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Accepted Article

Title: Neuron-Material Nanointerfaces: Surface Nanotopography Governs Neuronal Differentiation and Development

Authors: Hoon Kee Kim, Eunha Kim, Hongje Jang, Young-Kwan Kim, and Kyungtae Kang

To be cited as: *ChemNanoMat* 10.1002/cnma.201700063

Link to VoR: http://dx.doi.org/10.1002/cnma.201700063

A Journal of

ACES Asian Chemical Editorial Society

A sister journal of **Chemistry – An Asian Journal** and **Asian Journal of Organic Chemistry**

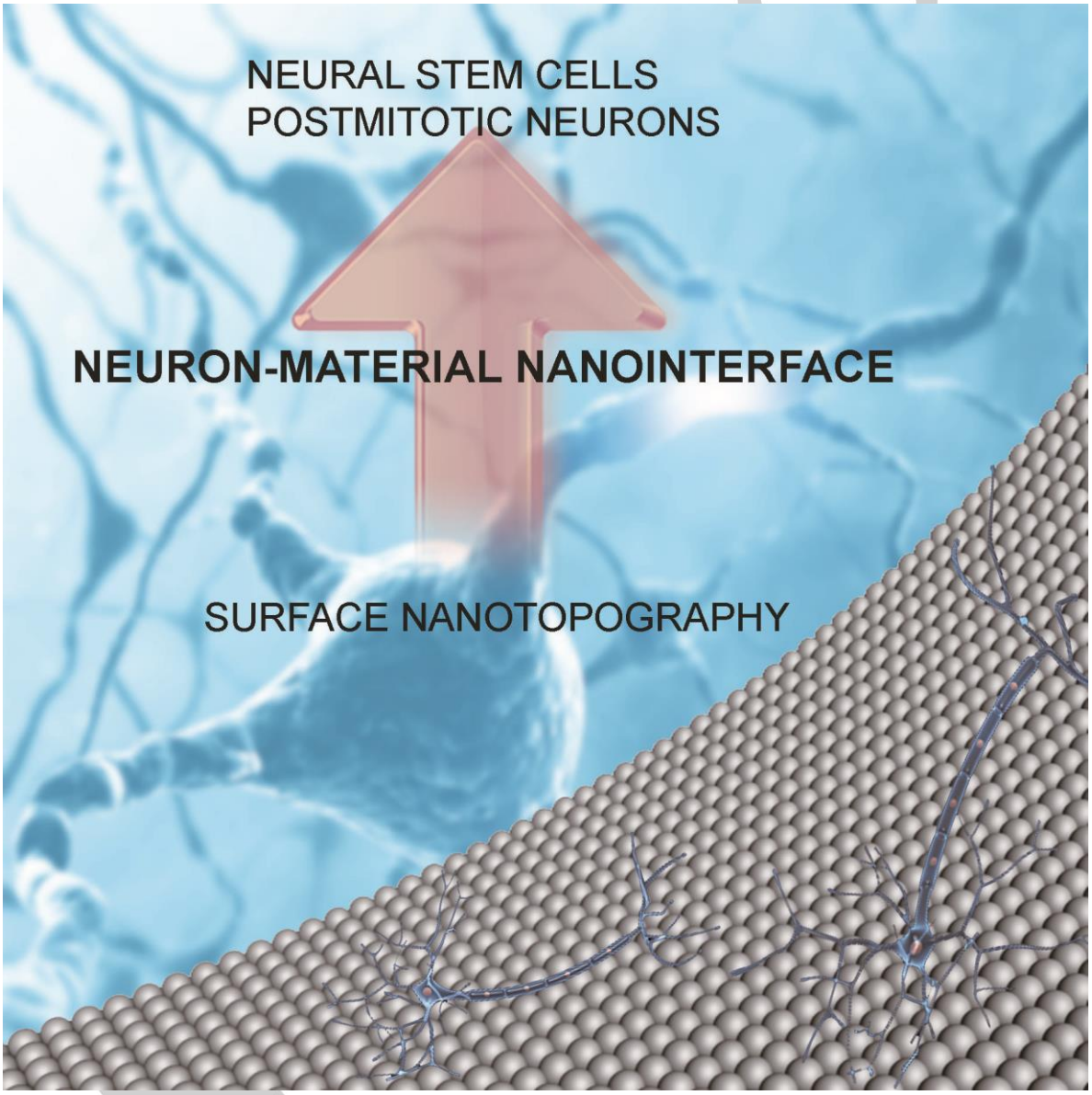
WILEY-VCH

10.1002/cnma.201700063
WILEY-VCH

FOCUS REVIEW

Neuron-Material Nanointerfaces: Surface Nanotopography Governs Neuronal Differentiation and Development

Hoon Kee Kim,[a] Eunha Kim,[b] Hongje Jang,[c] Young-Kwan Kim,[d] and Kyungtae Kang*



Abstract: This review summarizes recent experimental results regarding influences of surface nanotopography on stem cell differentiation into neurons and neuronal development. Collections of observations and their interpretations are provided. We specifically focus on the *in vivo* implications of the results, by suggesting that: (i) neural cells use their intracellular machineries to actively recognize surface nanotopography; (ii) the preferential differentiation of stem cells into neurons and the developmental acceleration of neurons occur on a similar range of nanotopographical features, implying that they have a similar biological background; and (iii) surface nanotopography, therefore, is deeply involved in the development of brain. We believe that this review will provide biological insights to the field of nano-neuron interface and encourage interdisciplinary efforts capable of addressing unsolved biological problems and increasing engineering needs.

