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Review Article

Electrical stimulation affects neural stem cell fate and function in vitro

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ABSTRACT

Electrical stimulation (ES) has been applied in cell culture system to enhance neural stem cell (NSC) proliferation, neuronal differentiation, migration, and integration. According to the mechanism of its function, ES can be classified into induced electrical (EFs) and electromagnetic fields (EMFs). EFs guide axonal growth and induce directional cell migration, whereas EMFs promote neurogenesis and facilitates NSCs to differentiate into functional neurons. Conductive nanomaterials have been used as functional scaffolds to provide mechanical support and biophysical cues in guiding neural cell growth and differentiation and building complex neural tissue patterns. Nanomaterials may have a combined effect of topographical and electrical cues on NSC migration and differentiation. Electrical cues may promote NSC neurogenesis via specific ion channel activation, such as SCN1α and CACNA1C. To accelerate the future application of ES in preclinical research, we summarized the specific setting, such as current frequency, intensity, and stimulation duration used in various ES devices, as well as the nanomaterials involved, in this review with the possible mechanisms elucidated. This review can be used as a checklist for ES work in stem cell research to enhance the translational process of NSCs in clinical application.

1. Introduction

Neural stem cell (NSC) is a type of self-renewing stem cell that possesses multidifferential ability to produce neurons and glial cells in the nervous system. NSCs are the most promising candidates in the field of tissue engineering and regenerative medicine due to their tissue formation and remodeling capability at neuronal injury sites. NSC therapies have been used to treat stroke, traumatic brain injury, spinal cord injury, and even neurodegenerative diseases. Endogenous electrical activity stimulates tissue regeneration, induces NSC recruitment to the wound site, and enhances wound healing (Spitzer, 2006). Exogenous electrical stimulation (ES) is the process of artificially stimulating action potentials (APs) by inducing electrical charge to the cell; this method is a flexible, nonchemical, and feasible technique in vivo and in vitro (Lee et al., 2018). ES significantly increases fetal NSC proliferation and differentiation into neuronal cells (Yamada et al., 2007; Chang et al., 2011), induces guided cell migration and integration (Park et al., 2011), and promotes human NSC and embryonic stem cell differentiation (Du et al., 2018). ES frequency, duration, voltage, and current vary according to the stem cell types and the aim of the treatment. The biophysical changes are triggered at the cell surface, thereby affecting membrane protein functions, such as enzyme activity, membrane receptor complex, and ion-transporting channels, by altering the charge distribution.

Given the intrinsic electrical characteristics of neurons, the electroactive biomaterials are useful to NSCs when exposed to ES. Conductive biomaterials can directly deliver electrical, electromechanical, and electrochemical stimulations to cells (Lakard et al.,

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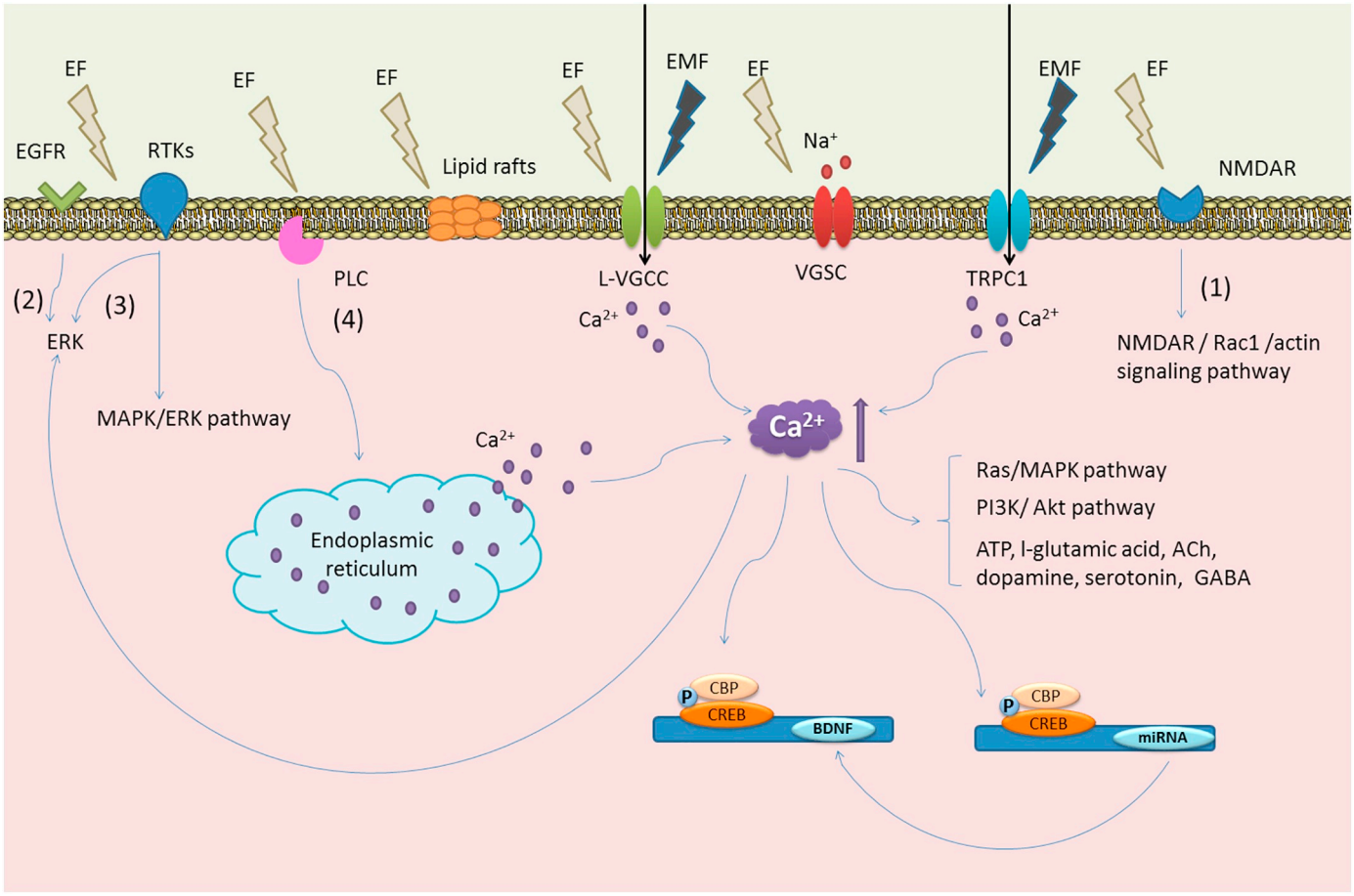

Fig. 1. Potential mechanism of electrical stimulation effect on NSCs.
Electrical stimulation (ES) causes the reorganization of the cytoskeletal filaments and polarization of lipid raft structures. ES can (1) affect the *N*-methyl-D-aspartate receptor (NMDAR) ligand gate and then activate NMDAR/Rac1/actin signaling pathway. ES (2) activates epidermal growth factor receptor (EGFR), followed by ERK. (3) Tyrosine phosphorylation activation in receptor tyrosine kinases (RTKs) contributes to MAPK/ERK pathway and ERK phosphorylation activation. (4) Phospholipase C (PLC) activation induces the increase in intracellular Ca^{2+} from the endoplasmic reticulum. Ca^{2+} influx induced by direct EFS and EMFS on L-type voltage-gated Ca^{2+} channels (VGCCs), or upregulated transient receptor potential canonical 1 (TRPC1) expression induces the phosphorylation-dependent activation of the transcription factor, that is, cAMP response element binding protein (CREB). The accumulation of Cav1-dependent CREB phosphorylation at Ser133 promotes neuronal gene (NeuroD1 and neurogenin1) expression and recruits histone acetyltransferase CBP. pCREB can also bind to a series of miRNA promoters to regulate their expression; CREB and its epigenetic mechanisms can affect the expression of BDNF. Changes in the cytoplasmic Ca^{2+} further affect the produced ATP, L-glutamic acid, acetylcholine, dopamine, serotonin, and GABA concentrations.

2009; Rivers et al., 2002). The family of electroactive biomaterials includes conductive polymers (CPs), metal nanoparticles (NPs), and C-based materials (Min et al., 2018). CPs are organic polymers with a conjugated single- or double-bond backbone formed by dopants. The conjugated backbone in CPs is capable of the discharge of ions, making these polymers as conductive as metals (Park et al., 2015). CPs, such as polypyrrole (PPy), polyaniline (PANI), polythiophene (PT), poly(3,4-ethylene dioxythiophene) (PEDOT), and poly(*p*-phenylene vinylene), support cell growth, adhesion, proliferation, and differentiation with or without ES (Mawad et al., 2012; Kaur et al., 2015). Metal NPs and C-based materials are widely reported in tissue engineering application because of their electrical properties (Harrison and Atala, 2007; Shevach et al., 2014). The advantage of electroactive biomaterials is that it can provide both physical and electrical properties, which are important for localized NSC differentiation and maturation. These biomaterials have high electrical conductivity, good biocompatibility, and few side effect when applied to construct scaffolds in seeding NSCs (Park et al., 2015). Functional scaffolds provide mechanical support and biophysical cues to guide neural cell growth and differentiation and form complex neural tissue patterns.

