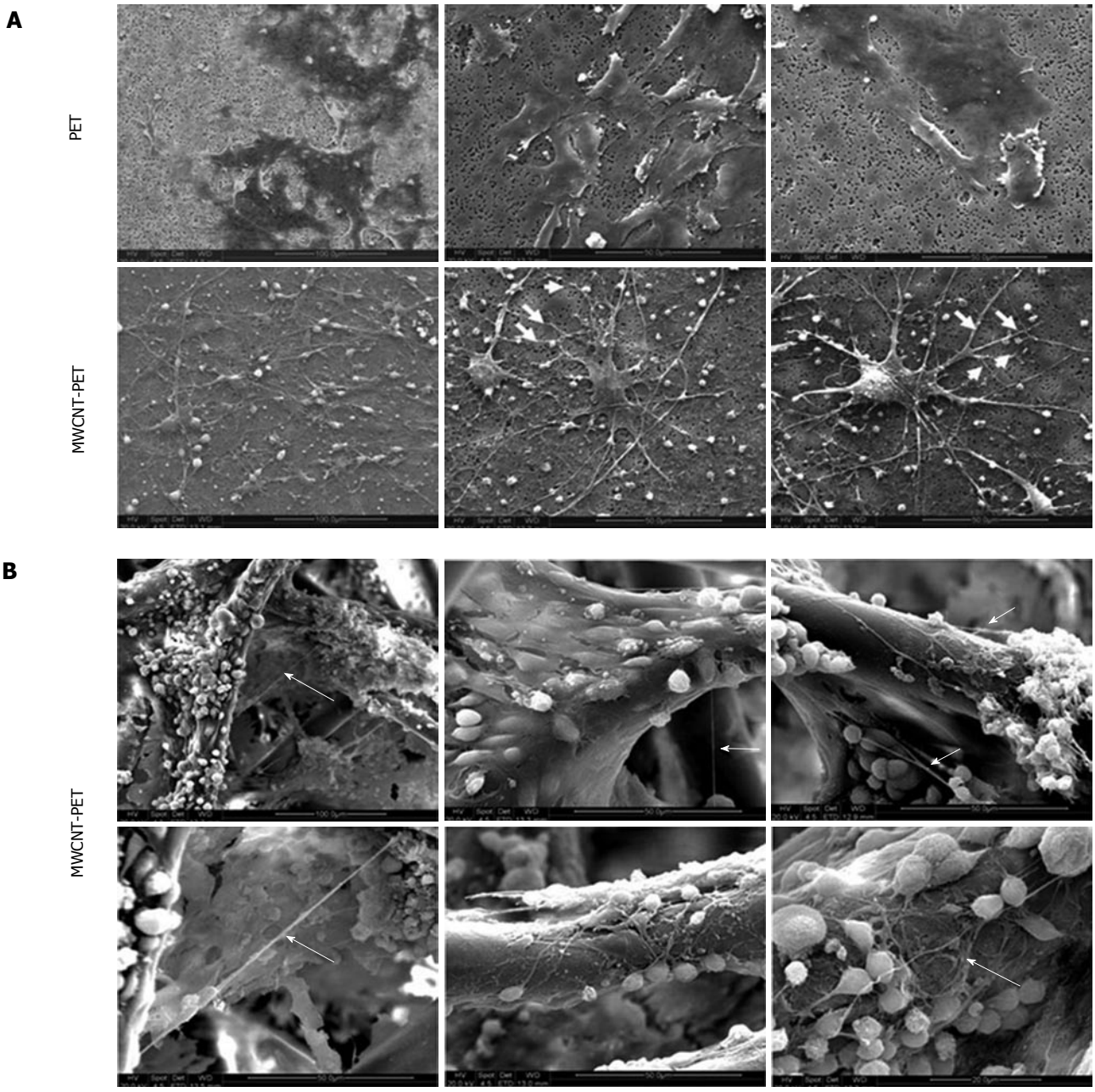

Figure 3 Neural differentiation of pluripotent stem cells. A: Neural cells derived from murine embryonic stem cells (mESCs) cultured on 2-D PET surface with or without multiwalled carbon nanotube (MWCNT) coating; B: Neural cells derived from mESCs cultured in 3-D PET scaffolds with MWCNT coating. Arrows point to neurite fibers. Images are adapted from Zang *et al*[83].