1. Introduction

Interactions between neurons—including those differentiated from stem cells and those primarily dissected from a tissue—and their environment are complicated and multifaceted. In a brain, neurons constantly encounter various types of surface and have ability to recognize their molecular-scale (chemical) and macroscale (physical) characteristics for proper development and functioning. The biological influences of these chemicals attached to the surface (e.g., small molecular cues, neuro-active proteins, and surface charges)[1] or floating in solution (e.g., neurotrophic factors and neurotransmitters)[2] on neuronal behaviors have been studied intensively and known to rely on many intracellular signaling cascades. However, those of physical aspects of surface (e.g., micro- and nanotopography, stiffness, and flexibility) have been regarded relatively marginal and thus gained less interests than the former.

This asymmetry is in part because that the interactions between the physical aspects of surface and neurons might give impression that they are manifested in a somewhat biologically less relevant way; the observed cellular responses to surface may seem too simple and passive to rely on sophisticated biological mechanisms, and/or less analogous to what occurs *in vivo*. For example, initial observations in this direction included enhanced adhesion on topographically rough surfaces or polarized neurite outgrowth guided by microgrooves, which could be inferred as simple adaptations of cytoskeletal structures to the morphological attributes of the surface, lacking systematic intracellular changes. In addition, the initial studies in this direction were mainly conducted by chemists or materials scientists, and biological implications from those studies could not expand toward the realm of neural cell biology. Nonetheless, in the design of neuron-material interfaces, the physical aspects of surface are already an indispensable factor to consider; regulation of surface topography does critically affect the viability and functions of neurons on top whether or not biologically understood.

In fact, studies on interactions between neural cells and surface topography are producing an increasing number of biologically meaningful results, as summarized by comprehensive reviews published recently.[3] Among a large number of results related to neuron-material nanointerfaces, this review will focus specifically on the influences of surface nanotopography on the differentiation and development of neural cells (incorporating stem cells, neural precursor cells, and postmitotic neurons), since we suspect that these would have biological relevance to *in vivo* neurogenesis more than others do. In particular, we will illuminate a possible connection between published responses of stem cells and neurons to surface topography, which likely implies the presence of common intracellular machineries or signaling cascades. Developmental changes are, in some ways, more interesting than other changes are, because they can be a direct evidence that neurons are capable of actively recognizing surface topography. The specific purpose of this review is, thus, to emphasize the biological relevance of the recent results and to suggest biological contexts possibly related to them, which we believe to deserve additional interests.

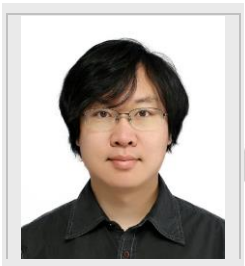
[a] Hoon Kee Kim and Prof. Dr. Kyungtae Kang
Department of Applied Chemistry
Kyung Hee University
1732 Deogyeong-daero, Giheung, Yongin, Gyeonggi 17104, Korea
E-mail: kkang@khu.ac.kr
[b] Prof. Dr. Eunha Kim
Department of Molecular Science and Technology
Ajou University
Suwon 16499, Korea
[c] Prof. Dr. Hongje Jang
Department of Chemistry
Kwangwoon University
20, Gwangwoon-ro, Nowon-gu, Seoul, Korea
[d] Dr. Young-Kwan Kim
Carbon Composite Materials Research Center, Institute of Advanced Composite Materials
Korea Institute of Science and Technolgy
San 101, Eunha-ri, Bongdong-eup, Wanju-gun, Jeollabuk-do, 565-905 Korea

Hoon kee Kim received his B.S. (2011) from the Department of Chemistry at Iowa State University. Under the supervision of Prof. Kyungtae Kang, he is working on topographical studies on cell differentiation and neurite outgrowth.

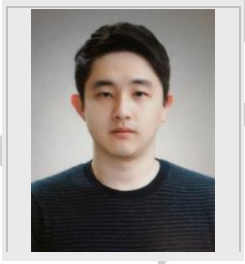
Prof. Eunha Kim studied Chemistry in Seoul National University and received his B.S. in 2005 and Ph.D. in 2011 under the supervision of Prof. Seung Bum Park. After postdoctoral research under the guidance of Prof. Ralph Weissleder at Massachusetts General Hospital/ Harvard Medical School, he was appointed as an Assistant Professor at the Department of Applied Chemistry and

Biological Engineering of Ajou University in 2015. He took a joint faculty position at the Department of Molecular Science and Technology of Ajou University in 2016. His research interests include fluorescent bioprobe development, molecular imaging, molecular diagnosis, and chemical biology.

Dr. Young-Kwan Kim received his Ph. D. in chemistry at Korea Advanced Institute of Science and Technology (KAIST) in 2012 and worked as a postdoctoral fellow at the University of Massachusetts, Amherst. He joined the Carbon Composites Research Center at Korea Institute of Science and Technology as a Senior Researcher in 2015. His research interests focus on the chemistry of carbon nanomaterials, their self-assembly and nanocomposites for the applications in laser desorption/ionization mass spectrometry, surface enhanced Raman scattering and the generation of high-performance nanocomposites.



Prof. Hongje Jang received Ph. D. in the Department of Chemistry at Korea Advanced Institute of Science and Technology (KAIST) in 2013. After postdoctoral research fellow at Georgia Institute of Technology with Prof. Mostafa A. El-Sayed, he has been working as an assistant professor at the Department of Chemistry of Kwangwoon University from 2016. His research interests can be represented as design and synthesis of nanostructures, functionalization, drug delivery and cancer therapy.



Prof. Kyungtae Kang is an assistant professor in the Department of Applied Chemistry at Kyung Hee University. He received his B.S. in chemistry at KAIST, Korea and his Ph.D. in chemistry at KAIST with Prof. Insung S. Choi and Prof. Yoonkey Nam. After completing his doctoral study, he conducted postdoctoral research at Harvard University with Prof. George M. Whitesides. His research interests include protein biochemistry, bioorganic chemistry, and neurochemistry.

