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INVITED REVIEW

Material and mechanical factors: new strategy in cellular neurogenesis

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Abstract

Since damaged neural circuits are not generally self-recovered, developing methods to stimulate neurogenesis is critically required. Most studies have examined the effects of soluble pharmacological factors on the cellular neurogenesis. On the other hand, it is now recognized that the other extracellular factors, including material and mechanical cues, also have a strong potential to induce cellular neurogenesis. This article will review recent data on the material (chemical patterning, micro/nano-topography, carbon nanotube, graphene) and mechanical (static cue from substrate stiffness, dynamic cue from stretch and flow shear) stimulations of cellular neurogenesis. These approaches may provide new neural regenerative medicine protocols. Scaffolding material templates capable of triggering cellular neurogenesis can be explored in the presence of neurogenesis-stimulatory mechanical environments, and also with conventional soluble factors, to enhance axonal growth and neural network formation in neural tissue engineering.

Key Words: *neural regenerative medicine; cellular neurogenesis; material cue; mechanical factor; soluble signal*

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Neurogenesis is a very important topic due to its relevance to nerve injuries and neurological disorders. Since the loss of functional circuits rarely displays spontaneous recovery, finding successful methods to stimulate neurogenesis is very useful and important. Due to the fact that the body is run by an abundance of biochemical cascades, it is no secret there are many soluble factors that could influence neurogenesis. Studies have therefore tested the effects of various soluble factors on the neurogenesis of neural precursor cells. On the other hand, it is becoming increasingly apparent that other extracellular cues, such as material and mechanical factors, can also influence cellular neurogenesis (Bani-Yaghoub et al., 2005; Higgins et al., 2013). This is very exciting for neuronal regenerative medicine as it allows to explore multiple stimulatory cues for neurogenesis. It also allows for a combination of multiple stimuli, soluble and non-soluble, administered together to be tested. That soluble factors, substrate cues, and mechanical stimuli all have the capability to influence neurogenesis may enlighten how we deal with neural damages and disorders.

Various soluble factors, including nerve growth factor (NGF), retinoic acid (RA), brain-derived neurotrophic factor, neuropathiazol, *etc*., have been identified to positively influence neurogenesis. NGF is responsible for increasing the survival and differentiation of sensory and sympathetic neurons and there is evidence that endogenous NGF helps protect neurons and promotes their repair (Sofroniew et al., 2001). RA stimulates cellular neurogenesis through pathways *via* activating retinoic acid receptors (RARs) and also peroxisome proliferator-activated receptor β/δ (Yu et al., 2012). These soluble factors have been shown to stimulate the neurogenesis in many *in vitro* and *in vivo* studies, and therapeutic ways to utilize the soluble reagents for neuro regeneration have been exploited. We will now highlight key findings on the material and mechanical control of cellular neurogenesis.

It is established that cell growth substrate affects cell adhesion, proliferation, and differentiation. The substrate control of cells has been applied to neuronal cells using chemical and topographic micropatterns. Chemical micropatterning produces *via* photo- and soft lithography techniques chemical/biomolecular cell-adhesive micropatterns and cell-repellent background. Cell attachment and growth can be geometrically confined within cell-adhesive micropatterns, so the cell size, shape, and interconnectivity can be systematically manipulated (see details in our review: Poudel et al., 2012). Topographic micropatterning produces physical microscale structures (ridges/grooves, islands/protrusions, *etc*.). Lithographically produced master or its inverse replica can be

used as topographic patterns. These topographies have also been shown to direct cell morphology and behavior (see the review: Lim, 2009). Micropatterned surfaces, both chemical and topographic, provide useful templates *via* which cell physiology under varying extracellular milieus could be systematically investigated *in vitro*.

When applied to neuronal cells, chemical micropatterns could affect neurite outgrowth and neuronal cell orientation/migration (Yang et al., 2005; Doyle et al., 2009). These studies took advantage of the chemical micropatterning that cells could be positioned within predetermined extracellular matrix (ECM) protein patterns *via* direct recruiting of integrins through patterned peptide sequence (such as Arg-Gly-Asp, RGD). In our recent study (Poudel et al., 2013), when patterned within narrow (5 and 10 μm wide) collagen-I lanes, SH-SY5Y neuroblastoma cells showed preferred nucleus orientation along the patterned lanes and exerted significantly longer neurite extension (one of the apparent markers of cellular neurogenesis) relative to unpatterned control. Interestingly, we also observed that the neurite extension for patterned cells without soluble RA stimulation was even greater than that of unpatterned but RA-treated sample. This may suggest that extracellular cue from chemical patterning could provide even stronger signal for cellular neurogenesis than the conventional soluble cue. Another study also demonstrated that the neurite growth and axonal specification in hippocampal neurons could be controlled by the scale of chemical micropatterning (Tomba et al., 2014).