secreting neurons were derived from hiPSCs and used for screening anti-Aβ drugs for the treatment of Alzheimer's disease[52]. β-secretase inhibitor and γ-secretase inhibitor were shown to inhibit Aβ40 and Aβ42 secretion from hiPSC-derived neuronal cells. Overexpressing synuclein in hESC-derived dopamine neurons led to the selective cell death; thus drugs interacting with this process or reducing the accumulation of synuclein in cells can be used to treat Parkinson's disease[91]. Quantitative analysis of neural cells derived from hiPSCs harboring mutations associated with neurodegenerative disorders (*e.g.*, Parkinson's, ALS and schizophrenia) indicated the defects in cell growth, migration, and function compared to healthy donors[90]. These disease-relevant cells are more suitable for assessing the outcome of drug treatment. For examples, anti-psychotic drug loxapine has been shown to improve neuronal connectivity in Schizophrenia models established from hiPSCs[92]. The selective loss of motor neurons derived from iPSCs of spinal muscular atrophy patients was also decreased by treating with drugs such as valproic acid and tobramycin[91].

High-throughput analysis and high-content imaging platforms need to be developed for efficient screening. Various automated platforms, including IN Cell Analyzer (GE Healthcare), Cellomics Arrayscan (ThermoFisher), and ImageXpress (Molecular Devices), have been devel-

oped to collect information about cell physiology and function, including cell viability and apoptosis, cell number and proliferation, cell migration *etc*[90]. 3-D culture conditions are necessary to recreate the phenotype better representing *in vivo* neural tissues. The main challenge of hPSC-based drug screening is that the cells generated from hPSCs are developmentally immature[91]. Thus, functional maturation of hPSC-derived cells is being actively pursued in the field. Compared to 2-D platform of drug screening, 3-D ECM scaffold-based screening has been shown to be more predictive in terms of cell sensitivity to the drugs[93]. Hence, efficient 3-D neural differentiation systems that can enhance neural cell functions are in a great demand. High-throughput electrophysiology is also a critical component in drug screening because it can provide functional readouts during the screening. Therefore, the pharmaceutical industry is developing the platform such as PatchXpress to assess the effect of ion channel modulators. Given the challenges in cell therapy and transplantation, disease modeling and drug screening have been considered as two immediate applications of hPSCs.

CONCLUSION

Neural cells (including oligodendrocyte progenitors and neural progenitors) derived from hPSCs have great potential in drug screening, disease modeling, and regenerative medicine. High-purity neural cells can be derived from hPSCs induced by various biological and biochemical cues. Natural and synthetic ECMs, including their composition, mechanical properties, and physical structures play important roles in regulating cell survival, proliferation, migration, and differentiation. Therefore, there is an urgent need to optimize ECMs for efficient neural differentiation and functional maturation, especially 3-D ECM scaffolds, which can interact with other niche factors (*e.g.*, cytokines, accessory cells and nutrients) and provide the physiologically relevant microenvironment to guide neural tissue development. Understanding the biochemical and biomechanical interactions of hPSCs and the ECMs should accelerate the applications of hPSCs, especially in the immediate applications in drug screening.

REFERENCES

1 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147 [PMID: 9804556 DOI: 10.1126/science.282.5391.1145]

2 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]

3 **Wu SM**, Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nat Cell Biol* 2011; **13**: 497-505 [PMID: 21540845 DOI: 10.1038/ncb0511-497]

4 **Metallo CM**, Azarin SM, Ji L, de Pablo JJ, Palecek SP. Engineering tissue from human embryonic stem cells. *J Cell Mol Med* 2008; **12**: 709-729 [PMID: 18194458 DOI: 10.1111/j.1582-4934.2008.00228.x]

5 **Lebkowski JS**. Interview: Discussions on the development of human embryonic stem cell-based therapies. *Regen Med* 2009; **4**: 659-661 [PMID: 19761390 DOI: 10.2217/rme.09.49]

6 **Alper J**. Geron gets green light for human trial of ES cell-derived product. *Nat Biotechnol* 2009; **27**: 213-214 [PMID: 19270655 DOI: 10.1038/nbt0309-213a]

7 **Wang S**, Bates J, Li X, Schanz S, Chandler-Militello D, Levine C, Maherali N, Studer L, Hochedlinger K, Windrem M, Goldman SA. Human iPSC-derived oligodendrocyte progenitor cells can myelinate and rescue a mouse model of congenital hypomyelination. *Cell Stem Cell* 2013; **12**: 252-264 [PMID: 23395447 DOI: 10.1016/j.stem.2012.12.002]