2. General behaviors of neurons on surface topography and their applications

Controlling surface topography to manipulate neuronal behaviors is now popular in the design of neuro-active surfaces, with support of advances in micro- and nanofabrication technology that allowed systematic variations in the size and morphology of surface micro/nanostructures. A few recent comprehensive reviews[3a, 4] on the interactions between neurons and surface topography suggested three categories (broadly defined) of changes in neuronal behaviors elicited by surface micro- and nanotopography (Figure 1a): (i) enhanced or decreased adhesion and viability; (ii) guided (perpendicular or parallel to the direction of anisotropic topographical features) growth of neurites; and (iii) developmental acceleration. In general, responses of neurons to a given surface topography are often unpredictable, and their exact biological mechanisms are, as mentioned previously, understood incompletely. Nonetheless, some of them listed below have been studied enough to be utilized for the design of neuro-active surfaces.

2.1. Contact Guidance

Guiding the direction of neurite growth is the most studied topic among those regarding neuron-surface topography interactions. Achieving this holds great promise in neurobiology/engineering, since the direction of neurite reflects the location of synapses (polarized junctions between an axon and a dendrite). As a result, directional control over the formation of neurites—and synapses between them—is critical for a variety of biomedical applications related to neural functions. In treating nerve injuries, for instance, building artificial nerve conduits that control the direction of neurite outgrowth ensures effective regeneration of damaged nerve fibers. Also, for designing an *in vitro* neuronal network as a mesoscopic (network-scale) model for brains, controlled formation of synapses is the most important, and difficult task.

The prevalence of observations of topographically guided neurite growth is likely due to the fact that it is intuitionally easy to predict; neurons are adhesive cells and they tend to physically follow features on surface. Since initial observations of neurite guidance using lithographically fabricated microgrooves,[5] a plethora of surface topographies (Figure 1b-d), including micro- and submicro-scale grooves, channels, and fibers, have been incorporated to study neurite growth of primary hippocampal/cortical/ganglia neurons,[6] neurons derived from neuroblasts,[7] and tissue explants.[8] These approaches included numerous systematic variations in experimental conditions such as the width, depth, and space of the topographical features, addition of chemical cues, and cell types, producing many results. The results, however, sometimes were contradictory, and, thus, suggested that the biological mechanism of topographical neurite guidance is complicated and multifaceted, which made them still remain incompletely understood, albeit abundant research efforts made in past decades.

In most of the cases, neurites tended to follow linear topographical features in parallel in a size-dependent manner (aligned better as the features became thinner), which enabled numerous applications of anisotropic micro-nanostructures to designing artificial nerve conduits. There were some unusual, intuitively unpredictable cases: for example, primary hippocampal neurons occasionally aligned perpendicularly to surface microgrooves, depending upon the vertical depth of the grooves[5b, 9] or age of the neurons,[5b] and similar results appeared for neuroblasts cultured on anisotropic microstructures. Contradicting trends also appeared for the location of neurites (whether they grow on the

ridge or in the groove) or the optimal dimension for the maximized guidance effect. These puzzling results are very interesting, but remain unexplained, prompting further systematic studies along with thorough biochemical investigations..

2.2. Nanotopography as a Tool for Designing Neuro-Active Devices

Advances in micro- and nanofabrication techniques rapidly enabled utilizing surface nanostructures for manipulating various neuronal behaviors other than guided neurite outgrowth. Vertically grown nanowires (made of Si, GaP, Au, etc.) were reported to support the survival and growth of neurons, despite their unnatural and bizarre-looking morphology (Figure 1e).[10] Neurons grown on the nanowire substrates, like other mammalian cells grown so,[11] were penetrated partially or entirely by the sharp tips of the nanowires, but, surprisingly, exhibited normal cellular functions. This interesting phenomenon was utilized by succeeding studies to fix the location of cell bodies on electrode surface,[10d] to deliver biomolecules inside the cytoplasm of neurons,[10e] or to measure the strength of interaction forces between neurons and sharp structures on the surface.[10b]

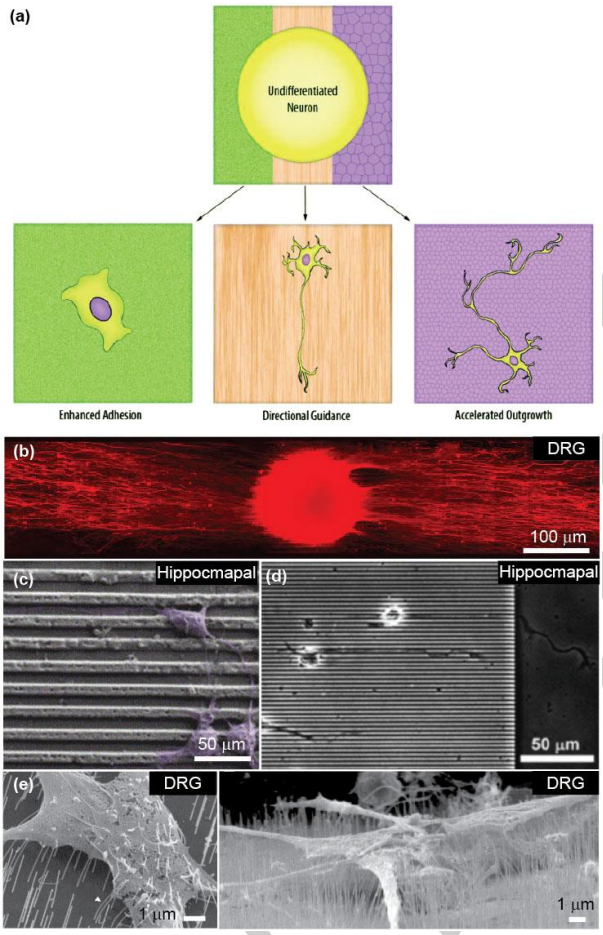


Figure 1. (a) Categorization of responses of neurons to surface nanotopography. Adopted from [3a] with permission. (b) DRGs on aligned polymeric fibers. NF160 (a marker for axons) was immunostained. Adopted and modified from [6c] with permission. (c) Scanning electron microscopy (SEM) image and (d) phase contrast image of hippocampal neurons cultured on PDMS microchannels with immobilized nerve growth factor, and quartz microgrooves, respectively. Adopted and modified from [9a] and [9b] with permission. (e) SEM images showing interactions between GaP nanowires and DRG neurons. Adopted and modified from [10a] with permission.

