Tissue Engineering and Regenerative Medicine, Vol. 10, No. 2, pp 39-46 (2013)
DOI 10.1007/s13770-013-0384-6

| Feature Article |

Culture of Neural Cells and Stem Cells on Graphene

Seungmi Ryu[1] and Byung-Soo Kim[2,3]*

[1]*Interdisciplinary Program for Bioengineering, Seoul National University, Gwanak-gu, Seoul, 151-744 Korea*
[2]*School of Chemical and Biological Engineering, Seoul National University, Gwanak-gu, Seoul, 151-744 Korea*
[3]*Institute of Bioengineering, Seoul National University, Gwanak-gu, Seoul, 151-744 Korea*

(Received: December 21st, 2012; Accepted: January 31st, 2013)

Abstract : Graphene, as a new potential biocompatible biomaterial, retains some unique properties such as high electrical conductivity, elasticity and good molecule absorption. It has tremendous potential for a wide variety of biomedical applications. Even though studies on graphene-based nanomaterials are still at a nascent stage, graphene has been a matter of common interest in tissue engineering and regenerative medicine. In this review, we summarize the characteristics of graphene that could impose biological effects on cells. We discuss in detail the exploration of graphene when applied on neural cells and stem cells; how it affects cell behavior and differentiation. We then present the results from cytotoxicity studies on graphene and its derivatives.

Key words: *graphene, neural cell, mesenchymal stem cell, induced pluripoten stem cell, neural stem cell, cytotoxicity*

1. Introduction

Graphene is a planar nano-composite that consists of rings of carbon atoms with a hexagonal lattice structure.[1] It is a basic building block for other graphitic materials such as few-layer graphene, graphene oxide (GO), and reduced graphene oxide (rGO). Few-layer graphene is a flake-like stack of 2-10 graphene layers. When graphene undergoes oxidation, it becomes a chemically-modified graphene, graphene oxide. This graphene oxide can be further reduced to produce reduced graphene oxide (Fig 1). Over the last several years, graphene has emerged as a promising nano-platform with enormous potential for biomedical applications and translational research because of its physical, chemical, and mechanical properties, which make graphene an ideal candidate for many biomedical applications such as tissue engineering[2-4] and drug delivery.[5] In this review article, we will first summarize the characteristics of graphene that could affect cells biologically. Then we will review studies that correlate graphene with cells, which current trend mostly focused on neural cell and stem cell. Despite the great enthusiasm about biomedical applications of graphene-based nanomaterials, there are some concerns about the potential toxicity and biocompatibility of these nanocomposites. Therefore, toxicity studies done on graphene-based nanomaterials will also be discussed in this review.

*Corresponding author
Tel: +82-2-880-1509; Fax: +82-2-888-1604
e-mail: byungskim@snu.ac.kr (Byung-Soo Kim)

2. Characteristics of Graphene

Although graphene is categorized as carbon allotropes, it possesses distinct properties that no other carbon molecules, such as benzene and other allotropes, has, With such, graphene has provided advantages in tissue engineering. Its notable properties, such as electrical conductivity, elasticity and adsorption of protein and low molecular weight substances, may alter the direction of stem cell differentiation and neural cell proliferation.

2.1 Electrical Conductivity

Excellent electrical conductivity of graphene is explained by how sp2-hybridized carbons are put together. Three out of four electrons of the outermost carbon atoms form σ bonds with three-neighboring electrons. The remaining one electron of each carbon forms a π bond. In accordance with the Pauli Exclusion Principle, the outermost shells of the carbon atoms are filled. σ bond forms a solid and stable bond. Meanwhile in π bond, as the remaining electrons are singular, only half of the each carbon atoms p orbital is filled. The unique electrical conductivity of graphene happens to be invoked due to such

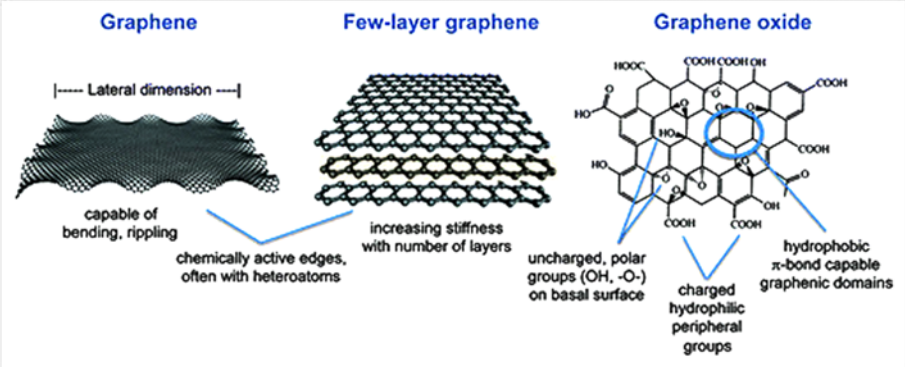

Figure 1. A schematic diagram of the graphene nanomaterial family and its properties relevant to biological interactions.[46]

bond formations. The interaction between graphene electrons is weak and the mean free path is remarkably long that when compared to other metals or semiconductors. Graphene presents unique electrical phenomena. Several ways to modify graphene's electrical properties were introduced; stacking it into multiple layers, altering its geometric structure, or processing it chemically.[6] Pristine graphene shows ~ 200,000 cm^2/V electron mobility at room temperature,[7] representing superior current density and electrical conductivity compared to other substances. For example, graphene obtained by hydrogen arc discharge exfoliation method shows electrical conductivity of up to 2×10^3 S/cm.[8]