In this review, we first summarize the roles of two different but widely used electrical stimuli, namely, electric fields (EFs) and electromagnetic fields (EMFs), in NSC growth, differentiation, and maturation in vitro. In particular, we focus on the emerging progress of electroactive biomaterials in NSCs. Details are provided on the various methods of stimulate devices, current and voltage intensities, duration, and main results. We also address the potential mechanisms of ES on NSCs (Fig. 1). Then, we discuss the future perspective of ES on NSCs in clinical transformation. We hope that this review will provide insight for researchers into the current state of research in this field quickly and choose appropriate experimental conditions on the basis of their own experimental requirements combined with existing literature.

2. Effect of ES on NSCs in vitro

Generally, ES on NSCs in vitro can be divided into two categories, that is, EFs and EMFs. Both categories affect NSC's fate and function in previous studies. In the following sections, we will discuss the positive effects of EFs and EMFs on NSC proliferation and differentiation in vitro.

2.1. Effect of EFs on NSCs in vitro

EFs show profound effects on cell proliferation, migration,

differentiation, and axonal outgrowth through ion channel distribution regulation. In contrast to biochemical methods, EF has the advantages of instant application time and direction control, precise frequency and voltage magnitude, and no chemical residuals left in the system (Feng et al., 2012). EFs have been used as an effective replacement to biochemical and physical cues in regulating NSC migration and differentiation to improve the therapeutic strategies for CNS repair (Li et al., 2014).

2.1.1. Types of EFs in NSC research

EFs generate from external power sources and are typically applied to biological cells/tissues via electrodes. According to the time-various current directions of EFs, these currents can be categorized as direct (DCEFs), alternating (ACEFs), pulse (PCEFs), and biphasic electrical currents (BECs). Direct current refers to the current whose magnitude and direction do not change with time, while alternating current refers to the current whose magnitude and direction change periodically with time. Pulsed current is a unidirectional or bidirectional current flow for a brief duration. The methods to induce EFs, treatment duration and stimulation parameters of EFs in various NSC species, and their main findings and mechanism involved are listed in detail in Table 1.

2.1.2. Influence of EF on NSC migration

EFs play a critical role in the migration direction and speed (Nishimura et al., 1996; Chao et al., 2000; Farboud et al., 2000). DCEFs guides the migration of adult rat hippocampal NS/PCs (Ariza et al., 2010), embryonic-derived NPCs (Li et al., 2008), neonatal neurons (Yao et al., 2008), and human embryonic stem cell (hESC) (Zhang et al., 2011) to the cathode. Low-field strength DCEF considerably allows NSCs to move toward the cathode. The voltage-dependent field strength with time affects the migration direction and distance. The migration distance increases significantly when EFs increased from 50 mV/mm to 100 mV/mm (Li et al., 2014). EFs can also significantly promote embryonic and adult NPCs directedness and cathodal migration displacement when the field strength ranges between 250 and 437 mV/mm (Li et al., 2014; Ariza et al., 2010).

EF also affects the directional migration velocity of NSCs. One study reported that the adult rat hippocampal NPC migration rate was slower (approximately 16 μm/h) than that of neonatal hippocampal cells (Yao et al., 2008). This rate was extremely similar to the embryonic rat NPC migration speed when exposed to the physiological strengths of EFs (Li et al., 2008). EFs also increased the NSPC migration rate in a voltage-dependent manner between 0 and 250 mV/mm. However, Li et al. (2014) reported that EFs did not change the embryonic stem cell migration velocity when the voltages increased from 50 mV/mm to 100 mV/mm. Combined with these results, the DCEFs in the range of 16–437 mV/mm can guide NSCs toward the cathode and enhance the migration distance and rate in a strength-dependent manner. The physiological strengths of EFs may aid in migrating NSCs to the injured site.

2.1.3. Influence of EF on NSC proliferation

Chang et al. (2011) showed that BEC at a calculated current density of 4 or 8 $\mu\text{Å}/\text{cm}^2$ for 200 μs at 100 Hz significantly increased fetal NSC proliferation. PCEFs can improve the viability of NSCs cultured on cathodes exposed to 20 Hz and 100 μs pulse at a potential gradient of 200 mV/mm (Du et al., 2018). Matos and Cicerone (2010) also reported that ACEFs with a frequency of 1 Hz can increase the NSC viability during the culture time, while others showed that ACEFs with 46 mV/mm and 0.5 mHz had insignificant influence on the cell proliferation or viability compared with control conditions (Ariza et al., 2010). The decrease in cell density when treated with 437 mV/mm DCEFs was also significant (Ariza et al., 2010). These results show that EFs have controversial effects on NSC proliferation. Although PCEFs and ACEFs (1 Hz) can enhance NSC viability, high-strength DCEFs remarkably decrease proliferation. The possible reason for this result is that long-term and high-intensity constant current may exert certain damage to the cells, and pulse and alternating currents may decrease the time of stimulating cells in one direction and maintain cell viability.

2.1.4. Influence of EF on NSC alignment

The overall NSC alignment is perpendicular to the DCEF vector, which agrees with the results of primary rat brain cells, such as astrocytes (Alexander et al., 2006; Borgens et al., 1994) and neurons (Rajnicek et al., 1992). Comparing DCEFs (437 mV/mm) and ACEFs (46 mV/mm, 0.5 mHz) showed a significant difference in NPC alignment (Ariza et al., 2010). The cell response in the ACEFs did not align with the EF vector in any direction and was similar to those in no-EF groups. The presumptive axons of rat hippocampal neurons derived from 18-day-old fetuses also aligned perpendicularly to those between 29 and 290 mV/mm EFs. Compared with the alternating and pulse currents, constant current may induce NSC alignment and become perpendicular to the EF vector. Constant current can form a relatively stable ionic distribution of the cell membrane. The alignment mechanism is most likely involved in the movement of membrane receptor because of electrophoresis and electro-osmosis (competing forces) (Kaur et al., 2015).

2.1.5. Influence of EF on NSC differentiation

DCEFs can promote adult NS/PC differentiation and enhance the neurite growth and rapid neural extension of induced neurons by regulating the Ca ion level. The short-duration (10 min/days) DCEFs of NSCs in combination with EGF/FGF or IFN-c can obtain longer neurite growth and morphologically mature neurons than those without stimulation (Kobelt et al., 2014). DCEFs combined with Cu can improve the differentiation of human adipose-derived stem cells (hADSCs) toward neuronal lineage. DCEFs also upregulated the expression of neuron-specific genes and proteins of hADSCs (Jaatinen et al., 2015). EFs also showed a significant increase in the proportion of neuronal differentiation on rat filum terminal-derived neural progenitor cells (FT-NPCs) in vitro (Dong et al., 2017). EFs not only induced FT-NSC neurogenesis but also modified their neurite outgrowth and orientations. The addition of 150 mV/mm EFs also increased the length of neurite processes of Tuj1- and MAP-2-positive neuronal cells. BEC and PCEFs can also enhance neuronal differentiation (Chang et al., 2011; Du et al., 2018). However, ACEFs showed insignificant differences in NSC differentiation (Ariza et al., 2010). These results showed that DCEFs, BEC, and PCEFs were beneficial in terms of neurite growth, neural differentiation, and NSC maturation.

DCEFs show greater advantages in directional migration, cell alignment, neural extension, and neural differentiation than the different but widely used EF forms. Direct current stimulation, which is used to repair nerve regeneration and neurite growth through injury or ischemia, is the most widely studied EF stimulation model to date (Thrivikraman et al., 2018). However, direct current stimulation can cause electrothermal and electrochemical hazards in cells and tissue, especially stable direct EFs, should not be ignored. PCEFs and BEC are also beneficial in NSC cell viability and neural differentiation. PCEFs and BEC can also save electricity, energy, and reduce directional stimulation to cells when compared with DCEFs. ACEFs cannot increase cell viability, migration, and neuronal differentiation, which will be the least use in future study. Extremely long stimulation time or high field strengths may lead to negative effects, such as shortened neurite length or unorganized morphology.

In conclusion, EFs not only have strong biological effect, that is, axonal growth guidance and directional cell migration induction but also can enhance NSC neural differentiation and maturation (Feng et al., 2012; Rajnicek et al., 2006a). EFs can be developed as a practical therapeutic strategy for brain repair by directing NSC migration to the injured brain regions to replace loss cells (Yao et al., 2011).

Table 1
Effects of electrical fields (EFs) on neuronal stem cells and induced neurons in vitro.