3. Regulation of the Development and Differentiation of Neural Cells by Surface Topography

Neurogenesis—the process by which neurons are differentiated from neural stem cells—and the subsequent development/maturation of neurons are key processes in the development of a brain and the formation of neural networks therein. Many recent studies showed that surface nanotopography could perturb these complex biological processes, but how neural cells recognize surface nanotopography and adopt intracellular states according to it is poorly defined. Through this section, we provide collections of results addressing this issue and emphasize that the relationship between surface nanotopography and neural cell biology deserves further biological interests.

3.1. Developmental Acceleration of Neurons

Development of neurons is a well-defined topic in neural cell biology; the five-step developmental pathway of primary hippocampal neurons described by Banker *et al.* still remains as a global standard in studies regarding the *in vitro* development of neurons (Figure 2a).[12] In this developmental pathway, a neuron first sprouts multiple indistinguishable neurites for sensing the surrounding area. One of the neurites is then selected by the neuron to be an axon, at which point the neurons is regarded ‘polarized’. Such a sequence of processes, however, seems not conserved in neuronal development *in vivo*. Evidences indicate that neurons developing *in vivo* first need to migrate over a quite long distance at earlier than E18, and during this migration the neurons already have protrusions elongated in a polarized fashion (Figure 2b). When the neuron reaches to its destination, the protruded part subsequently develop into an axon before dendrites start to develop.[13]

A satisfactory explanation that addresses this discrepancy between *in vitro* and *in vivo* neuronal developments has not been made; it is immensely difficult to look into molecular details of developmental processes (especially *in vivo*), because it relies on too many factors that are not tractable. Yet, one important fact is the above-mentioned *in vitro* developmental pathway was discovered by using chemically treated coverslips (i.e., a flat 2D surface) and neurons dissociated from a developing embryonic tissue. This can be unneglectable—particularly when one wants to study *in vivo* neuronal development by extrapolating from the results obtained *in vitro*—because of two reasons: (i) neurons at E18 stage (18 days old in the embryonic state) already contain polarized morphology and neurites. Therefore, neurons

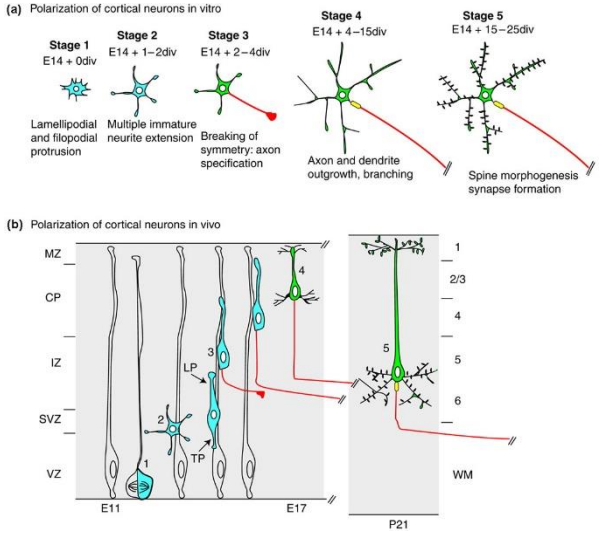


Figure 2. (a) Developmental pathway of primary hippocampal neurons cultured on chemically treated coverslips, described by Dotti and Banker (1988). (b) The formation of the axon–dendrite polarity of pyramidal neurons in a developing brain. Neurons are first polarized with the emergence of the trailing (TP) and leading processes (LP), which precedes the development of dendrites. Adopted from [13] with permission.

dissociated at this stage may have completely different intracellular (generic and proteomic) states. (ii) Neurons developing in brains are surrounded by a soft and 3D environment (described more specifically later in this paper), which is largely different from the surface of coverslips. Role of physical properties of surface—including topography—in neuronal development, in this sense, may be critical in elucidating the observed differences between *in vitro* and *in vivo* neuronal development.

The most frequently observed phenomenon in neuronal development on nanotopographical features is acceleration: at a given age of culture, neurons on nanotopographical surface contain longer neurites than those on flat surface. Such developmental acceleration was initially found—not intentionally in many cases—in reports using anisotropic nanofeatures for guiding the direction of neurite elongation. These reports simply focused on promoted neurite elongation (i.e., longer neurites), but did not perceive their observations as accelerated development of the entire neurons. Here, aligned electrospun nanofibers have been frequently used as a facile method to generate linear nano-featured surface. Electrospun poly(L-lactide) (PLLA), poly(ε-caprolactone), or poly(D,L-lactic-co-glycolic acid) nanofibers with or without growth factors chemically attached in various ways were used as culture substrates for dorsal root ganglia (DRG) tissue explants,[14] primary DRG neurons,[15] PC12,[16] and SH-SY5Y cells.[17] Despite some variances in experimental conditions (the fibers in some cases were composited with carbon materials or decorated with biomolecules), all the results were consistent in that the presence of aligned nanofibers (whose thickness ranged from 200 to 500 nm) promoted neurite extension in neurons cultured on top. Similar promoted neurite elongation appeared also on other types of nanofeatures (e.g., silicon nanopillars,[6d] polymeric nanogratings,[18] nanoparticle-polymer composites,[19] and carbon nanotube-coated surface[20]), indicating that such effect generally occurs at hundreds of nanometers-range, regardless of the surface morphology. Interestingly, the promoted neurite extension did not occur—or, at least was not examined—on a variety of microscale surface topographies, which had been studied earlier than the nano-scale substrates.[5]