2.2 Elasticity

Other characteristic that makes graphene ideal in regenerative medicine is its superb elasticity. So far graphene has the highest Young's modulus compared to other identified substances. Its value is reported to be 0.1-1TPa.[9] Solely according to its high elastic force, it remains unbroken and stably preserved. Without changing its properties graphene is bendable, which makes it convenient to be coated onto even a non-uniform surface.[10] This native elastic property of graphene is known to influence cell growth and its fate.

2.3 Adsorption of Proteins and Low Molecular Weight Substances

Another important characteristic of graphene that can be utilized for tissue engineering is its ability to adsorb protein and low molecular weight chemicals. In order to either grow or communicate with neighboring cells, cells secrete various substances. Such substances are adsorbed onto graphene surface and affect cell proliferation and differentiation.[3] There are several basic principles that allow adsorption of proteins and low molecular weight substances on graphene surface, such as ionic bonds and hydrophobic interactions between molecules. Among those, the most prominent is the π-π stacking caused by van der Waals forces. The attractive π-π stacking forces are created by the consecutive sp2 bonding of graphene molecules and benzene rings that some amino acids possess.[11] For instance, bone morphogenetic protein, a well-known protein that enhances osteogenic differentiation, was found to bind well on graphene and graphite surface.[12,13] The same principle is applied to rationalize how trypsin[14], heparin[15], lysozyme[16], and peptides[17] also bind to graphene oxide and graphite. The π electron cloud carried on graphene is speculated to interact with hydrophobic protein, resulting in strong non-covalent bond between them.[3] Findings on the effect of graphene on osteogenic and adipogenic differentiation inferred to be caused by the adsorption of small molecule dexamethasone and beta-glycerolphosphate protein on graphene. Moreover, insulin, which is known to enhance adipogenic differentiation gets denatured on graphene surface which in turn results in inhibition of adipogenic differentiation.[3] Hence as protein and low molecular weight chemical gets adsorbed and deformed by graphene depending on their hydrophobicity, graphene can impose a great influence on cells.

3. Culture of Neural Cells on Graphene

Graphene has been considered to be a new effective electrode material with high conductivity. It has high transmittance and excellent conductivity as mentioned. On that account, emergence of graphene offers an ideal breakthrough for nervous system model in biomedical applications. First, chemically stable nanostructured graphene is suitable for neural cell adhesion. Because neural cells are electro-active and function based on electrical activities, good electrical conductivity imposed by graphene is ideal for neural model. A noble and innovative method of graphene/ polyethylene terephthalate (PET) film stimulator applied for modulating neural cell-to-cell interactions

was proposed by Heo *et al*.[18] The high two-dimensional conductivity of graphene contributed to *in vitro* stimulation. Moreover, the transparency of graphene allows real time optical microscopic examination of the neural cell's morphological changes. Transient non-contact electric field stimulation was found to enhance and effectively shape cell-to-cell interaction *in vitro*. A significant increase in new cell-to-cell coupling among the cultured neural cells was observed and cell-to-cell coupling was further strengthened. The graphene/PET film substrate was discovered to enhance cell viability and cell proliferation compared to the conventional culture dish substrate. The majority of neural cells underwent morphological changes at the cellular level when electrical stimulation was applied. The electrical stimulation was observed to increase the cell mobility by overexpressing filamentous actin (F-actin). As the stimulation resulted in cells filopodia extension, focal adhesions between the cells and PET substrate were weakened. Simultaneously, fibrillar adhesions transferred from broader sites in the cytoplasm to perinuclear locations. It indicates that the electrical stimulation actively influences and modifies the cellular edge. Thus, the new model of graphene/PET substrate applied together with electric field can modulate neural cell adhesion, interaction and mobility.

In another study, Li *et al*.[19] examined the mechanism of how neurites are affected by graphene alone. This study demonstrated that graphene exhibits excellent biocompatibility with neural cells. Moreover, viability of the neural cells and average neurite length on graphene substrate were significantly enhanced compared to the conventional polystyrene tissue culture substrates, indicating that graphene could be a neuron-favorable material. Further analysis indicated graphene enhanced expression of growth-associate protein-43(GAP43), resulting in the boost of neurite sprouting and outgrowth. The neurite sprouting and outgrowth is one of the signs of nervous system development. Mainly two causes for such phenomena. First, the surrounding matrix of neurons may have been mimicked by the topography of wrinkled and rippled chemical vapor deposition (CVD)-grown graphene substrate, which may assist neuron growth. Second, the high electrical conductivity of graphene could also account for better neurite outgrowth, as in previous study it has been shown that the electrical conductivity of carbon nanotube (CNT) does promote neuron growth.[20,21]

4. Culture of Stem Cells on Graphene

Stem cells play a pivotal role in human body because of their ability for continual growth and renewal. They are critical for numerous groundbreaking therapies in the field of regenerative medicine and tissue engineering. Intensive research has been carried out on stem cells to understand the myriad of environmental factors that organize their complex molecular and cellular events. A few studies on the interrelationship between stem cell and graphene have drawn tremendous interests. Studies done so far can be summed up by the main target stem cell lines used, such as bone marrow-derived mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs) and neural stem cells.