8 **Engle SJ**, Puppala D. Integrating human pluripotent stem cells into drug development. *Cell Stem Cell* 2013; **12**: 669-677 [PMID: 23746976 DOI: 10.1016/j.stem.2013.05.011]

9 **Deshmukh RS**, Kovács KA, Dinnyés A. Drug discovery models and toxicity testing using embryonic and induced pluripotent stem-cell-derived cardiac and neuronal cells. *Stem Cells Int* 2012; **2012**: 379569 [PMID: 22654918]

10 **Di Giorgio FP**, Boulting GL, Bobrowicz S, Eggan KC. Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. *Cell Stem Cell* 2008; **3**: 637-648 [PMID: 19041780 DOI: 10.1016/j.stem.2008.09.017]

11 **Dimos JT**, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 2008; **321**: 1218-1221 [PMID: 18669821 DOI: 10.1126/science.1158799]

12 **Chambers SM**, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* 2009; **27**: 275-280 [PMID: 19252484 DOI: 10.1038/nbt.1529]

13 **Li Y**, Gautam A, Yang J, Qiu L, Melkoumian Z, Weber J, Telukuntla L, Srivastava R, Whiteley EM, Brandenberger R. Differentiation of oligodendrocyte progenitor cells from human embryonic stem cells on vitronectin-derived synthetic Peptide acrylate surface. *Stem Cells Dev* 2013; **22**: 1497-1505 [PMID: 23249362 DOI: 10.1089/scd.2012.0508]

14 **Alsanie WF**, Niclis JC, Petratos S. Human embryonic stem cell-derived oligodendrocytes: protocols and perspectives. *Stem Cells Dev* 2013; **22**: 2459-2476 [PMID: 23621561 DOI: 10.1089/scd.2012.0520]

15 **Solozobova V**, Wyvekens N, Pruszak J. Lessons from the embryonic neural stem cell niche for neural lineage differentiation of pluripotent stem cells. *Stem Cell Rev* 2012; **8**: 813-829 [PMID: 22628111 DOI: 10.1007/s12015-012-9381-8]

16 **Ma W**, Tavakoli T, Derby E, Serebryakova Y, Rao MS, Mattson MP. Cell-extracellular matrix interactions regulate neural differentiation of human embryonic stem cells. *BMC Dev Biol* 2008; **8**: 90 [PMID: 18808690 DOI: 10.1186/1471-213X-8-90]

17 **Kraehenbuehl TP**, Langer R, Ferreira LS. Three-dimensional biomaterials for the study of human pluripotent stem cells. *Nat Methods* 2011; **8**: 731-736 [PMID: 21878920 DOI: 10.1038/nmeth.1671]

18 **Sart S**, Agathos SN, Li Y. Engineering stem cell fate with biochemical and biomechanical properties of microcarriers. *Biotechnol Prog* 2013; **29**: 1354-1366 [PMID: 24124017 DOI: 10.1002/btpr.1825]

19 **Keirstead HS**, Nistor G, Bernal G, Totoiu M, Cloutier F, Sharp K, Steward O. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. *J Neurosci* 2005; **25**: 4694-4705 [PMID: 15888645 DOI: 10.1523/JNEUROSCI.0311-05.2005]

20 **Ogawa S**, Tokumoto Y, Miyake J, Nagamune T. Induction of oligodendrocyte differentiation from adult human