On the other hand, there was a slightly different view on such promoted neurite elongation on nanotopographies: we and others suggested that the acceleration driven by nanotopography is not restricted to neurites, but it occurs throughout entire neurons. In order to prove this, primary neurons cultured on various nanotopographies were categorized based on the *in vitro* developmental stages mentioned in the previous section. The results (from primary motor neurons on aligned PLLA nanofibers[21] or primary hippocampal neurons on anodized aluminum oxide substrates[22]) clearly showed that the polarization of neurons—i.e., major neurite determination but not just neurite extension—was accelerated on nanotopographic substrates (Figure 3a). Interestingly, it was found that such 'developmental acceleration' selectively occurred on nanotopographical features that had a pitch (i.e., the distance between adjacent vertexes) higher than 200 nm, as shown on

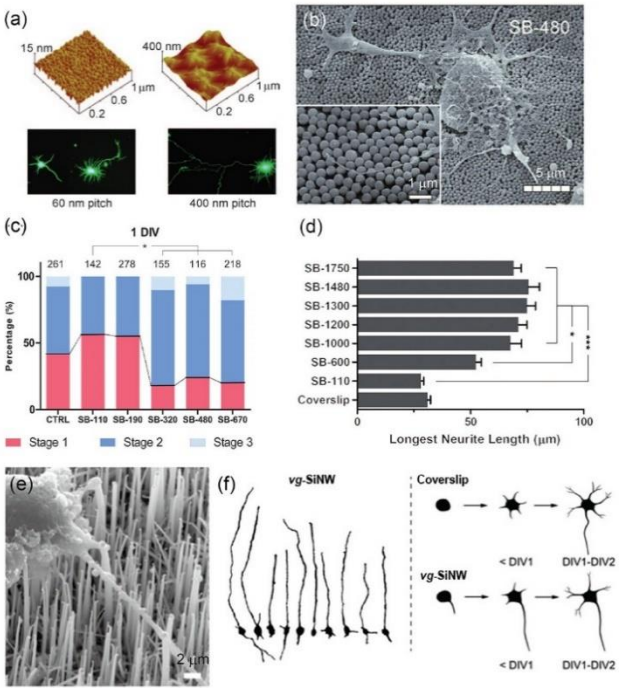


Figure 3. (a) Pitch-dependent developmental acceleration of hippocampal neurons on anodized aluminum oxide substrates. Adopted from [22] with permission. (b) SEM image of a neuron cultured on densely packed silica nanobeads. Adopted from [23] with permission. (c) Distribution of neurons at each *in vitro* developmental stage on silica nanobead-substrates, showing that developmental acceleration selectively occurred on nanobeads bigger than 200 nm. Adopted and modified from [23] with permission. (d) Lengths of longest neurites on larger silica nanobead-substrates. Adopted and modified from [24] with permission. (e) SEM image of a hippocampal neuron adhered on vertically grown silicon nanowires, and (f) the newly found developmental pathway of hippocampal neurons on Si nanowires. Adopted and modified from [25] with permission.

monolayers of assembled beads with different diameters (Figure 3b and c).[23] This pitch-dependent developmental acceleration was more pronounced as surface nanotopographical features enlarged, leveling off at 1000 nm-pitch (Figure 3d).[24] These results are interesting, since they imply strongly that neurons have ability to recognize differences in surface topography at nanoscale, and to subsequently response to them. Supporting this, the reports above also showed that F-actin dynamics—an intracellular machinery responsible for pathfinding and regulating forward/backward neurite proceed—is deeply involved in the neuronal recognition of surface nanotopography: biochemical inhibition of F-actin dynamics resulted in neurons developed into indistinguishable morphologies on different nanotopographies. In a more recent work, primary hippocampal neurons were shown to adopt a completely new developmental pathway on densely packed, vertically grown silicon nanowires (Figure 3e).[25] Here, a major neurite developed first right after plating, and it elongated amazingly fast, resulting in an unusual neuronal morphology. The development of minor neurites occurred afterwards, and this twisted development did not impede the maturation of neurons and their functionality, implying possible relevance of the newly found developmental pathway to that occurring *in vivo* (Figure 3f).

3.2. Preferential Differentiation of Stem Cells into the Neuronal Lineage on Nanotopography

Pluripotent stem cells are capable of differentiating into various types of cells, and thus are extremely critical in biogenesis. In the process of neurogenesis, a certain subset of stem cells—defined as neural stem cells (NSCs)—have to properly differentiate into neural cells (e.g., neurons and glial cells) prior to neuronal development and network formation. Here, the location, time, rate, and direction of such differentiation should be manipulated delicately for the successful formation of a neural network, as in cases of other tissues. Surface nanotopography, again, is known to largely influence differentiation and other behaviors (e.g., adhesion and proliferation) of stem cells.[26] This direction of research—topographical control of stem cell fate—has gained much more intensive interests than the one related to the development of postmitotic neurons, since the ability to determine stem cell fate precisely would pave ways for a myriad of regenerative biomedical applications.

