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Graphene platform for neural regenerative medicine

Graphene is a material composed of a single layer of carbon atoms arranged in a two-dimensional honeycomb lattice. The unique electrical, optical, thermal, and mechanical properties of graphene are extensively exploited for various applications in electronics, energy, and sensors. Studies also proposed the potential of graphene for biomedical applications. The intrinsic characteristics of graphene and its availability for chemical and physical modifications make graphene promising vehicle for various biomedical applications including drug delivery, bioimaging, disease diagnostics, etc. The chemical structure of graphene and, in turn, its functionality, can be altered by attaching functional groups, which not only modify the properties of graphene but also allow its conjugation with antibodies, peptides, ligands, contrast agents, drugs, and genes for various biomedical applications (John et al., 2015).

Graphene can also be used as a cell-contacting biomaterial for tissue engineering and regenerative medicine. Recent studies have shown that graphene substrates can support the adhesion, proliferation, and differentiation of mesenchymal stem cells (MSCs), induced pluripotent stem cells, and other mammalian cells. Specifically for neural regenerative medicine, graphene has demonstrated that it can perform as an effective culture platform compatible with neural cells and their precursors. Hippocampal cells and neural stem cells, nucleated on graphene substrates showed significantly enhanced neurogenesis, as assessed by neurite sprouting and overall cell proliferation compared with control glass substrate, both in neurite outgrowth and neurite growth even in the absence of soluble neurogenesis signal from RA. Further, specific neuronal markers, microtubule-associated protein 2 (MAP2) and neurofilament light chain (NFL), were observed to be responsive to mechanical stretch. Combined data strongly suggest that extracellular mechanophysical signal can be a possible route for neuronal regenerative medicine.

Our group recently explored the effect of graphene substrate culture on the induction of cellular neurogenesis. While several studies have tested cellular behaviors on graphene-covered surfaces, some studies did not have a good control over the quality of the graphene substrate with respect to the number of graphene layers or substrate coverage. For instance, cell cultures on partly graphene-covered substrate with inconsistent graphene layer numbers were used. In our recent study (Lee et al., 2015), we could successfully fabricate a monolayer film of high-quality graphene on 15×15 mm² glass substrate, which was sufficiently large to assess various cell behaviors. This prompted a study to test the effect of dynamic loading on neurogenesis. We applied cyclic stretch to human neuronal cells seeded on collagen-coated elastic membranes without or with RA (Higgins et al., 2013). For cells exposed to sinusoidal equi-axial stretching at 10% strain and 0.25 Hz frequency for 120 min per day for 7 days, both neurite extension and outburst number were significantly increased in comparison with unstretched control. Echoing the result on the static micropatterning study described above, dynamic mechanical stretch was also found to induce neurite outgrowth even in the absence of soluble neurogenesis signal from RA. Further, specific neuronal markers, microtubule-associated protein 2 (MAP2) and neurofilament light chain (NFL), were observed to be responsive to mechanical stretch. Combined data strongly suggest that extracellular mechanophysical signal can be a possible route for neuronal regenerative medicine.

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Much of our group’s effort has been centered on regulating cell function and fate via the integration of biomaterial and mechanical cues. While conventional neural bioscience researches have mostly depended on soluble neurogenesis triggering factors such as nerve growth factor, we have been exploring the potential of mechanophysical cues to induce neurogenesis (see our review: Stoll et al., 2014). First, we made an attempt to utilize the capacity of geometric cell function control via micropatterning. Cell patterning can geometrically organize cells, enabling precise regulation of the cell size, shape, and interconnectivity. By investigating geometry-dependent intra- and intercellular signaling, the mechanism of morphology-directed cellular function change can be systematically studied. For neural regenerative medicine, we tested the hypothesis that micropatterning of neuronal cells within narrow lane shapes may increase neurogenesis (Poudel et al., 2013). This was to simply mimic the anisotropic architecture of the axons. Further, by testing the interplay between geometric and biochemical signals, the microenvironment could be optimally tailored for enhanced neuronal regeneration. We observed that SH-SY5Y human neuroblastoma cells seeded on collagen-I micropatterned lanes responded to the micropatterning dimension synergistically with the treatment of retinoic acid (RA), a soluble neurogenic factor for neuroblastoma cells. Cells confined within narrow (5- and 10-μm-wide) lanes exhibited greater neurite extension and preferred nucleus orientation along the anisotropic lane direction, relative to unpatterened control, which effect was strengthened under RA. Neurite extension was significantly greater for microlane-patterned but RA-untreated cells compared with RA-treated but unpatterned cells, indicating that the geometric cell confinement alone may induce neurogenesis to a greater degree than the conventional soluble induction.