fibroblast-derived induced pluripotent stem cells. *In Vitro Cell Dev Biol Anim* 2011; **47**: 464-469 [PMID: 21695581 DOI: 10.1007/s11626-011-9435-2]
21 **Nistor GI**, Totoiu MO, Haque N, Carpenter MK, Keirstead HS. Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. *Glia* 2005; **49**: 385-396 [PMID: 15538751 DOI: 10.1002/glia.20127]
22 **Watson RA**, Yeung TM. What is the potential of oligodendrocyte progenitor cells to successfully treat human spinal cord injury? *BMC Neurol* 2011; **11**: 113 [PMID: 21943254 DOI: 10.1186/1471-2377-11-113]
23 **Zhang YW**, Denham J, Thies RS. Oligodendrocyte progenitor cells derived from human embryonic stem cells express neurotrophic factors. *Stem Cells Dev* 2006; **15**: 943-952 [PMID: 17253955 DOI: 10.1089/scd.2006.15.943]
24 **Kang SM**, Cho MS, Seo H, Yoon CJ, Oh SK, Choi YM, Kim DW. Efficient induction of oligodendrocytes from human embryonic stem cells. *Stem Cells* 2007; **25**: 419-424 [PMID: 17053214 DOI: 10.1634/stemcells.2005-0482]
25 **Sher F**, Balasubramaniyan V, Boddeke E, Copray S. Oligodendrocyte differentiation and implantation: new insights for remyelinating cell therapy. *Curr Opin Neurol* 2008; **21**: 607-614 [PMID: 18769257 DOI: 10.1097/WCO.0b013e32830f1e50]
26 **Hu BY**, Du ZW, Zhang SC. Differentiation of human oligodendrocytes from pluripotent stem cells. *Nat Protoc* 2009; **4**: 1614-1622 [PMID: 19834476 DOI: 10.1038/nprot.2009.186]
27 **Sundberg M**, Hyysalo A, Skottman H, Shin S, Vemuri M, Suuronen R, Narkilahti S. A xeno-free culturing protocol for pluripotent stem cell-derived oligodendrocyte precursor cell production. *Regen Med* 2011; **6**: 449-460 [PMID: 21749203 DOI: 10.2217/rme.11.36]
28 **Sundberg M**, Skottman H, Suuronen R, Narkilahti S. Production and isolation of NG2+ oligodendrocyte precursors from human embryonic stem cells in defined serum-free medium. *Stem Cell Res* 2010; **5**: 91-103 [PMID: 20538536 DOI: 10.1016/j.scr.2010.04.005]
29 **Kerr CL**, Letzen BS, Hill CM, Agrawal G, Thakor NV, Sterneckert JL, Gearhart JD, All AH. Efficient differentiation of human embryonic stem cells into oligodendrocyte progenitors for application in a rat contusion model of spinal cord injury. *Int J Neurosci* 2010; **120**: 305-313 [PMID: 20374080 DOI: 10.3109/00207450903585290]
30 **Czepiel M**, Balasubramaniyan V, Schaafsma W, Stancic M, Mikkers H, Huisman C, Boddeke E, Copray S. Differentiation of induced pluripotent stem cells into functional oligodendrocytes. *Glia* 2011; **59**: 882-892 [PMID: 21438010]
31 **Tokumoto Y**, Ogawa S, Nagamune T, Miyake J. Comparison of efficiency of terminal differentiation of oligodendrocytes from induced pluripotent stem cells versus embryonic stem cells in vitro. *J Biosci Bioeng* 2010; **109**: 622-628 [PMID: 20471604 DOI: 10.1002/glia.21159]
32 **Miura K**, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S. Variation in the safety of induced pluripotent stem cell lines. *Nat Biotechnol* 2009; **27**: 743-745 [PMID: 19590502 DOI: 10.1038/nbt.1554]
33 **Pouya A**, Satarian L, Kiani S, Javan M, Baharvand H. Human induced pluripotent stem cells differentiation into oligodendrocyte progenitors and transplantation in a rat model of optic chiasm demyelination. *PLoS One* 2011; **6**: e27925 [PMID: 22125639 DOI: 10.1371/journal.pone.0027925]
34 **Liu Y**, Jiang P, Deng W. OLIG gene targeting in human pluripotent stem cells for motor neuron and oligodendrocyte differentiation. *Nat Protoc* 2011; **6**: 640-655 [PMID: 21527921 DOI: 10.1038/nprot.2011.310]
35 **Hu J**, Deng L, Wang X, Xu XM. Effects of extracellular matrix molecules on the growth properties of oligodendrocyte progenitor cells in vitro. *J Neurosci Res* 2009; **87**: 2854-2862 [PMID: 19472225 DOI: 10.1002/jnr.22111]