The determination of stem cell fate is intrinsically complex; too many chemical and physical factors are involved in manifesting differentiation, and they are prone to work in contextual and combinatorial fashions. Furthermore, previous works have used a variety of stem cells that reside in different positions in differentiation lineages, and different surface topographies made by multiple materials ranging from micro- to nanoscale, which sometimes produced contradictory results.[3c, 27]

Research efforts have been mainly made for three types of stem cells: embryonic stem cells (ESCs), NSCs, and mesenchymal stem cells (MSCs). Interestingly, general trends observed over the cells were roughly consistent; the presence of anisotropic nanotopography facilitated the formation of focal adhesion complex, upregulated neuronal expression, and downregulated

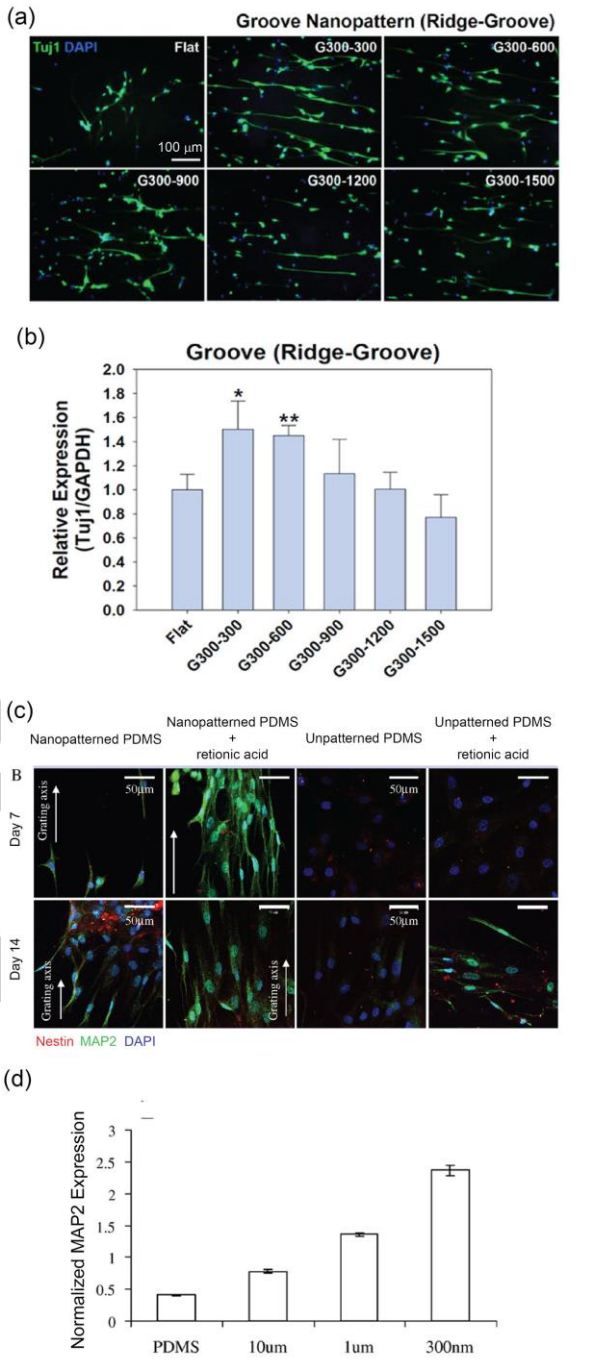


Figure 4. (a) Fluorescence images of human NSCs immunostained for Tuj1 (neuronal marker) and DAPI. The cells were cultured on polymeric nanogratings. (b) qRT-PCR results on human NSC expression of Tuj1 on nanogratings with different lengths of ridge. (a) and (b): adopted and modified from [36] with permission. (c) Fluorescence images of human MSCs immunostained for MAP2, Nestin, and DAPI. The cells were cultured on nano-patterned, and unpatterned PDMS, with or without retinoic acid (RA). (d) Thinner nanogratings show better ability to guide the differentational direction oh MSCs into neurons. (c) and (d): adopted and modified from [41] with permission.

the expression to other cells. For example, in a work by Yim *et al.*, human ESCs preferentially differentiated into neurons on anisotropic micro/nanogratings made of polydimethylsiloxane (PDMS), and into glial cells on isotropic features of the same sort.[28] The same type of cells differentiated into the neuronal lineage preferentially on 250-nm electrospun nanofibers[29] and 350-nm ridge/groove patterns without the use of chemical inducing agents.[30] NSCs are more specialized than ESCs, since the range of their differentiation is restricted to neurons and glial cells. Still, inducing preferred differentiation into one among them is a challenging goal, and, thus, gained intensive interests as well. As for ESCs, anisotropic features promoted neuronal differentiation (as opposed to glial differentiation) of NSCs. This trend appeared when NSCs grew on electrospun polycaprolactone (PCL),[31] PLLA,[32] or poly(ethersulfone)[33] fibers, photolithographically fabricated silicon microstructures,[34] PDMS micropatterns,[35] polymeric nanogrooves,[36] TiO_2 nanostructured surfaces,[37] graphene-nanoparticle hybrid systems,[38] and 3D graphene foam (Figure 4a).[39] More interesting finding was that this type of induced neuronal differentiation also occurred for MSCs, which normally differentiate into the skeletal lineage. When MSCs adhered on anisotropic fibrillary hydrogel, neuronal markers were upregulated significantly.[40] Similar results were obtained by another work using PDMS nanogratings.[41] This tendency was more pronounced as the nanogratings became thinner, and 350 nm was shown to be the best width to induce neuronal differentiation (Figure 4b). By an extension study, the critical role of focal adhesion kinase (FAK) in topography-mediated neuronal differentiation was revealed.[42]