As another attempt exploiting mechanophysical cues, we tested the effect of mechanical loading. This was to test whether neuronal cell function could be altered by dynamic mechanical environments, although neurons have not been normally recognized as mechanoresponsive. If mechanical loading results in improved neurogenesis, even though it is not physiologically relevant, the method can be practically considered as useful for in vitro neural tissue engineering. This prompted a study to test the effect of dynamic loading on neurogenesis. We applied cyclic stretch to human neuronal cells seeded on collagen-coated elastic membranes without or with RA (Higgins et al., 2013). For cells exposed to sinusoidal equi-axial stretching at 10% strain and 0.25 Hz frequency for 120 min per day for 7 days, both neurite extension and outburst number were significantly increased in comparison with unstretched control. Echoing the result on the static micropatterning study described above, dynamic mechanical stretch was also found to induce neurite outgrowth even in the absence of soluble neurogenesis signal from RA. Further, specific neuronal markers, microtubule-associated protein 2 (MAP2) and neurofilament light chain (NFL), were observed to be responsive to mechanical stretch. Combined data strongly suggest that extracellular mechanophysical signal can be a possible route for neuronal regenerative medicine.

In regard to molecular mechanism of cell-graphene interaction, our data for the first time revealed that focal adhesion kinase (FAK) and p38 mitogen-activated protein kinase (MAPK) may play...
mediatory roles in the graphene triggering of neurogenesis (Lee et al., 2015). In the presence of FAK and p38 inhibitors, graphene triggering of neurite extension was significantly impaired, suggesting that focal adhesion formation on graphene and environmental stimulation from graphene culture may be mainly responsible for the enhanced neurite development on graphene. It was found that the other important sensors, such as extracellular signal regulated kinase (ERK) or RhoA kinase (ROCK), may not majorly participate in the graphene control of neuronal cell functioning.

Despite promising early results on the graphene induction of cell neurogenesis, there remains much more to improve for accomplishing graphene-based neural regenerative medicine. First, the studies on neuronal cell-graphene interaction have only dealt with plain cell culture using mono- or multilayered graphene films, and there has been very little effort to incorporate other vital cell-stimulatory cues such as geometric, topographic, etc. Considering our data on the geometric (micropatterning) stimulation of neurogenesis, an incorporation of the geometric cue with the graphene control of neuronal cells may provide potential synergistic effects. Also, there has been relatively little effort to actively take advantage of the unique properties of graphene to stimulate neural cells, specifically, its excellent electrical conductivity. Keeping in mind that electrical stimuli can be beneficial for neurogenesis, more systematic attempts to exploit the electrical stimulation capability of graphene are recommended. Fundamentally, while studies reported positive neuronal cell-stimulatory effects from graphene, very little has been determined about the molecular mechanisms on how neuronal cells adapt to graphene. Our data suggested that focal adhesion (FAK) and environmental stress (p38) signaling may play governing roles in neural cell-graphene interaction. FAK, a linker protein that connects transmembrane integrin to cytoskeleton at the focal adhesion site, mediates a range of cell behaviors including adhesion, growth, differentiation, and mechanical and substrate sensitivity. p38 MAPK responds to environmental stimulatory signals, such as substrate and soluble factors, and may also be activated by an electrical signal. A more in-depth signaling pathway studies including the up- and downstream effectors of FAK and p38, respectively, may enhance the graphene stimulatory effects on neurogenesis. Taken all together, a combinatorial approach that simultaneously utilizes identified stimulatory factors (geometric, electrical, sensitized FAK and p38, etc.) may maximize the effect of graphene induction of neurogenesis. The combined approach may help develop novel graphene-based neural regenerative medicine protocols.

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