Utilizing the capability of chemical micropatterning, it was also shown that neural network formation could be manipulated. These studies adopted combinations of cell-adhesive protein patterns and cell-non-adhesive back-filling. For example, 10^4 μm^2 area poly-L-lysine squares were utilized for neural stem cell patterning with polyethylene oxide cell-repellent background (Ruiz et al., 2008). If the patterns were connected with 15 μm wide lanes, they could successfully guide axonal outgrowth along the connection lanes to ultimately form well-communicated neural network. In another study, PCC7-MzN neuroblastoma cells formed neural network with a nodal compliance of 86% when patterned on 20 μm diameter laminin dots interconnected by 4 μm wide lanes and with non-tissue culture grade polystyrene background (Lauer et al., 2001). Also, different shapes of cell patterning (circle, square, triangle) may affect neurite initiation, soma shape, axonal outgrowth, and network formation (Jang and Nam, 2012). Improved cell-cell communication achieved *via* chemical patterning-directed network formation may further enhance neuronal cell differentiation and even the commitment of stem cells into neuronal cells (Solanki et al., 2010; Béduer et al., 2012).

Topographic substrate modification has also been utilized to direct neuronal cell behavior. Most of the effort has been based on the well-established phenomenon, so-called contact guidance. Cells cultured on anisotropic topographies such as ridges and grooves tend to exhibit cell alignment/elongation along the anisotropic direction of the topography (although it is not through direct RGD recruiting of integrin as was for chemical patterning). Following the anisotropic cell shaping, related and downstream cell behaviors including nucleus orientation, focal adhesion and cytoskeletal formation, and even cell migration, proliferation, and terminal differentiation are anisotropically directed (Lim, 2009). It is only natural to imagine that such an anisotropic control of cell shape and behavior by ridge and groove could be well applied to formulate neuronal cell functions, considering that axonal structure of neuronal system is intrinsically anisotropic. While it is still under investigation what geometrical shape and size (ridge and groove width, ridge height, pitch all at both micro and nanoscale) are most effective in directing the contact guidance for various cell types (Guilak et al., 2009), the use of anisotropic topographies constitute one of the important non-soluble approaches to guide neuronal cell behavior.

An earlier study showed that Xenopus neurites grew along the grooves as deep as 14 nm and as wide as 1 μm and neurites determined where to emerge from somas depending on the groove substrates (Rajnicek et al., 1997). Other studies using varying dimensions of ridges and grooves also demonstrated that neural cell orientation and neurite/axonal growth were aligned following the anisotropic direction but to a different degree depending on the size of the ridges and grooves (Sørensen et al., 2007; Fozdar et al., 2010). According to a recent study (Su et al., 2013), rat adrenal pheochromocytoma (PC-12) cells cultured on ridge and groove patterns (800 and 100 nm width respectively, 1,200 nm depth) displayed not only greater bipolar elongation and neurite extension but also higher expression of growth-associated protein-43 compared with cells on planar surfaces. However, groove topography decreased the neuronal cell movement speed, illustrating how topography may have a positive effect on certain aspects of neurogenesis and a negative effect on others. Another study showed that alignment of cellular elements from inner ear neural system increases with increasing ridge height and with decreasing periodicity (Tuft et al., 2013). In addition to neurite alignment, anisotropic surface topographies were also demonstrated to induce bone marrow-derived mesenchymal stem cell (MSC) differentiation toward a neuronal fate (D'Angelo et al., 2010).

Data on chemical and topographic patterning imply that researchers are now able to gain more control over the desired cellular neurogenesis. This is important because it allows neural cell shape, neurite/axonal outgrowth, and terminal cellular neurogenesis to be manipulated at a more intricate level. This is expected to ultimately lead to advance the strategy to deal with neuronal injuries and disorders. For example, synthetic neurochips with fully functional neural network developed from chemical and topographic micropatterning may be used to replace damaged neural system (Bani-Yaghoub et al., 2005).