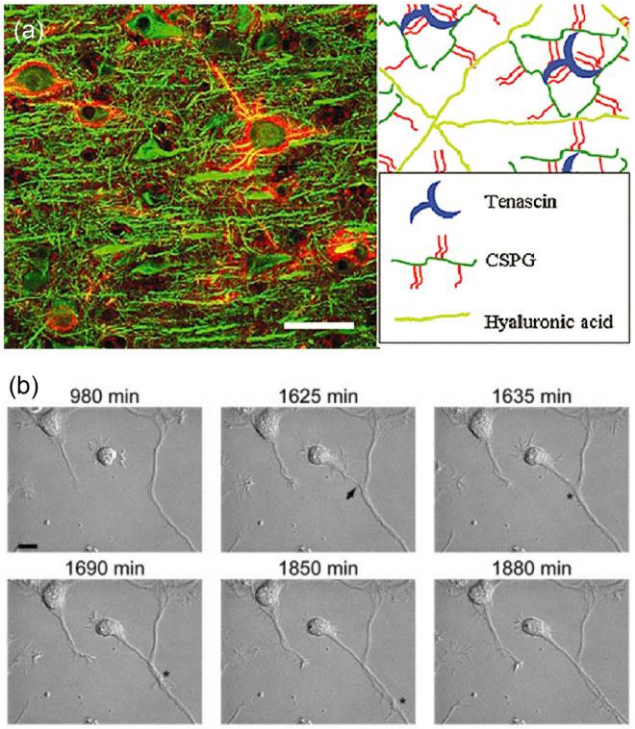


Figure 5. (a) Fluorescence image and illustration of perineuronal nets (PNNs). Macaque brain stained with microtubule-associated protein 2 (MAP2) (red) and Wisteria floribunda agglutinin (WFA) (green). Adopted and modified from [44a]. (b) Interactions between multipolar cells and preexisting axons observed by time-lapse imaging analysis of the overlay culture. Asterisks indicate growth cones. Adopted from [46].

4. Biological Mechanisms and Their In Vivo Relevance

In our recent publication that discovered a new developmental pathway of primary hippocampal neuron on silicon nanowires,[25] we suggested—although not biologically proved—that the newly found developmental pathway triggered by surface nanotopography may reflect what occurs during *in vivo* brain development. Considerations analogous to this are slowly emerging in the interfacing field of nanoscience and neurobiology, with a premise that neurons can 'spontaneously' sense surrounding surface topography, and have corresponding intracellular machineries for doing so. The mechanism by which neurons sense physical aspects of external surface, however, remains incompletely understood, so does its role in neurogenesis and development.

4.1. Surface Topography that Neurons Encounter in Brains

Extracellular matrix (ECM)—space feeling the vicinities of cells and scaffolds—forms the very first environment that neurons (and other cells) encounter in a living organism. Its composition, organization, and physical properties are extremely heterogeneous and dynamic; they are tissue-specific, and vary constantly, reflecting the biological status of a tissue.[43] Generally, ECM is composed mostly of protein-based fibers, but for nervous systems, a critical difference exists in the composition of ECM: a main component of brain ECM, also called perineuronal net (PNN), is not primarily made of proteinaceous fibers (Figure 5a).[44] Particularly, collagen fibers, which occupy about 30% of the total protein in an organism, barely exist in brain ECM. Instead, PNN is filled with a reticular mixture of proteoglycans (proteins decorated with glycosaminoglycans, represented by heparan sulfate and chondroitin sulfate), polysaccharides (hyaluronic acid), and few other glycoproteins (tenascin and laminin), which overall form an elastic hydrogel with nanotopographically complex surface, decorated with neuro-active molecules.

Apart from ECM materials, neural cells also recognize surfaces of other cells (neural cells themselves or glial cells) as 'physicochemical environment' during their development. Cell-cell interaction often plays important roles in many biological contexts in neurons, as represented by the functions of glycoproteins, such as neural cell adhesion molecules.[45] During brain development, a pioneering axon develops first and acts as a guiding scaffold for axons developing later, which in the end fasciculate (aggregate) together (Figure 5b).[46] The mechanism by which axons follow the surface of another axon is not completely understood, but it is

likely that both chemical cues presented on cellular surface and its surface topography *per se* cooperatively guide the growth of following axons.

Interactions between neurons and glial cells (e.g., astrocytes, oligodendrocytes, and microglia for the central nervous system; Schwann cells for the peripheral nervous system) have a much more multifaceted and complicated nature;[47] they were repeatedly shown to govern the survival, development, and metabolism of neurons and also the formation and plasticity of synapses. Although a comprehensive list of roles of glial cells yet remains to be unveiled, the importance of glial cells in neuronal development is obvious. Many research efforts have been devoted to elucidate the roles of extracellular soluble signals (e.g., neurotrophic factors) secreted by glial cells or direct glial-neuron interactions in neurodevelopment. However, there are a fewer, but increasing number of evidences indicating that the topography generated by the surface of glial cells also provide important physical stimuli for a broad range of important neuronal processes including survival, adhesion, and development. Bruder *et al.* provided a demonstration of this, by using substrates molded from fixed Schwann cells—that is, only taking the morphology of them, but not the secreted/attached chemicals—and proved that only the morphology of the Schwann cells were enough to improve the adhesion and survival of primary neurons.[48] The supportive ability of surface nanotopography mimicking that of glial cells were further tackled by Blumenthal *et al.*, who showed that neurons recognize the nanotopography with similar roughness to astrocyte surface via mechanosensing cation channels.[49]

4.2. Role of Focal Adhesion and Its Connection to Mechanotransduction

Surprisingly, the dimensional ranges of nanotopographical features observed to elicit the preferential neuronal differentiation of stem cells and the facilitated development of primary neurons largely overlap. Anisotropic structures that are approximately 200-500 nm thick were commonly observed to be most effective for both cases. Therefore, it is tempting to suspect that the two processes—completely different processes occurring in completely different cells—rely, at least partially, on the same set of intracellular machineries and their relevant signaling cascades. In general, non-neuronal cells (including stem cells) are better understood than neurons in their biological recognition of the physical attributes of extracellular surface. It is broadly accepted that topographical sensing in non-neuronal cells is based on the surface-dependent formation of integrin-based focal adhesion complexes. Proteins involved in a focal adhesion complex (e.g., talin and vinculin) can exert tension on intracellular cytoskeletal fibers, which then reorganize cytoskeletal network, and cellular/nuclear morphology. These types of physical changes can trigger biochemical signal cascades and derive genetic changes—this series of processes are called mechanotransduction. When stem cells encounter topographic features, mechanotransduction seems to play an important role in determining the direction of differentiation. In the work by Yim *et al.*,[42] FAKs in MSCs were preferentially phosphorylated on PDMS nanogratings that promoted the neuronal differentiation of the MSCs (Figure 6a). In another work, the expression of vinculin and FAK—core components of a focal adhesion complex—increased on smaller nanogrooves, which also promoted the expression of a neuronal maker.[36] They also suggested that MEK/ERK pathway—a downstream intracellular signaling pathway to FAK phosphorylation—correlates with neuronal differentiation of the stem cells (Figure 6b).