Electrical field methods	Cell model	Electrodes	Stimulation parameters	Stimulation duration	Main results of study	Potential mechanism	Reference
DCEFs	Rat NSPCs	Agar–salt bridges were used to connect Ag/AgCl electrodes in the beakers containing Steinberg's solution.	50, 100, and 250 mV/mm. For long-term observations, 30 mV/mm.	3 h, images were acquired every 10 min. For long-term observations, the exposure time is 10 h.	Guide NSPC migration toward the cathode.	NMDAR/Rac1/actin signal transduction pathway in mediating EF-induced NSPC migration.	Li et al., 2008
DCEFs	Rat NSPCs	Pt electrodes adhered to the silicon frame.	0.53 or 1.83 V/m (applied the power supply settings of 1.2 and 2.5 V).	10 min/days for 2 days with the total differentiation time of 3 days.	Promote differentiation and mature neurons with long neurite and rapid neural extension.	Regulated Ca^{2+} level in the cell	Kobelt et al., 2014
DCEFs	Rat NSC	Agar–salt bridges filled with Steinberg's solution gelled with Ag/AgCl electrodes	For migration analysis, 250 mV/mm. For EF polarity reversal, 500 mV/mm.	Lasted for 145 min or 3 h. Image was obtained every 5 min.	Enhance NSC migration toward the cathode.	LY294002 and PI3K inhibitors regulate the orientation and parameters related to changes in direction, respectively.	Arocena et al., 2010
DCEFs and ACEFs	NPCs	Agar–salt bridges with Ag/AgCl electrodes	437 mV/mm for 16–24 h a day; 46 mV/mm for 30 min.	For the first 3 days; 0.5 mHz with square bipolar wave form.	Guide NS/PC migration toward the cathode. Enhance the neurite growth and differentiation while decreasing viability. From DCEFs, no effect on alignment, neurite growth, viability, migration, and differentiation. From ACEFs	PI3K-Akt pathway regulation	Ariza et al., 2010
DCEFs	Human embryonic stem cell line H9	Agar–salt bridges filled with Steinberg's solution.	16, 50, 100, and 300 mV/mm.	10, 20, 30, 40, 50, 60, 80, 100, 120, 140, and 160 min.	Guide NSC migration.	Rho-kinase pathway inhibit EF-guided directional migration	Feng et al., 2012
DCEFs	Mouse embryonic stem cell	Agar–salt bridges filled with Steinberg's solution and Ag/AgCl electrodes.	50–100 mV/mm.	24 h, images recorded every 3 min for 1 h.	Enhance the directedness and displacement of the cathodal migration; have no influence on cell migration velocity.	PI3K/AKT and cell surface receptors and actin	Li et al., 2014
DCEFs	Human ADSCs	A three-electrode system and a Ag wire electrode coated with Cu.	35 mV/mm in Cu + 1 mÁ, 53 mV/mm in Cu + 1.5 mÁ, and 155 mV/mm in 1 mÁ.	4, 7, and 14 days.	Morphological changes and neuron-specific gene and protein upregulation.		Jaatinen et al., 2014
DCEFs	Rat FT-NPCs	Agar–salt bridges filled with Steinberg's solution.	50, 100, 150, 200, and 250 mV/mm.	12 h stimulation for 7 or 14 days.	Improve the neuronal differentiation rate of rat FT-NPCs.		Dong et al., 2017
PCEFs	Human NPCs	A hand-made electrotactic chamber via agar–salt bridges and Ag/AgCl electrodes.	250 mV/mm, pulse width (pw) of 0.45 ms at 1000 Hz, pw 4.5 ms at 100 Hz, pw 0.45 s at 1 Hz.	6 h on the days 1 and 10. Pictures were taken every 3 min during a 6–12 h period.	The migration distance increases, has decreased effect on differentiated human NPC migration, and no change in survival.	Intracellular Ca^{2+} signaling	Hayashi et al., 2016
PCEFs	Human NCSCs	Top electrodes (99.999%) had pure Au wires cultured on cathodes, and the bottom electrode plate had a thin layer of PDMS.	Potential of 200 mV/mm potential and duration of 100 μs.	1, 3, or 8 h of pulsatile electrostimulations with 2, 20, and 100 Hz.	Enhance neuronal differentiation and survival.		Du et al., 2018
Biphasic current electric fields	Mouse NSCs	Biphasic current stimulator chip with ITO electrodes.	100 Hz with the magnitudes of 4, 8, 16, and 32 mÁ/cm^2 with the pulses of 50 and 200 ms in a continuous manner.	1,2, 3, 4, and 7 days.	Promote proliferation and neuronal differentiation.	HSP-mediated differentiation	Chang et al., 2011
BES	Rat olfactory bulb NPCs	The upper and lower FOT (an electrically conductive glass) plates; electrode wires were fixed at the edge of the FTO glass plates.	25 mV/mm and 50 mV/mm with a pulse-burst pattern and 8 ms pulses (20% duty cycle).	12 h	Exerts a protective effect against growth factor-deprived apoptosis, enhances cell survival, and decreases the apoptotic/necrotic rate. p-Akt expression and BDNF secretion increase.	BES prevents growth factor-deprived apoptosis through BDNF-PI3K/Akt signaling.	Wang et al., 2013

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Table 1 (*continued*)

Electrical field methods	Cell model	Electrodes	Stimulation parameters	Stimulation duration	Main results of study	Potential mechanism	Reference
ACEFs	Mouse NSCs	The Ni-coated wire electrodes were 8.5 cm apart from each other at the sides of the cell and 6 cm apart across the cell.	0.1, 0.5, 1, and 10 Hz; field amplitudes of 2, 4, and 16 V/m.	0, 1, 7, 14, and 21 days	Neural stem cell viability increases, while neuronal differentiation remains unchanged		Matos et al., 2010

Abbreviations (Table 1): DCEFs: direct current electric fields, NSPCs: neural stem/progenitor cells, NSCs: neural stem cells, FT-NPCs: rat filum terminale-derived neural progenitor cells, NCSCs: human neural crest stem cells, ADSCs: adipose-derived stem cells, ACEFs: alternating current electric fields, PCEFs: pulsed current electric fields, NPCs: neural progenitor cells, HSP: heat shock protein, ITO: indium tin oxide, FTO: F-doped tin oxide, BES: biphasic electrical stimulation.

2.2. Effect of EMFs on NSCs in vitro

EMFs are noninvasive stimulus that have profound research in the brain. Transcranial magnetic field stimulation promotes adult hippocampal neurogenesis and increases the numbers of newborn neurons in the granule cell layer of the dentate gyrus in vivo (Cui et al., 2017; Cuccurazzu et al., 2010). In vitro, EMFs exert a positive effect on NSC proliferation and differentiation. Here, we will summarize the effect of EMFs on the process of inducing neuronal growth, differentiation, and maturation of NSCs and other induced neurons. Additional details about stimulation parameters of EMFs are listed in Table 2.

2.2.1. Effect of EMF on NSC proliferation

Extremely low-frequency (ELF) EMF (50 Hz, 1 mT) exposure can increase cell proliferation in different cell models (Grassi et al., 2004; Wolf et al., 2005). For example, exposure to ELF-EMFs (50 Hz, 0.4 mT) significantly enhanced the proliferation capability of hippocampal NPCs cultured from embryonic and adult ischemic brains (Cheng et al., 2015). Repetitive transcranial magnetic stimulation (rTMS) affected NSC proliferation in vivo via microRNA-106b-25 cluster. Then, Liu et al.(Liu et al., 2015) focused on the effects of rTMS on the proliferation of neonatal rat NSCs in vitro. Rat NSCs were exposed to rMS (200/400/600/800/1000 pulses per day, with 10 Hz frequency and 50% maximum machine output) over a 3-day period. The results showed that rMS can promote NSC proliferation in a dose-dependent manner. Meanwhile, Ma et al.(Ma et al., 2016) exposed eNSCs to ELF-EMFs (50 Hz, 1 mT) for 4 h per day for 1, 2, and 3 days and found that eNSC proliferation and maintenance were significantly improved compared with the no-EMF group in the proliferation medium. However, another paper of Ma et al.(Ma et al., 2014) suggested that intermittent 50 Hz ELF-EMF (5 min on and 10 min off) exposure did not change eNSCs proliferation or the ratio of neurons and astrocytes derived from eNSCs, which was similar to the results of to Nikolova (Nikolova et al., 2005)'s study, wherein intermittent exposure to ELF-EMF (50 Hz, 2 mT, 5 min on and 30 min off) did not affect cell cycle and cell proliferation in ES-derived neural progenitor cells. The increased proliferation rate of NSCs by EMF exposure only showed the undifferentiated NSCs (Kim et al., 2013). When NSCs began to differentiate, proliferation significantly decreased instead of increases, which also agreed with other findings that EMFs (50 Hz, 1 mT, for 12 days) can significantly decrease the proliferation rate of bone mesenchymal stromal cells (BMSCs) and downregulated the early neuronal marker (Kim et al., 2013; Haghighat et al., 2017a). These results showed that EMFs can increase NSC proliferation at low frequency (50 Hz) and magnetic intensity (1 mT), and the exposure time should be continuous and not intermittent. When NSCs start to differentiate, the function of EMFs becomes changeable, thereby inhibiting proliferation.