Additionally, recent developments in nanotechnology have introduced new classes of nanomaterials that can be used to trigger cellular neurogenesis. Carbon nanotube (CNT)-based nano-scaffolds were effective in cell and nuclear shaping

of human MSCs and triggering gene expressions related to neurogenesis including voltage-gated ion channel formation (Park et al., 2013). Another type of nanomaterial that is of significant interest for cellular neurogenesis includes graphene, 2-dimensional monolayer of hexagonal carbon atoms, in addition to its huge applications in electronic devices. Several recent studies strongly suggest the potential of graphene to stimulate neurite sprouting (Li et al., 2011), to enhance electrical signaling in neural networks (Tang et al., 2013), and to induce MSC fate toward neuronal lineage (Wang et al., 2012).

Mechanical factors, both static and dynamic, may also play an important role in regulating neurogenesis. It is now gathering much attention that neural and axonal development and cellular neurogenesis may be driven by mechanical cues (Franze, 2013). As regards static mechanical factor (such as substrate stiffness), a pioneering study on MSC fate reported that on soft gel MSCs tend to differentiate toward neuronal cells unlike myogenesis and osteogenesis favored on stiffer gels (McBeath et al., 2004). Another study also found that soft environments promote early neurogenesis of human pluripotent stem cells while decreasing their self-renewal (Keung et al., 2012). Similarly, it was shown that differentiated neurons prefer soft substrates as their growth milieu (Georges et al., 2006). It was proposed that growing neurons could detect substrate stiffness and avoid stiff portions of the substrate as with an observation of neuronal retraction and re-extension at static stress over 274 ± 41 pN/μm^2 (Franze et al., 2009). These data may give an indication as to which static mechanical condition is ideal for neurogenesis, and therefore suggest practical knowledge on what matrix stiffness will be required for neuronal tissue regeneration.

Dynamic mechanical stimulation, tension or shear, also has a potential to affect the neurogenesis. Based on traditional understanding on the role of tension in axonal elongation or retraction (Heidemann et al., 1995), studies attempted to apply direct tension to axonal growth cone for regenerative medicine purpose. Continuous mechanical tension (3.5 μm per 5 minutes) could induce stretch-induced growth (1 cm growth after 10 days of stretch) of central nervous system axons (Smith et al., 2001). Later studies reported even more effective axonal growth cone stretch and achieved up to a growth rate of 10 mm/day (Loverde et al., 2011; Pfister et al., 2004). In our recent study, instead of direct pulling of anchored axons, we examined the stretching of neuronal cells seeded on a stretchable membrane. We observed that SH-SY5Y neuroblastoma cells exposed to 10% stretch (equiaxial, 0.25 Hz, 120 minutes/day for 7 days) even without soluble stimulatory factor (RA) displayed significantly longer and more neurites than unstretched control (Higgins et al., 2013), suggesting the competitive importance of mechanical signal relative to soluble stimulation. One particular study tested the effects of fluid flow-induced shear stresses (0.1 to 1.5 Pa) on neurite outgrowth, in which PC-12 cells showed the longest neurite length at 0.25 Pa shear (Kim et al., 2006). These unconventional data on dynamic mechanical (stretch, fluid shear) stimulations of neurogenesis may suggest a new strategy to treat neuronal injuries and disorders.

The process of neurogenesis is incredibly complex and there is still much to learn. However, it is becoming increasingly evident that not only are the conventional soluble factors influencing neurogenesis important, but also the substrate cues and mechanical stimuli. The implications this knowledge has are crucial for neuronal regenerative medicine. Although much of the evidences so far are from *in vitro* studies, it can be possibly applied to deduce optimal *in vivo* environments, material and mechanical, that should be provided to accomplish successful neural regeneration. For example, a scaffold with cells can be maintained *in vitro* then implanted *in vivo* for treating spinal cord injury, which should be able to provide a structural platform capable of inducing axonal growth and neural network formation. Obtained knowledge from chemical/topographic patterning and nanomaterials combined with mechanical stimulations may be applied to this situation to exert the best neural regeneration outcome. The more information that is acquired regarding not only the soluble factors but the material and mechanical factors as well, the more successful neurogenesis will be achievable in treating neural damages and diseases.

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