36 **Gil JE**, Woo DH, Shim JH, Kim SE, You HJ, Park SH, Paek SH, Kim SK, Kim JH. Vitronectin promotes oligodendrocyte differentiation during neurogenesis of human embryonic stem cells. *FEBS Lett* 2009; **583**: 561-567 [PMID: 19162023 DOI: 10.1016/j.febslet.2008.12.061]
37 **Blaschuk KL**, Frost EE, ffrench-Constant C. The regulation of proliferation and differentiation in oligodendrocyte progenitor cells by alphaV integrins. *Development* 2000; **127**: 1961-1969 [PMID: 10751184]
38 **Aizman I**, Tate CC, McGrogan M, Case CC. Extracellular matrix produced by bone marrow stromal cells and by their derivative, SB623 cells, supports neural cell growth. *J Neurosci Res* 2009; **87**: 3198-3206 [PMID: 19530164 DOI: 10.1002/jnr.22146]
39 **Mekhail M**, Almazan G, Tabrizian M. Oligodendrocyte-protection and remyelination post-spinal cord injuries: a review. *Prog Neurobiol* 2012; **96**: 322-339 [PMID: 22307058 DOI: 10.1016/j.pneurobio.2012.01.008]
40 **Jagielska A**, Norman AL, Whyte G, Vliet KJ, Guck J, Franklin RJ. Mechanical environment modulates biological properties of oligodendrocyte progenitor cells. *Stem Cells Dev* 2012; **21**: 2905-2914 [PMID: 22646081 DOI: 10.1089/scd.2012.0189]
41 **Leipzig ND**, Shoichet MS. The effect of substrate stiffness on adult neural stem cell behavior. *Biomaterials* 2009; **30**: 6867-6878 [PMID: 19775749 DOI: 10.1016/j.biomaterials.2009.09.002]
42 **Volpato FZ**, Führmann T, Migliaresi C, Hutmacher DW, Dalton PD. Using extracellular matrix for regenerative medicine in the spinal cord. *Biomaterials* 2013; **34**: 4945-4955 [PMID: 23597407 DOI: 10.1016/j.biomaterials.2013.03.057]
43 **Boulting GL**, Kiskinis E, Croft GF, Amoroso MW, Oakley DH, Wainger BJ, Williams DJ, Kahler DJ, Yamaki M, Davidow L, Rodolfa CT, Dimos JT, Mikkilineni S, MacDermott AB, Woolf CJ, Henderson CE, Wichterle H, Eggan K. A functionally characterized test set of human induced pluripotent stem cells. *Nat Biotechnol* 2011; **29**: 279-286 [PMID: 21293464 DOI: 10.1038/nbt.1783]
44 **Kim DS**, Lee JS, Leem JW, Huh YJ, Kim JY, Kim HS, Park IH, Daley GQ, Hwang DY, Kim DW. Robust enhancement of neural differentiation from human ES and iPS cells regardless of their innate difference in differentiation propensity. *Stem Cell Rev* 2010; **6**: 270-281 [PMID: 20376579 DOI: 10.1007/s12015-010-9138-1]
45 **Hicks AU**, Lappalainen RS, Narkilahti S, Suuronen R, Corbett D, Sivenius J, Hovatta O, Jolkkonen J. Transplantation of human embryonic stem cell-derived neural precursor cells and enriched environment after cortical stroke in rats: cell survival and functional recovery. *Eur J Neurosci* 2009; **29**: 562-574 [PMID: 19175403 DOI: 10.1111/j.1460-9568.2008.06599.x]
46 **Koch P**, Opitz T, Steinbeck JA, Ladewig J, Brüstle O. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. *Proc Natl Acad Sci USA* 2009; **106**: 3225-3230 [PMID: 19218428 DOI: 10.1073/pnas.0808387106]
47 **Nistor G**, Siegenthaler MM, Poirier SN, Rossi S, Poole AJ, Charlton ME, McNeish JD, Airriess CN, Keirstead HS. Derivation of high purity neuronal progenitors from human embryonic stem cells. *PLoS One* 2011; **6**: e20692 [PMID: 21673956 DOI: 10.1371/journal.pone.0020692]
48 **Gerrard L**, Rodgers L, Cui W. Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling. *Stem Cells* 2005; **23**: 1234-1241 [PMID: 16002783 DOI: 10.1634/stemcells.2005-0110]
49 **Axell MZ**, Zlateva S, Curtis M. A method for rapid derivation and propagation of neural progenitors from human embryonic stem cells. *J Neurosci Methods* 2009; **184**: 275-284 [PMID: 19715727 DOI: 10.1016/j.jneumeth.2009.08.015]
50 **Stover AE**, Brick DJ, Nethercott HE, Banuelos MG, Sun L, O'Dowd DK, Schwartz PH. Process-based expansion and