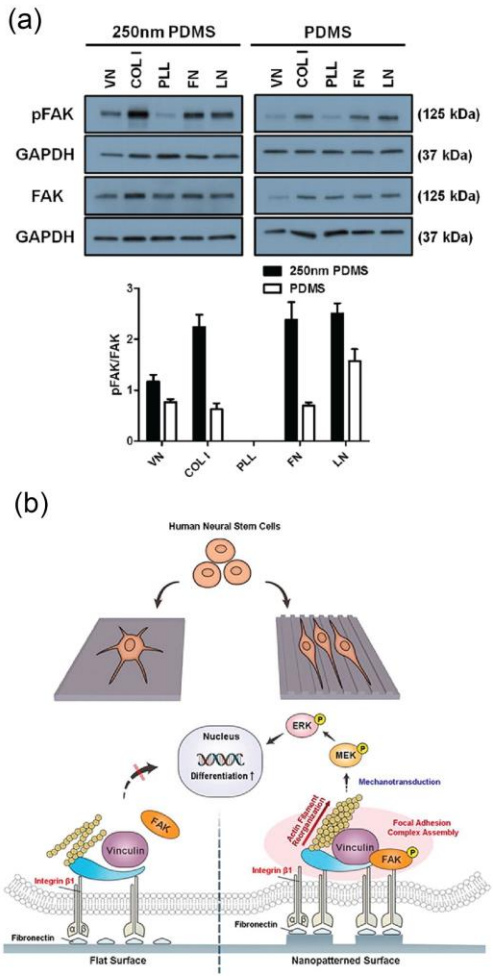


Figure 6. (a) Western blot analysis showing that FAK phosphorylation preferentially occurs for human MCSs cultured for three days on nanogratings coated with various proteins (VN: vinculin, COLI: collagen I, PLL: poly-L-lysine, FN: fibronectin, LN: laminin). Adopted and modified from [42]. (b) Suggested mechanism (through MEK-ERK pathway) for nanotopographical manipulation of the focal adhesion signaling pathway and differentiational regulation of human NSCs. Adopted and modified from [36].

Yet, it may be not safe to project the findings from studies on stem cell differentiation directly to neuronal development—but it is obviously worth considerations. Stem cells are largely different from postmitotic neurons, particularly in the extent to which focal adhesions occur and regulate cellular behaviors. Focal adhesions in non-neuronal cells regulate a range of cellular behaviors including adhesion, migration, and proliferation.[50] Focal adhesions also appear in neurons, while their morphology and functions are quite different; emerging evidence supports the

involvement of focal adhesions—and their downstream signalling cascades—in neurite development, growth cone pathfinding, and synapse formation.[51] Therefore, this type of 'adhesion-triggered' signalling seems to occur in both contexts, but in different forms. In our previous publication, we also found that F-actin dynamics is deeply involved in the neuronal recognition of surface nanotopography, but the observed developmental acceleration was only partially governed by Rho/ROCK pathway.[24] The involvement of FAK phosphorylation and MEK/ERK pathway in development of neurons on nanotopography, however, remains unexplored.

5. Conclusions and Perspectives

The research on the differentiation/development of neural cells on nanotopographical features is now in its transition from an exploring phase that produced a large number of observations from different experiments to a more biologically and fundamentally oriented phase that can derive a general rule from the observations and can build a solid biological explanation. We emphasize that this stream of studies is not only an interesting interdisciplinary approach of multiple fields (nanochemistry, materials chemistry, and neurochemistry) any more, but it is rather a fresh view on complicated, unsolved biological problems pertaining to neurogenesis and development. In this regard, recruiting academic interests from the neural cell biology field is essential; it will not only reinforce the understanding of the observations, but also largely help deriving their biological mechanisms, which can be directly utilized to build sophisticated *in vitro* models for studying neurobiology, and to design highly advanced neuro-regenerative devices.

This review illuminated the overlapping aspects of stem cells and neurons in their responses to surface nanotopography. Both directions of studies produced numerous interesting results for decades, but they, strangely, were not often conducted together, nor were their results used to help one another. Therefore, we suggest: clarifying this somewhat foggy relationship between topographical regulation of neuronal differentiation and development, and their underlying biological backgrounds is one of the most interesting and indispensable future directions of this field.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (no. 2016R1C1B2011414).

Keywords: Cell adhesion • Nanostructures • Neurite development • Neurochemistry • Stem cell differentiation

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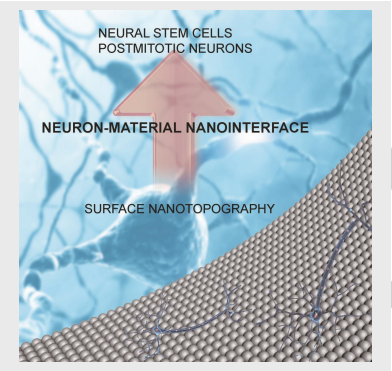
FOCUS REVIEW

This review summarizes recent experimental results regarding influences of surface nanotopography on stem cell differentiation into neurons and neuronal development. Collections of observations and their interpretations are provided. We believe that this review will provide biological insights to the field of nano-neuron interface and encourage interdisciplinary efforts.



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