2.2.2. Effect of EMFs on NSC differentiation

The cell differentiation of NSCs is another pivotal process during NSC neurogenesis. Previous ELF-EMF exposure (50 Hz, 1 mT, 24 h per day for approximately 6–10 days in vitro; 50 Hz, 1 mT, 3.5 h per day for 12 days in vivo) exerted a positive effect on the neurogenesis of postnatal and adult NSCs (Cuccurazzu et al., 2010; Wang et al., 2000). For example, ELF-EMFs (50 Hz, 1 mT, continuously) enhanced NSC neurogenesis and differentiation by upregulating voltage-gated Cav1-channel expression and activity. The percentage of cells displaying immunoreactivity for neuronal markers (β-III-tubulin, MAP2) and Cav1.2 and Cav1.3 channels significantly increased (Piacentini et al., 2008). Meanwhile, another study group evaluated the effects of rTMS on the differentiation of NS/PCs dissolved from the subventricular zone (SVZ) of adult mouse brain. These researchers found that rTMS (1 or 30 Hz) treatment increased NS/PC proliferation and neuronal differentiation compared with no-EMFs (Abbasnia et al., 2015). Bai et al.(Bai et al., 2013) reported that EMFs (50 Hz, 5 mT, 60 min per day for 12 days) can significantly facilitate rat BMSCs (rBM-MSCs) to

Table 2
Effects of electromagnetic fields (EMFs) on neuronal stem cells and induced neurons in vitro.

Stimulus mode	Cell model	Magnetic field excitation	Stimulation parameters	Stimulation duration	Main results of study	Potential mechanism	Reference
ELF-EMFs	Mouse NSCs	Alternating magnetic field in the solenoid.	50 Hz,1 mT, continuously.	Up to 12 days	Neuronal markers, namely, MAP2, and Cav1.2 and Cav1.3 channels increase.	Regulate Cav1 channel activity and pCREB.	Piacentini et al., 2008
ELF-EMFs	NPCs from mouse embryonic and adult ischemic brains	ELF-EMF was generated by a solenoid.	50 Hz, 0.4 mT.	7 days	Enhance neurogenesis and promote proliferation and neuronal differentiation	Regulated by Akt pathway	Cheng et al., 2015
ELF-EMFs	Mouse eNSCs	sXc-ELF exposure system, a vertical EMF.	0.5, 1, and 2 mT for 3 days; or 2 mT for 1, 2, and 3 days with 5 min on/10 min.	1/2/3 days	No change in cell proliferation and increased Tuj1-positive cells.	Decrease in Sox 2 and Math1, Math3, Ngn1. and Tuj1 upregulation at the mRNA level.	Ma et al., 2014
ELF-EMFs	Mouse eNSCs	sXc-ELF exposure system (IT'IS Foundation, Zurich, Switzerland).	50 Hz and 1 mT for 1, 2, and 3 days with 4 h per day.	3 days	Increase the ratio of differentiated neurons and promoted neurite outgrowth.	Upregulate the expression levels of TRPC1 and proneural genes (NeuroD and Ngn1).	Ma et al., 2016
ELF-EMFs	Mouse embryonic stem cells	Two magnetically shielded four-coil systems.	50 Hz and 2 mT.	48 h	Affect the apoptosis-related bcl-2, box, and cell cycle regulation. No change in neural-specific genes in mRNA levels.	Trigger EMF responses at the transcript level of cell cycle regulation and apoptosis-related genes in neural progenitor cells derived from pluripotent ES cells in vitro.	Nikolova et al., 2005
ELF-EMFs	hBM-MSCs	Coils produce a vertical magnetic field.	50 Hz and 1 mT, continuously.	12 days	Decrease proliferation and enhance neural-like morphology and downregulated expression levels of MT1 and MT3 and intracellular Zn concentration.	Regulate (Zn)-metallothionein-3	Aikins et al., 2017
ELF-EMFs	rBM-MSCs	Coils produce a sinusoidal EMF.	50 Hz and 5 mT per day.	12 days	Express neuronal-specific markers and genes, form synaptic junction, and decrease G0/G1 ratio.	Regulate the expression of neural cell markers and cell cycle	Bai et al., 2013
ELF-EMFs	BMMSCs	Device was provided by Hankyong National University.	50 Hz and 1 mT.	12 days	Increase the levels of neuronal differentiation marker (MAP2), downregulate early neuronal marker (Nestin), and decrease proliferation rate.	Include Ca^{2+} regulation	Kim et al.,2013
HIPEMFs	NSCs from newborn SD rats	HMF-S20-type pulsed magnetic field device.	0.5, 1.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 10.0 T; 0.1 Hz.	24 h, 48 h, 72 h, and 7 day	Promote NSC proliferation.		Meng et al., 2009
rTMS	NSCs from 3-day-old SD rats	rTMS, which is a 90 mm figure-of-eight coil.	200/400/600/800/1000 pulses per day,10 Hz, and interval time 10s.	3 days	Enhance NSC proliferation in a dose-dependent manner.	miR-106b/p21/cdks/cyclins pathway	Liu et al., 2015
rTMS	Harvested NS/PCs from normal mice	rTMS, which is a 100 mm circular coil.	1 Hz:150 pulses/day (5 s train, 10 s pause) in 450 s; 30 Hz: 150 pulses/day (1 s train, 5 s pause) in 30 s.	7 or 14 days	Increase NS/PC proliferation and neuronal differentiation.	Increase BDNF protein level and release neurotransmitters	Abbasnia et al., 2015
Low-frequency and high-intensity EMFs	P19-derived neuronal cells	Magnetic fields were produced by the acrylic supported.	50 Hz, 1 or 10 mT, sinusoidal.	21 days	Influence the neuronal differentiation process and promote the functional neuronal network		Saito et al., 2009
Pulsed electromagnetic fields	hBM-MSCs	Helmholtz coil.	60 Hz, 10 mT, pulsed.	1, 3, and 5 days	Increase the expression levels of neural markers, such as NF-L, NeuroD1, and Tau and decrease cell death in dose- and time-dependent manners.	PI3K/Akt/Bad signaling pathway	Urnukhsaikhan et al., 2016
Low-frequency and high-intensity EMFs	rBM-MSCs	The MF generator was made of two coils.	50 Hz, 20 mT and NO with the high and low concentration of 1 and 10 mM, respectively.	24, 48, and 72 h; 1 week	Decrease cell viability and increase neurite outgrowth and percentage of cells expressing Map2 marker. Enhance the entry of Ca ion.	Further explore intracellular calcium ion	Haghighat et al., 2017a

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Table 2 (*continued*)

Stimulus mode	Cell model	Magnetic field excitation	Stimulation parameters	Stimulation duration	Main results of study	Potential mechanism	Reference
Low-frequency and high-intensity EMFs	rBM-MSC	The magnetic field generator consisted of two coils.	50 Hz, 20 mT, with low (50 μM) and high NO concentrations (1 mM).	10 days	Decrease cell proliferation and the expression of neuronal differentiation in Nestin and DCX markers and change cell morphology.		Haghighat et al., 2017b

Abbreviations (Table 2): ELF-EMFs: extremely low-frequency electromagnetic fields, NSCs: neural stem cells, hBM-MSCs: human bone marrow mesenchymal stem cells, rBM-MSCs: rat bone marrow mesenchymal stem cells, eNSCs: embryonic NSCs, HIPEMFs: high-intensity pulsed electromagnetic stimulation, rTMS: repetitive transcranial magnetic stimulation, NO: nitric oxide, and MT1: metal response element transcription factor 1.

differentiate into functional neurons, thereby altering the expression of neuronal-specific markers and genes. However, Ma et al. (2014). suggested that the ratio of neurons derived from eNSCs was unchanged by the intermittent exposure to ELF-EMF (50 Hz, 2 mT, 5 min on and 10 min off, 3 days). After 2 years, Ma et al.(Ma et al., 2016). reported that exposure to ELF-EMF (50 Hz, 1 mT, 4 h per day, 3 days) increased the differentiation of eNSC-derived neurons. ELF-EMF exposure (50 Hz, 10 mT, sinusoidal) on the neuronal differentiation process of P19 embryonal carcinoma cells (P19 cells) significantly increased the percentage of MAP2 positive cells and spike frequencies detected by MEA (Saito et al., 2009). All these results showed that ELF-EMFs played a critical role in NSC differentiation. These results also suggested that magnetic field strength and exposure duration may result in differences in NSC differentiation. Low frequency (50 Hz), low magnetic field strength (< 2 mT), and continuous time stimulation may be required for NSC differentiation. Meanwhile, for other cell types, such as rBM-MSCs and P19 cell, these conditions can also facilitate to differentiate neuronal cells when exposed to EMFs, and they may use a stronger magnetic field strength than NSCs. Although EMFs have considerable effects on neurogenesis and differentiation in vitro and in vivo, further studies are still needed to verify this effect in vivo and clarify the possible mechanism (Ma et al., 2014).

2.2.3. Effect of EMFs on NSC neurite outgrowth

The outward growth of neurites is another important process in brain development that is related to nerve fiber projection, synapse formation, and neuron maturation. ELF-EMF exposure can enhance many aspects of the neurite outgrowth of PC12 cell and dorsal root ganglia; these aspects include the percentage of neurite-bearing cells, the average length of neuritis, and the direction of neurite outgrowth (Zhang et al., 2006; Macias et al., 2000).