neural differentiation of human pluripotent stem cells for transplantation and disease modeling. *J Neurosci Res* 2013; **91**: 1247-1262 [PMID: 23893392 DOI: 10.1002/jnr.23245]
51 **Hu BY**, Zhang SC. Differentiation of spinal motor neurons from pluripotent human stem cells. *Nat Protoc* 2009; **4**: 1295-1304 [PMID: 19696748 DOI: 10.1038/nprot.2009.127]
52 **Yahata N**, Asai M, Kitaoka S, Takahashi K, Asaka I, Hioki H, Kaneko T, Maruyama K, Saido TC, Nakahata T, Asada T, Yamanaka S, Iwata N, Inoue H. Anti-Aβ drug screening platform using human iPS cell-derived neurons for the treatment of Alzheimer's disease. *PLoS One* 2011; **6**: e25788 [PMID: 21984949 DOI: 10.1371/journal.pone.0025788]
53 **Oki K**, Tatarishvili J, Wood J, Koch P, Wattananit S, Mine Y, Monni E, Tornero D, Ahlenius H, Ladewig J, Brüstle O, Lindvall O, Kokaia Z. Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain. *Stem Cells* 2012; **30**: 1120-1133 [PMID: 22495829 DOI: 10.1002/stem.1104]
54 **Polentes J**, Jendelova P, Cailleret M, Braun H, Romanyuk N, Tropel P, Brenot M, Itier V, Seminatore C, Baldauf K, Turnovcova K, Jirak D, Teletin M, Côme J, Tournois J, Reymann K, Sykova E, Viville S, Onteniente B. Human induced pluripotent stem cells improve stroke outcome and reduce secondary degeneration in the recipient brain. *Cell Transplant* 2012; **21**: 2587-2602 [PMID: 22889472 DOI: 10.3727/096368912X653228]
55 **Jensen MB**, Yan H, Krishnaney-Davison R, Al Sawaf A, Zhang SC. Survival and differentiation of transplanted neural stem cells derived from human induced pluripotent stem cells in a rat stroke model. *J Stroke Cerebrovasc Dis* 2013; **22**: 304-308 [PMID: 22078778 DOI: 10.1016/j.jstrokecerebrovasdis.2011.09.008]
56 **Chen SJ**, Chang CM, Tsai SK, Chang YL, Chou SJ, Huang SS, Tai LK, Chen YC, Ku HH, Li HY, Chiou SH. Functional improvement of focal cerebral ischemia injury by subdural transplantation of induced pluripotent stem cells with fibrin glue. *Stem Cells Dev* 2010; **19**: 1757-1767 [PMID: 20192839 DOI: 10.1089/scd.2009.0452]
57 **Popescu IR**, Nicaise C, Liu S, Bisch G, Knippenberg S, Daubie V, Bohl D, Pochet R. Neural progenitors derived from human induced pluripotent stem cells survive and differentiate upon transplantation into a rat model of amyotrophic lateral sclerosis. *Stem Cells Transl Med* 2013; **2**: 167-174 [PMID: 23413376 DOI: 10.5966/sctm.2012-0042]
58 **Daadi MM**, Steinberg GK. Manufacturing neurons from human embryonic stem cells: biological and regulatory aspects to develop a safe cellular product for stroke cell therapy. *Regen Med* 2009; **4**: 251-263 [PMID: 19317644 DOI: 10.2217/17460751.4.2.251]
59 **Tate CC**, Shear DA, Tate MC, Archer DR, Stein DG, LaPlaca MC. Laminin and fibronectin scaffolds enhance neural stem cell transplantation into the injured brain. *J Tissue Eng Regen Med* 2009; **3**: 208-217 [PMID: 19229887 DOI: 10.1002/term.154]
60 **Sart S**, Ma T, Li Y. Extracellular Matrices Decellularized from Embryonic Stem Cells Maintained Their Structure and Signaling Specificity. *Tissue Eng Part* A 2013 Aug 15; Epub ahead of print [PMID: 23848515 DOI: 10.1089/ten.tea.2012.0690]
61 **Raghavan S**, Gilmont RR, Bitar KN. Neuroglial differentiation of adult enteric neuronal progenitor cells as a function of extracellular matrix composition. *Biomaterials* 2013; **34**: 6649-6658 [PMID: 23746858 DOI: 10.1016/j.biomaterials.2013.05.023]
62 **Haque A**, Yue XS, Motazedian A, Tagawa Y, Akaike T. Characterization and neural differentiation of mouse embryonic and induced pluripotent stem cells on cadherin-based substrata. *Biomaterials* 2012; **33**: 5094-5106 [PMID: 22520296 DOI: 10.1016/j.biomaterials.2012.04.003]
63 **Earls JK**, Jin S, Ye K. Mechanobiology of human pluripotent stem cells. *Tissue Eng Part B Rev* 2013; **19**: 420-430 [PMID: 23472616 DOI: 10.1089/ten.teb.2012.0641]
64 **Gonzalez-Rodriguez D**, Guevorkian K, Douezan S, Brochard-Wyart F. Soft matter models of developing tissues and tumors. *Science* 2012; **338**: 910-917 [PMID: 23161991 DOI: 10.1126/science.1226418]
65 **Sun Y**, Villa-Diaz LG, Lam RH, Chen W, Krebsbach PH, Fu J. Mechanics regulates fate decisions of human embryonic stem cells. *PLoS One* 2012; **7**: e37178 [PMID: 22615930 DOI: 10.1371/journal.pone.0037178]
66 **Eroshenko N**, Ramachandran R, Yadavalli VK, Rao RR. Effect of substrate stiffness on early human embryonic stem cell differentiation. *J Biol Eng* 2013; **7**: 7 [PMID: 23517522 DOI: 10.1186/1754-1611-7-7]
67 **Musah S**, Morin SA, Wrighton PJ, Zwick DB, Jin S, Kiessling LL. Glycosaminoglycan-binding hydrogels enable mechanical control of human pluripotent stem cell self-renewal. *ACS Nano* 2012; **6**: 10168-10177 [PMID: 23005914 DOI: 10.1021/nn3039148]
68 **Saha K**, Keung AJ, Irwin EF, Li Y, Little L, Schaffer DV, Healy KE. Substrate modulus directs neural stem cell behavior. *Biophys J* 2008; **95**: 4426-4438 [PMID: 18658232 DOI: 10.1529/biophysj.108.132217]
69 **Nemir S**, West JL. Synthetic materials in the study of cell response to substrate rigidity. *Ann Biomed Eng* 2010; **38**: 2-20 [PMID: 19816774 DOI: 10.1007/s10439-009-9811-1]
70 **Keung AJ**, de Juan-Pardo EM, Schaffer DV, Kumar S. Rho GTPases mediate the mechanosensitive lineage commitment of neural stem cells. *Stem Cells* 2011; **29**: 1886-1897 [PMID: 21956892 DOI: 10.1002/stem.746]
71 **Keung AJ**, Asuri P, Kumar S, Schaffer DV. Soft microenvironments promote the early neurogenic differentiation but not self-renewal of human pluripotent stem cells. *Integr Biol* (Camb) 2012; **4**: 1049-1058 [PMID: 22854634 DOI: 10.1039/c2ib20083j]
72 **Zoldan J**, Karagiannis ED, Lee CY, Anderson DG, Langer R, Levenberg S. The influence of scaffold elasticity on germ layer specification of human embryonic stem cells. *Biomaterials* 2011; **32**: 9612-9621 [PMID: 21963156 DOI: 10.1016/j.biomaterials.2011.09.012]
73 **Sun Y**, Chen CS, Fu J. Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment. *Annu Rev Biophys* 2012; **41**: 519-542 [PMID: 22404680 DOI: 10.1146/annurev-biophys-042910-155306]
74 **Levenberg S**, Burdick JA, Kraehenbuehl T, Langer R. Neurotrophin-induced differentiation of human embryonic stem cells on three-dimensional polymeric scaffolds. *Tissue Eng* 2005; **11**: 506-512 [PMID: 15869429 DOI: 10.1089/ten.2005.11.506]
75 **Lu HF**, Lim SX, Leong MF, Narayanan K, Toh RP, Gao S, Wan AC. Efficient neuronal differentiation and maturation of human pluripotent stem cells encapsulated in 3D microfibrous scaffolds. *Biomaterials* 2012; **33**: 9179-9187 [PMID: 22998816 DOI: 10.1016/j.biomaterials.2012.09.006]
76 **Ylä-Outinen L**, Joki T, Varjola M, Skottman H, Narkilahti S. Three-dimensional growth matrix for human embryonic stem cell-derived neuronal cells. *J Tissue Eng Regen Med* 2012 May 18; Epub ahead of print [PMID: 22611014 DOI: 10.1002/term.1512]
77 **Mahairaki V**, Lim SH, Christopherson GT, Xu L, Nasonkin I, Yu C, Mao HQ, Koliatsos VE. Nanofiber matrices promote the neuronal differentiation of human embryonic stem cell-derived neural precursors in vitro. *Tissue Eng Part A* 2011; **17**: 855-863 [PMID: 20973749 DOI: 10.1089/ten.tea.2010.0377]
78 **Willerth SM**, Arendas KJ, Gottlieb DI, Sakiyama-Elbert SE. Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells. *Biomaterials* 2006; **27**: 5990-6003 [PMID: 16919326 DOI: 10.1016/j.biomaterials.2006.07.036]
79 **Kuo YC**, Huang MJ. Material-driven differentiation of induced pluripotent stem cells in neuron growth factor-grafted poly(ε-caprolactone)-poly(β-hydroxybutyrate) scaffolds. *Bio-*