Subsequently, Ma et al. (2016) measured the neurite outgrowth of eNSC-derived neurons after ELF-EMF exposure and found that the total length of neurites per cell and the number of branch points per cell significantly increased. Meanwhile, low-frequency EMF (50 Hz, 20 mT) combined with high concentration (1 mM) of NO can significantly increase the length of cell neurites and upregulate the neuronal markers on rBM-MSC differentiation process (Haghighat et al., 2017b,c). However, when exposed to high frequency (1800 MHz), RF-EMF can impair the neurite outgrowth of the eNSC-derived neurons (Chen et al., 2014).

In conclusion, EMFs can promote neurogenesis and significantly facilitate NSCs to differentiate into functional neurons. However, stimulation parameters, such as magnetic field strength, frequency, duration, and number of sessions, vary according to different studies and species. Optimizing the EMF parameters needed to obtain to a balance between beneficial and adverse effects is required. EMFs can be useful in orienting NSC differentiation undergoing in vitro expansion prior to in vivo transplantation.

2.3. Effect of conductive nanomaterial scaffold ES on NSC

NSCs are generally introduced to a target region by mixing with or being seeded on a functional scaffold to support proper cell functions. Functional scaffolds provide mechanical support and biophysical cues in guiding neural cell growth and differentiation and forming complex neural tissue patterns. Several neural tissue-engineering studies have developed biomaterial substrates and scaffolds by using an electro-CP or metal deposits that can provide stem or progenitor cells with ES to enhance neuronal differentiation (Pires et al., 2015; Ramadass et al., 2016; Thrivikraman et al., 2014; Seidlits et al., 2008). Additional information is listed in Table 3.

2.3.1. Conductive conjugated polymers

Conductive conjugated polymers made up of alternating double and single bands in the main backbone has attracted considerable attention because of their response to light and electrical stimuli (Ravichandran

Table 3
Nanomaterial electrical stimulation on neuronal stem cells and induced neurons in vitro.

Conductive scaffolds	Cell model	Nanomaterial characteristics	Conductive Biomaterials		Stimulation parameters	Stimulation duration	Main results of study	Potential mechanism	Reference
			Advantages	Disadvantages					
A crosslinked PEDOT:PSS substrate	hNSCs	Noncytotoxic PEDOT:PSS (xPEDOT:PSS)	High stability High conductivity Biocompatibility Water solubility (doped with PSS)	Relatively low mechanical strength	100 Hz, pulsed DCES, 1 V with 10 ms pulses	1, 2, 3, 4,8, and 12 days	Enhances neurite outgrowth and differentiation.		Pires et al., 2015
A conductive PPy (DBS)-laminin platform	hNSCs	Laminin coated with electroactive PPy containing DBS	High stability High conductivity Biocompatibility High mechanical strength	Fragile Susceptible to irreversible oxidation Insoluble in water	± 0.25 mA/cm^2 by using a biphasic waveform of 100 μs pulses with 20 μs interphase	8 h per 24 h period for 3 days, 6 days in total	Long neurites, considerable branching, and enhanced differentiation.		Stewart et al., 2015
A conductive nanofibrous PANI/PG scaffolds	NSCs	Mixing 10 and 15 wt% PANI with poly (e-caprolactone-gelatin) (PG; 70:30) solution (PANI/PG) by electrospinning	High stability High conductivity Water solubility	Lack of plasticity Poor cell adhesion and growth Low solubility	1.5 V for 15, 30, and 60 min.	1, 3, and 5 days	Enhance cell proliferation and neurite outgrowth.		Laleh et al., 2009
A conductive PPy scaffold system	hNSCs	Ppy plates with pieces of polydimethylsiloxane PDMS	High stability High conductivity Biocompatibility High mechanical strength	Fragile Susceptible to irreversible oxidation Insoluble in water	+1 V to −1 V square wave at 1 kHz for 1 h.	1, 2, 3, 5, and 7 days	Alters the transcriptome and is involved in cell survival, inflammatory response, and synaptic remodeling.	Regulates VEGF-A pathway.	George et al., 2017
A PLLA/PANi nanofibers scaffold	NSCs	PLLA with PANi at a ratio of 85:15	High stability High conductivity Water solubility	Lack of plasticity Poor cell adhesion and growth Low solubility	100 mV/mm for 60 min	2, 4, 6, and 8 days	Extends neurite outgrowth.		Prabhakaran et al., 2011
A Ti-coated nanopatterned substrate	hNSCs	A thin layer of Ti with nanograting topographies (150–300 nm groove/ridge)	High stability High conductivity High optical signal	Cytotoxicity High price	1 Hz, 30 min, two times daily, 20 V, 5 μA	5 days	Enhances neuronal differentiation and functional maturation.	Ca ion influx, activates voltage-gated Ca channels, and promotes pERK1/2.	Yang et al., 2017
A biphasic triboelectrically and polymer nanoparticles platform	PMEFs	Conductive Ti-deposited Si with triboelectrical stimulator	High stability High conductivity High optical signal	Cytotoxicity High price	1 Hz, 60 min/day	Daily; days 3–14, 21, and 30	Promotes the neuronal maturation of iNeurons, and upregulates neuronal-related genes.	Induces Ca^{2+} mobilization and protein kinase C activation and ERK1/2 signaling pathways.	Jin et al., 2016
Multilayered PANI-GNP films	hBMSCs	Electroactuated GNPs as nanomanipulators	High stability Low cytotoxicity in initial step	Relatively weak optical signal Long-term cytotoxicity High price	100 mV/cm, 15 min	Daily	Long filopodial extensions and high mRNA expression level for neural-specific markers.	G0/G1 cell cycle arrest and intracellular Ca^{2+} signaling.	Greeshma et al., 2015
A PEG-MNPs scaffold	hBMSCs	PEG-phospholipid encapsulated magnetite (Fe_3O_4) nanoparticles	Superparamagnetic property Low cytotoxicity Low-cost	Weak strength Low stability Toxic solvent is needed	1 mT, 50 Hz continuously	6 days	Improves neural differentiation and upregulates neural maker, NeuroD1, MBP, DCX, and MAP2.	p-CREB signaling activation.	Choi et al., 2014
3D conductive hydrogel-based microwell	ADSCs	Distinct cell spheres in PEG microwells and wide disks in PEG/PEDOT:PSS microwells	High stability High conductivity Biocompatibility Water solubility (doped with PSS)	Relatively low mechanical strength	1000 mV/sample of the steady-state direct current electric field	10 days	Positive neuronal differentiation.		Heo et al., 2018

(*continued on next page*)

Table 3 (*continued*)

Conductive scaffolds	Cell model	Nanomaterial characteristics	Conductive Biomaterials		Stimulation parameters	Stimulation duration	Main results of study	Potential mechanism	Reference
			Advantages	Disadvantages					
An electroconductive carbon nanotube rope substrate	NSC	A C nanotube rope-like structure with a diameter of 1 mm and length of 1.5 cm	High mechanical strength High conductivity Magnetic property	Oxidative stress Toxicity Hydrophobicity Additional synthesis step	10 mV, 0.75 mÅ; 1 mV, 0.03 mÅ; 5 mV, 0.5 mÅ, 25 ms intermittent stimulation	2, 9, 7, 14, and 21 days	Promoted neuronal maturity and the speed of neurite outgrowth.		Huang et al., 2012
A conductive annealed carbon nanofibrous scaffold	Mouse NSC	Annealing electrospun mats with biphasic electrical stimulation	High mechanical strength High conductivity Magnetic property	Oxidative stress Toxicity Hydrophobicity Additional synthesis step	100 μÅ and pulse rates of 100 Hz with 100 μs for another 24 h	24 h, 7 days	Improves neuronal differentiation and maturation.		Zhu et al., 2017
A conductive SWCNTs and laminins substrate	NSC	Layer-by-layer assembled SWCNTs and laminins	High mechanical strength High conductivity Magnetic property	Oxidative stress Toxicity Hydrophobicity Additional synthesis step	A series (~10–15) of 1 ms pulses spaced in 1–10 s intervals	24, 48, 72, and 120 h	NSC differentiation.		Kam et al., 2008
3D graphene scaffold	iPSC-derived hNPCs	C layers dip coated with poly (methyl methacrylate) (PMMA)	High mechanical strength High conductivity Easy synthesis	Oxidative stress Serious aggregation Toxicity hydrophobicity	A frequency of 1 Hz and an amperage of 10 μÅ for 30 min/day	First 3 days, 7,11, and 21 days	Enhances neurogenesis, increases maturation, improves cell morphology.		Nguyen et al., 2018

et al., 2010; Balint et al., 2014). CPs not only exhibit tunable conductivity but also offer other controllable physical and chemical properties, such as volume, color, and wettability (Park et al., 2015). CP processing is also low cost and relatively simple. Some CPs, such as PPy, PANI, poly (3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS), and P3HT, provide an acceptable biocompatibility in the mammalian cell system (Park et al., 2015; Balint et al., 2014). Thus, the application of CPs in biomedicine is more advantageous than in inorganic conductive materials. CPs with minimal toxic effects, high conductivity, and acceptable biocompatibility can be used in building scaffolds to support cells, especially in providing electrical stimulus.

2.3.1.1. PPy. Among conductive conjugated polymers, PPy is the most studied (Balint et al., 2014; Li et al., 2004). PPy exhibits many excellent qualities and stimulus-responsive properties, such as high conductivity, great chemical stability, and high mechanical strength (Min et al., 2018; Balint et al., 2014). Most importantly, PPy is relatively biocompatible in vitro and in vivo. PPy can be easily synthesized by chemical polymerization, which provides several advantages, including a large-scale low-cost production and flexibility in the formation of PPy/synthetic polymer composites with expected properties for biomedical applications (Min et al., 2018). However, the use of PPy alone as scaffold is restricted due to its inflexibility, infusibility, processability, nonbiodegradability, and poor long-term stability in electrical potential application (Min et al., 2018; Shi et al., 2004; Thomas et al., 2000).

PPy allows the temporal and spatial control of ES and produces minimal inflammatory responses; incorporating bioactive molecules can affect stem cell survival and differentiation (Herland et al., 2011; Lundin et al., 2011; Zhang et al., 2010). Electrically stimulated neural cells on the PPy-deposited nanofiber scaffolds exhibit enhanced neurite outgrowth compared with nonstimulated cells (George et al., 2017). Stewart (Stewart et al., 2015) demonstrated that a conductive PPy (dodecylbenzenesulfonate, DBS)-laminin platform combined with ES promotes neurite growth, increases the number of neuron branch, and improves the hNSC differentiation rate.

2.3.1.2. PANI. The second most studied CP after PPy is PANI, which is also known as aniline black (Balint et al., 2014). PANI exists in various forms on the basis of its oxidation level, namely, the fully oxidized pernigraniline, half-oxidized emeraldine, and fully reduced leucoemeraldine bases (Ghasemi-Mobarakeh et al., 2011; Zhou et al., 2010). Among these forms, PANI emaraldine is the most stable and conductive (Ghasemi-Mobarakeh et al., 2011; Zhou et al., 2010). PANI possesses many advantages, such as low cost, easy to synthesize, good chemical stability, and the ability to be electrically switched between its conductive and resistive states (Balint et al., 2014; Cullen et al., 2008; Borriello et al., 2011).

The conductive nanofibrous scaffolds containing PANI and its derivatives can support stem cell growth, adhesion, and proliferation, thereby gaining interest toward the biocompatibility of PANI in vivo for tissue-engineering applications (Ghasemi-Mobarakeh et al., 2009; Jeong et al., 2008; Bidez et al., 2006; Ghasemi-Mobarakeh et al., 2008). Prbhakaran et al.(Prabhakaran et al., 2011) fabricated a composite polymeric scaffold; they blended poly-L-lactide (PLLA) with PANI at a ratio of 85:15 by electrospinning and performed the ES of NSCs seeded on the electrospun nanofibers with the fiber diameter of 195 ± 30 nm. Then, NSCs cultured on PLLA/PANI nanofiber scaffold applied with an electric field of 100 mV/mm for 60 min resulted in an extended neurite outgrowth compared with the cells grown on a nonstimulated scaffold. The ES with conductive nanofibrous PANI/PG scaffold can considerably enhance the nerve cell proliferation and neurite outgrowth compared with PANI/PG scaffolds that were not subjected to ES (Ghasemi-Mobarakeh et al., 2009).

2.3.1.3. PEDOT:PSS. PEDOT:PSS is utilized in various studies because it provides biocompatibility characteristics that are similar to those of

PT derivatives and melanin, which are natural biological substances (Rad et al., 2014; Karagkiozaki et al., 2013; Luo et al., 2008). PEDOT is synthesized via the polymerization of the bicyclic monomer 3,4-ethylenedioxythiophene (Zhou et al., 2010). In general, PEDOT has been doped into PSS to obtain excellent film-forming ability and hydrophilic polyelectrolyte system (Groenendaal et al., 2000). The doped PEDOT:PSS adjusts the band gap to improve conductivity and provide thermal stability in the doping state (Schweizer, 2005). It has been actively used in biomedical fields, such as neural and nanofiber electrode coating, because of its high charge delivery capacity and as a substrate to mediate cell adhesion, proliferation, and neuronal cell signaling.

Pires et al. (2015) also reported that a crosslinked PEDOT:PSS substrate platform can enhance human NSC differentiation and increase neurite growth when exposed to electrical stimulus. The 3D conductive culture system of PEG/PEDOT:PSS microwells by ES exposure can mimic the architecture of cell niche and enhance the induced neural differentiation of hADSCs directly (Heo et al., 2018).

CPs are generally nontoxic in vitro and short-term in vivo application and not internalized by cells (Asplund et al., 2009; Miriani et al., 2008; Park et al., 2015). Biocompatible CPs, such as PEDOT and PPy, have been widely used in biomedicine application (Balint et al., 2014; Park et al., 2015; Abidian and Martin, 2008). PANI has also been favored in various applications due to its high conductivity and redox reversibility (Park et al., 2015). For disadvantages, PPy is fragile, susceptible to irreversible oxidation, and insoluble in water (Min et al., 2018; Park et al., 2015). PANI lacks plasticity, poor adhesive to cells, and has low solubility (Park et al., 2015). PEDOT:PSS has a higher electrochemical stability and better conductivity and thermal stability than PPy and PANI; thus, PEDOT:PSS is the most promising candidate for the long-term implantation of the central nervous system (Park et al., 2015).

2.3.2. Metal nanomaterials

Various metal NPs have been used in producing nanomaterials in the field of biomaterials, including Au (Xing et al., 2016), Ag (Xu et al., 2012a), and other noble metal NPs and metal oxide NPs, such as iron oxide (Paquet et al., 2011) and zirconia. Metal or metal oxide NPs are widely used in conductive scaffolding, electronic switches, actuators, and sensors due to their desired electrical conductivity, magnetic, and antibacterial properties (Min et al., 2018; Gutiérrez-Sánchez et al., 2012; Arvizo et al., 2012; Prasanthkumar et al., 2010).

2.3.2.1. Ti-coated nanopatterned scaffold (TNS). Ti NPs (TiNPs) are promising metal NPs that have been applied in various research applications. The electroconductive nanopatterned substrates were prepared by depositing a thin layer of Ti with nanograting topographies (150–300 nm of groove/ridge, grove thickness of −150 μm) onto polymer surfaces. TNS with ES significantly increased the neurite extension and upregulated the expression of the neuronal markers, such as Tuj1 and NeuN, in hNSCs. TNS also increased the induction rate of neuron-like cells exhibiting electrophysiological properties from hNSCs and promoted the differentiation into dopaminergic and glutamatergic neurons (Yang et al., 2017). Jin et al. (2016) showed that ES, which was connected to the TNS, can directly enhance the efficiency of fibroblast reprogramming to functional neuronal cells. The expression of neuronal lineage-related markers, such as nestin, Tuj1, and MAP2, increased significantly and promoted the neuronal maturation of induced neurons.

2.3.2.2. Au NPs (GNPs). GNPs are one of the most essential metal NPs that are widely applied in biomedical studies (Min et al., 2018). GNPs have also been favored in various fields because of their high stability, superconductivity, and low cytotoxicity in the initial step (Min et al., 2018; Sabella et al., 2011). However, GNPs are weak optical signals, high cost and long-term cytotoxicity (Sabella et al., 2011). A novel platform by which a uniform layer of GNPs electrostatically was embedded onto thin PANI films was designed to deliver electric stimuli to adhered hMSCs. EFs exerted by a PANI-GNP platform enhanced the neurogenic differentiation in hBM-MSCs, with long filopodial extensions, neural-like morphological changes, and upregulated neural-specific markers (Greeshma et al., 2015).

2.3.2.3. Magnetite NPs (MNPs). Iron oxide NPs have been appied in many in vivo fields, including the enhancement of magnetic resonance imaging contrast and treatment, tissue repair, immunoassay, fluid decontamination, and cell sorting (Xie et al., 2011; Xu et al., 2012b). Choi et al. (2014) synthesized PEG-phospholipid encapsulated MNPs used on hBM-MSCs to improve their intracellular uptake. MNPs were exposed to the cells under 50 Hz of EMFs to improve neural differentiation. The EMFs that were combined with MNPs enhanced neural differentiation and upregulated the neural makers, namely, NeuroD1 and MBP, DCX, and MAP2 in vitro.

2.3.3. Carbon nanomaterials (CNTs)

CNT surfaces have significant properties, including large surface area, mechanical toughness, and high electrical conductivity, as well as excellent charge injection ability and reduced interfacial electrode impedance. CNT has been widely studied as an effective electrode and substrate for the neural interface and can support neuronal adhesion and growth. Neural tissue extracellular matrix consists of various nanostructured components that directly interact with neural cells and stimulate cell growth and differentiation. Hence, the nanoscale features of CNTs, Carbon nanofibers (CNFs), and graphene may provide a biomimetic nanostructured environment, thereby making them superior to other conventional biomaterials in micro–macro dimension (Lovat et al., 2005; Matsumoto and Shimizu, 2013; Fattahi et al., 2014).