materials 2012; **33**: 5672-5682 [PMID: 22591608 DOI: 10.1016/j.biomaterials.2012.04.046]
80 **Carlberg B**, Axell MZ, Nannmark U, Liu J, Kuhn HG. Electrospun polyurethane scaffolds for proliferation and neuronal differentiation of human embryonic stem cells. *Biomed Mater* 2009; **4**: 045004 [PMID: 19567936 DOI: 10.1088/1748-6041/4/4/045004]
81 **Preynat-Seauve O**, Suter DM, Tirefort D, Turchi L, Virolle T, Chneiweiss H, Foti M, Lobrinus JA, Stoppini L, Feki A, Dubois-Dauphin M, Krause KH. Development of human nervous tissue upon differentiation of embryonic stem cells in three-dimensional culture. *Stem Cells* 2009; **27**: 509-520 [PMID: 19074418]
82 **Johnson PJ**, Tatara A, McCreedy DA, Shiu A, Sakiyama-Elbert SE.Tissue-engineered fibrin scaffolds containing neural progenitors enhance functional recovery in a subacute model of SCI. *Soft Matter* 2010; **6**: 5127-5137 [PMID: 21072248]
83 **Zang R**, Yang ST. Multiwall carbon nanotube-coated polyethylene terephthalate fibrous matrices for enhanced neuronal differentiation of mouse embryonic stem cells. *J Mater Chem B* 2013; **1**: 646-653 [DOI: 10.1039/C2TB00157H]
84 **Liu N**, Li Y, Yang ST. Microfibrous carriers for integrated expansion and neural differentiation of embryonic stem cells in suspension bioreactor. *Biochem Eng J* 2013; **75**: 55-63 [DOI: 10.1016/j.bej.2013.03.017]
85 **Liu N**, Ouyang A, Li Y, Yang ST. Three-dimensional neural differentiation of embryonic stem cells with ACM induction in microfibrous matrices in bioreactors. *Biotechnol Prog* 2013; **29**: 1013-1022 [PMID: 23657995]
86 **Kuo YC**, Chen CW. Inverted colloidal crystal scaffolds with induced pluripotent stem cells for nerve tissue engineering. *Colloids Surf B Biointerfaces* 2013; **102**: 789-794 [PMID: 23107957]
87 **Kuo YC**, Lin CC. Accelerated nerve regeneration using induced pluripotent stem cells in chitin-chitosan-gelatin scaffolds with inverted colloidal crystal geometry. *Colloids Surf B Biointerfaces* 2013; **103**: 595-600 [PMID: 23261585]
88 **Chan LY**, Birch WR, Yim EK, Choo AB. Temporal application of topography to increase the rate of neural differentiation from human pluripotent stem cells. *Biomaterials* 2013; **34**: 382-392 [PMID: 23083932]
89 **Xu XH**, Zhong Z. Disease modeling and drug screening for neurological diseases using human induced pluripotent stem cells. *Acta Pharmacol Sin* 2013; **34**: 755-764 [PMID: 23685955 DOI: 10.1038/aps.2013.63]
90 **Rajamohan D**, Matsa E, Kalra S, Crutchley J, Patel A, George V, Denning C. Current status of drug screening and disease modelling in human pluripotent stem cells. *Bioessays* 2013; **35**: 281-298 [PMID: 22886688 DOI: 10.1002/bies.201200053]
91 **Ebert AD**, Svendsen CN. Human stem cells and drug screening: opportunities and challenges. *Nat Rev Drug Discov* 2010; **9**: 367-372 [PMID: 20339370 DOI: 10.1038/nrd3000]
92 **Brennand KJ**, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH. Modelling schizophrenia using human induced pluripotent stem cells. *Nature* 2011; **473**: 221-225 [PMID: 21490598 DOI: 10.1038/nature09915]
93 **Li D**, Isherwood S, Motz A, Zang R, Yang ST, Wang J, Wang X. Cell-based screening of traditional chinese medicines for proliferation enhancers of mouse embryonic stem cells. *Biotechnol Prog* 2013; **29**: 738-744 [PMID: 23606670 DOI: 10.1002/btpr.1731]
94 **Sharp J**, Frame J, Siegenthaler M, Nistor G, Keirstead HS. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury. *Stem Cells* 2010; **28**: 152-163 [PMID: 19877167]

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