Conductive CNT rope substrates as carriers for NSC exposure to ES during culture significantly promote the outgrowth of neurites and their differentiation into mature neurons (Huang et al., 2012). The conductive annealed CNF scaffold that was combined with biphasic ES strongly promoted NSC proliferation, upregulated neuronal gene expression level, and increased MAP2 immunofluorescence, thereby demonstrating improved neuronal differentiation and maturation (Zhu et al., n.d.). Kam et al. (2009) demonstrated the conductive single-walled CNT-laminin substrate composited by layer-by-layer assembled single-walled CNTs and laminin, which were exposed to ES; this substrate was conducive to NSC growth and differentiation and suitable for AP excitation.

3D printing technology has been used to construct new conductive 3D complex scaffolds, which possess extremely intricate microarchitectures and controlled porosity. Porous 3D scaffolds can provide physical support and physiochemical cues for neural cell adhesion, proliferation, and migration. A previous study used 3D printer to fabricate a well-dispersed multiwalled CNT-hydrogel composite neural scaffold with a tunable porous structure that improved electrical properties and nanofeatures; consequently, an electroconductive MWCNT-incorporated 3D scaffold coupled with ES may have a synergistic effect on promoting NSC proliferation, differentiation, and neurite outgrowth (Lee et al., 2018). Another study presented a novel approach to support the effect of ES on the neurogenesis of human Rett-induced pluripotent stem cell (iPSC)-derived hNPC lines by using a 3D graphene scaffold system in vitro. The 3D graphene scaffold can significantly improve the adhesion, differentiation, and maturation of patient-specific iPSC-derived hNPCs under ES (Nguyen et al., 2018).

In summary, the application of electronic biomaterials on NSC neurogenesis and differentiation is highly promising given that these materials possess the required criteria, such as biocompatibility, high conductivity, and flexibility. However, when we apply the cellular studies into the animals, we need to be careful, especially with regard to chronic stimulus and recording, long term stability, toxicity, and biocompatibility. A previous review displayed that the long-term stability

of implantable CP-based microelectrodes for chronic recording or stimulation is still under investigation (Park et al., 2015). Ongoing research on CNTs and graphene may provide insight into their specific advantages for neural interfaces and tissue-engineering scaffolds in the near future. Nonetheless, effective strategies to prevent gliosis in implanted nerve devices still need to be developed. Metal nanomaterials, as inorganic conductive materials, biocompatibility and biodegradability are prolonged concerns for these materials to be implanted in vivo (Min et al., 2018; Park et al., 2015).

Abbreviation (Table 3): NSC: neural stem cell, PMEFs: primary mouse embryonic fibroblasts, HMSCs: human mesenchymal stem cells, ADSC: adipose-derived stem cells, SWCNTs: single-walled carbon nanotubes, MWCNTs: multiwalled carbon nanotubes, PPy: polypyrrole, PANI: polyaniline; GNPs: Au nanoparticles, DBS: dodecylbenzenesulfonate, PLLA: poly-L-lactide, Ti: titanium, and PEDOT:PSS: poly(3,4-ethylenedioxy thiophene) doped with polystyrene sulfonate.

3. Potential mechanism of effect of ES on NSCs

ES can affect NSC proliferation, differentiation, and migration in various ways. Electric fields have an important influence on cell directional migration and differentiation (Zhao et al., 1999), including microfilament recombination (Cho et al., 1996), cell-surface receptor (CSR) redistribution (Zhao et al., 1999), and changes in intracellular Ca^{2+} dynamics. Meanwhile, EMFs mostly affect NSC proliferation and differentiation through the Ca^{2+} signal pathway. We will briefly summarize the potential mechanisms as follows (Fig. 1).

3.1. Cell biomechanics

Cell biomechanics play an important role in many vital cellular processes, including proliferation, adhesion, motility, and differentiation (Huang et al., 2004), and can be modulated considerably by an externally applied EF. Cytoskeleton reorganization and plasma membrane movement are the main components of cell biomechanics.

Cytoskeleton is a complex network of interlinking filaments and tubules that extend throughout the cytoplasm from the nucleus to the plasma membrane. Cytoskeleton is involved in multiple cellular functions. The primary function of cytoskeleton is to impart cell shape and mechanical resistance to deformation and stabilize the tissue by binding to extracellular connective tissues and other cells (Herrmann et al., 2007). The cytoskeleton can also contract, thereby allowing cell migration by deforming the cell and cell environment (Fletcher and Mullins, 2010). Cytoskeleton is involved in many cell signaling pathways. Electric stimulation affects the cytoskeleton directly by transforming electrical stimulus into mechanical activity, thereby causing the reorganization of the cytoskeletal filaments and actin redistribution and influencing a series of cellular processes, especially migration.

Another important mechanical element of cell biomechanics is the plasma membrane, which functions as a cellular barrier that plays a crucial role in cell homeostasis. Plasma membrane participates in inward–outward trafficking, motility, and cell–cell interaction. The membrane mechanism of all functions is coordinated by its interaction with the cell cytoskeleton. Membrane is physically attached to actin cytoskeleton at the focal adhesion sites and specific linker proteins, such as myosin-I and ezrin/radixin/moesin proteins (Titushkin and Cho, 2009). Under an applied EF, glycolipids on the plasma membrane could be aggregated into nanodomains, that is, lipids rafts, by redistributing membrane lipids and proteins. Then, the recruitment of membrane lipids and proteins results in glycolipid congregation, which leads to an increase in raft size, thereby reducing raft motility. The consequent polarization of the raft structures causes directed cell migration, which is frequency dependent (Thrivikraman et al., 2018; Lin et al., 2017).

The external DC electric field could cause substantial actin cytoskeleton reorganization. This result may be due to the fact that DC electric field induces cell membrane redistribution, and many growth factors, such as EGF, FGF, and TGF-b1, can bind to appropriate receptors to trigger signaling pathways and produce local changes in actin dynamics. However, the electric field has insignificant effect on the intermediate filament or microtubule structure (Zhao et al., 1999; Titushkin and Cho, 2009).

One study using rat LGE in vitro model demonstrated that EFs at the range of physiologically relevant strengths (30–250 mV/mm) can promote the directional migration of NPSC, and the *N*-methyl-D-aspartate receptor (NMDAR)-activated NMDAR/Rac1/actin signaling pathway may be responsible (Li et al., 2008). NMDARs are ligand gated. Ca^{2+}-permeable ion channels mediate various physiological and pathological processes in the CNS, such as neuronal development and synapse plasticity (Dingledine et al., 1999; Choi, 1995), and play an important role in neuronal migration. The Rho GTPase Rac1, RhoA, and Cdc42 can affect the regulation of actin cytoskeletal by remodeling and influencing axon guidance, neurite outgrowth, and neuronal migration (Kawauchi et al., 2003; Wong et al., 2001). A signaling link between the Rac GTPase and transmembrane guanylyl cyclase mediates the growth factor-induced migration of fibroblasts (Guo et al., 2007). Rho, Rac, Cdc42, and their effectors play coordinated roles in growth cone guidance by EFs (Rajnicek et al., 2006b). Rho GTPases also interact with the cytoskeleton and growth cone dynamics in an EF (Rajnicek et al., 2006a). The protein–protein interactions of NMDARs with Rac1 signals and actin cytoskeleton may represent a common cellular and molecular mechanism, by which NMDAR mediates neuronal migration in the CNS (Li et al., 2008).

In summary, cell biomechanics play an important role in cell migration and can be modulated considerably by an externally applied electric field. The reorganization of the cytoskeletal filaments, actin redistribution and cell membrane, and lipid raft structure polarization may be responsible. NMDAR-activated NMDAR/Rac1/actin signaling pathway may play an important role in these cellular processes.

3.2. Component redistribution on cell exterior

Considering that ES causes plasma membrane reorganization, the relative electrophoretic movement of charged membrane proteins and lipids resulting in the redistribution of components, including CSRs and ion channels, on the cell exterior, thereby affecting the molecular signaling pathways associated with them (Thrivikraman et al., 2018; Poo and Robinson, 1977).

Applying EF can induce direct CSR activation and downstream signaling without extracellular ligands, thereby suggesting a ligand-independent activation phenomenon by EF (Wolf-Goldberg et al., 2013). An important receptor relative to EF is the epidermal growth factor receptor (EGFR). The EFs of physiological strength activate several signaling pathways, including EGFR, MAPK, ERK, Src, and PI3K signaling, only during long exposure (from a few hours to days). The short exposures of pulsed EF with amplitudes higher than physiological exposures can directly affect EGFR activation and signaling. This activation is EGFR-kinase-dependent and attributed to electrochemical products formed in the solution during electric stimulation (Zhao, 2009). Given that the binding of EGF to EGFR causes downstream ERK activation, ERK phosphorylation is also activated after being exposed to low EFs (LEFs). Meanwhile, the tyrosine phosphorylation of other receptor tyrosine kinases (RTKs) following exposure to LEF has also been demonstrated. RTK can activate downstream MAPK/ERK pathway and also contribute to LEF-stimulated ERK phosphorylation. ES can also induce the activation of plasma membrane receptors coupled to phospholipase C (PLC), thereby resulting in the release of Ca^{2+} from the endoplasmic reticulum by activating PLC signaling cascade (Wolf-Goldberg et al., 2013).

During the process of NSC differentiation, the expression and their states of ion channels lead to different electrophysiological characteristics. Therefore, the development of ion channels is an important

indicator for NSC growth and differentiation. As an immature cell type, NSCs are unable to produce AP due to the lack of voltage-gated Na^+ channels (VGSCs), particularly tetrodotoxin-sensitive Na + channels. Voltage-gated Ca^{2+} channels (VGCCs) are extensively expressed in neurons and play functional roles in early neuronal development (Lang et al., 2005). VGCCs are responsible for various fundamental Ca^{2+}-dependent intracellular events, such as differentiation, apoptosis, and proliferation (Scheffler et al., 2005). The expression levels of CACNA1C VGCC and SCN1α VGSC increased in hNSCs compared with cells without ES (Yang et al., 2017), thereby indicating that ES could enhance NSC neurogenesis via specific ion channel activation.

In summary, ES can cause the redistribution of components on the cell exterior, thereby inducing several cellular activities. The EGFRs, MAPK, ERK, Src, and PI3K signaling induced by them cause specific ion channel changes, which in turn affect NSC development and differentiation.

3.3. Increase in intracellular Ca^{2+}

Ca^{2+} is a key factor affecting the NSC functional activities, such as migration, proliferation, and differentiation. However, Ca^{2+} transients are rarely found in the majority of NSCs. No reports have detected a significant inward VGCC current in NPCs by using physiological recording methods (Cui et al., 2017). Intracellular Ca^{2+} can be increased by ES, including Ca^{2+} influx and intracellular Ca^{2+} release. PLC signaling activation cascade by an endogenous electric field results in the release of Ca^{2+} from the endoplasmic reticulum, while Ca^{2+} influx can be mediated by stretch-activated cation channels (Titushkin and Cho, 2009).

EF and EMF stimulation directly stimulate L-type VGCCs in the plasma membrane and induce the increase in intracellular Ca^{2+}. Except for VGCCs, ELF–EMF exposure also significantly upregulates transient receptor potential canonical 1 (TRPC1) expression to mediate Ca^{2+} influx and promote neurogenesis (Kong et al., 2008). The TRPC1 channel is also a VGCC that plays a key role in Ca^{2+} influx and NSC proliferation (Fiorio Pla et al., 2005).

Voltage-gated Ca^{2+} influx can activate the Ras/MAPK and PI3K/Akt pathways (Peltier et al., 2007). The Ca^{2+}-dependent Ras/MAPK pathway plays important roles in neuronal survival, differentiation, and plasticity. As a serine/threonine kinase, Akt is involved in many signaling pathways in regulating cell survival, proliferation, and differentiation (Peltier et al., 2007). Akt pathway may play a critical role in ELF–EMF-induced neurogenesis in cultured NSCs. The asymmetric redistribution of PI3K/AKT may be related to the effect of EF on the cell migration direction and the velocity of many cell types (Meng et al., 2011). The increased Ca influx induced by ES can promote ERK1/2 phosphorylation, which affects neuronal differentiation (Yang et al., 2017).

Ca^{2+} influx also induces the phosphorylation-dependent activation of the transcription factor cAMP response element binding protein (CREB). As a Ca^{2+}-dependent transcription factor, CREB can modulate the initiation of transcriptional programs, thereby exerting an important influence on adult neurogenesis (Jagasia et al., 2009; Merz et al., 2011). Exposure to ELF-EMF also results in the accumulation of Cav1-dependent CREB phosphorylation at Ser133 in differentiated NSCs, followed by the promotion of the expression of neuronal genes (i.e., NeuroD1 and Neurogenin1) and the recruitment of the histone acetyltransferase CBP. pCREB can also bind to a series of miRNA promoters to regulate their expression (Cui et al., 2017; Piacentini et al., 2008; Sinnegger-Brauns et al., 2009). CREB and its epigenetic mechanisms can affect the expression of BDNF, which plays a critical role in NSC activities. Ca also responds to depolarization, ATP, L-glutamic acid, ACh, dopamine, serotonin, and GABA. However, the identification of the specific neurotransmitter receptors involved requires additional studies (Chen et al., 2015; Young et al., 2011).

3.4. Energy metabolism

Mitochondria are the central organelles of energy metabolism. EF induces transmembrane potential across the mitochondrial membrane, thereby affecting the cellular metabolism in the mitochondrial inner membrane (Teissie et al., 1981), and transforms electrical energy into chemical bond energy of ATP to accelerate ATP synthesis and consumption (Vajrala et al., 2008).

The released ATP may exert mitogenic effects by purinergic receptor stimulation via autocrine and paracrine mechanisms, thereby leading to a transient intracellular Ca^{2+} elevation (Sauer et al., 2002). ATP-dependent P2X ligand-gated channels (Seegers et al., 2002) and morphologically sensitive stretch-activated cation channels can contribute to Ca^{2+} influx into the cell during an ES. In turn, Ca^{2+} influx may interfere with glycolysis in the cytoplasm and aerobic respiration in the mitochondria (Titushkin and Cho, 2009).

The EF stimulation of skeletal muscle cells leads to ROS generation through the discharge of extracellular ATP and incitement of P2Y1 receptors (Diaz-Vegas et al., 2015). Moderate intracellular ROS in stem cell population induces them to undergo differentiation (Owusu-Ansah and Banerjee, 2009). Increased intracellular ROS level in response to EF exposure acts as a signal transducer and could initiate various differentiation programs (Thrivikraman et al., 2018).

4. Conclusion and future prospects

In this review, we summarized the functions and potential mechanisms of two different but widely used electrical stimuli, that is, EFs and EMFs. EFs offer a strong biological effect on guiding cell migration and integration, while EMFs promote NSC neurogenesis, proliferation, and differentiation. Conductive nanomaterials as functional scaffolds combine topographical and biophysical cues to enhance NSC growth and differentiation.

EFs play various but crucial roles in NSC development and growth, as discussed in the first subsection of the second section and Table 1. Various strengths and different EF patterns can also affect NSC migration, proliferation, alignment, and differentiation. NSCs are sensitive to galvanotaxis and move toward to cathode when exposed to EFs. DCEF is the most widely studied among the EF stimulation devices to date and is used for nerve regeneration and neurite growth. A continuous DCEF can increase the differentiation rate in neurons at approximately 15% than that of EMFs on NPCs (Ariza et al., 2010). The continuous DCEFs also cause alignment, where none was seen in the ELF-EMFs. Compared with EMFs, EFs have unique advantages in the directed migration of nerve cells, particularly nerve grafting and regeneration in some necrotic and injured ischemic locations.

Through a noninvasive method, EMFs has been extensively studied in the brain and can promote stem cell proliferation, neurogenesis, and differentiation. Various magnetic stimulus equipment is used in existing studies, and various stimulation parameters and exposure durations generally affect NSC's fate and function, respectively, as summarized in Table 2. EMFs not only show considerable effect on NSCs in vitro but also affect SVZ neurogenesis in adult mouse brain. For example, rTMS at low (1 Hz) and high (30 Hz) frequencies significantly enhances NSC proliferation and differentiation in the adult murine intact brain. rTMS (> 5 Hz) improves neuronal excitability, thereby resulting in the modulation of brain activities, such as brain metabolism, neurotransmitter regulation, and ion channel distribution (Bilek et al., 2013; Lisanby et al., 2000). rTMS has considerable application value and been widely used in preclinical studies and clinical experiments. rTMS also modulates motor skills and cognitive function in healthy subjects and exhibits therapeutic effects for patients with neurological and psychiatric disorders (Xie et al., 2017). Daily prefrontal TMS was approved by Food and Drug Administration (FDA) in 2008 for the treatment of patients with major depressive disorder (George et al., 2013; Belmaker and Agam, 2008). Deep-brain magnetic stimulation with a modified

rTMS protocol has been developed and demonstrated to be effective for Parkinson's disease and neuropsychiatric disorders, including depression.

According to their own characteristics and advantages, conductive nanomaterials hold topography and biophysical support to promote NSC differentiation and maturation. These nanomaterials also enhance NSCs to differentiate into special neuronal subtypes (Yang et al., 2017). To provide both conductivity and biocompatibility, researchers studied multiple substances in confirming their effects (Vaitkuviene et al., 2014; Liu et al., 2016). A CNT-CP composite material can impart conductivity and show superior synergistic conductivity compared with CPs and CNT, according to the results of a recent research in developing a conductive platform (Min et al., 2018; Huyen, 2011). Conductive substrates composed of two or more conductive materials can potentially provide new possibilities on NSCs for future clinical transplantation and application.

In summary, ES comprising EFs, EMFs, and biomaterials ES, as a noninvasive treatment, is a promising clinical option for NSC transplantation therapies. We aim guide researchers in future studies on ES. However, an in-depth understanding of the underlying mechanisms that govern clinical success is still needed. The optimization of stimulation parameters in vitro studies, followed by large, prospective animal studies and then clinical studies, is still required to explore the full potential of ES.

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