4.1 Bone Marrow-Derived Mesenchymal Stem Cells

Graphene was observed to be able to control and accelerate osteogenic differentiation of human mesenchymal stem cells (hMSC). Nayak *et al*.[22] reported that graphene can accelerate cell differentiation towards bone cell when cultured even in osteogenic media lacking BMP-2, a commonly used additional growth factors. They studied the influence of graphene on stem cell growth using four substrates with variable stiffness and surface roughness; polydimethylsiloxane (PDMS), PET, glass slide and Si/SiO2 substrate. The study demonstrated that regardless of the underlying substrate, graphene is the driving force in bone cell formation. Moreover, all graphene coated substrates showed a strong increase in calcium deposits due to bone nodule formation. While the effect is more distinct on stiffer substrates, similar effect is observed on the softer PET and PDMS substrates, which are known to be less favorable toward osteoblasts.[23] The control group, represented by cover slips in osteogenic medium without graphene, showed that the osteogenic medium without BMP-2 was insufficient to induce differentiation within the 15-day time frame, suggesting that graphene can indeed accelerate osteogenic differentiation of hMSC. Interestingly, they also show that BMP-2-treated and graphene-coated substrates were able to induce cell differentiation at the same rate. To confirm that graphene is critical for the observed stem cell differentiation, amorphous carbon thin films and highly oriented pyrolytic graphite (HOPG) was set as the control groups. They observed that none of them were incapable of leading cell differentiation. The results point out mechanical properties and surface morphology as the decisive factors because CVD graphene which consists of many ripples and wrinkles on the micrometer scale was completely absent in the control groups. The large-scale disorder caused by the ripples in CVD graphene was suggested to play a role in protein adsorption, cell adhesion, proliferation, and differentiation. Furthermore, the ability of graphene to sustain lateral stress was also believed to provide sufficient amount of local cytoskeletal tension.

Lee *et al*.[3] investigated the correlation between graphene,

grapehe oxide (GO) and native PDMS on the adipogenic and osteogenic differentiation of hMSCs. The shapes of MSCs cultured on graphene, GO and PDMS only were distinctively different from one another. MSCs on PDMS appeared rounder and lacked the filopodia extensions. However, cells cultured on graphene and GO exhibited cellular protrusions. The cells on graphene films were homogenously dispersed and showed spindle-shape morphology, whereas those cultured on GO films were more widespread and larger. A direct correlation between the adsorption capacity of the substrate for serum proteins and the subsequent cell growth was deduced. It was found that graphene and GO adsorbed serum proteins up to 8% and 25%, respectively. Serum contains many extracellular matrix globular proteins and glycoproteins such as albumin and fibronectin.[24] The more graphene and GO adsorb serum proteins, the higher density of adhesion molecules is available for cell attachment and growth. The π-electron cloud in graphene is competent to interact with the inner hydrophobic cores of proteins. Due to the presence of oxygenated groups, the hydrophilic GO can bind to serum proteins via electrostatic interactions. After 12 days of cell culture in osteogenic induction media, 7-fold increase in the extent of mineralization in the MSCs cultured on graphene was observed compared to those cultured on PDMS. Lee *et al*.[3] were the first to find that graphene surface has the ability to preconcentrate dexamethasone and beta-glycerolphosphate, which are typical osteogenic inducers, via π-π stacking between the aromatic rings in the biomolecules and the graphene basal plane. Dexamethasone acts synergistically with beta-glycerolphosphate to synthesize new mineralized bone matrices. Mineral deposition elevates with the increased level of dexamethasone.[25] Meanwhile, GO possesses less affinity toward dexamethasone compared to graphene as it experiences larger electrostatic repulsion from phosphate ions. The fact that extensive mineralization was observed only in MSCs cultured with osteogenic induction media suggests that graphene's ability to stably bind chemicals-for this particular study, growth agents-is the key factor in accelerating MSC bone differentiation.

Interestingly, the situation gets reversed when MSCs were chemically induced to differentiate into adipocytes on graphene and GO; GO strongly enhanced adipogenesis with 5-fold higher lipid accumulation compared to graphene. Adipogenesis is enhanced on GO due to its high affinity for insulin, which is the main mediator for fatty acid synthesis. The ability of GO to participate in H-bonding and electrostatic interactions enhanced its binding capacity for insulin. Circular Dichroism data showed immobilized insulin on graphene surface was denatured due to strong π-π stacking. This study shows that the origin of the osteogenic differentiation is traced to the ability of graphene to act as a preconcentration platform for osteogenic inducers. Furthermore, GO's high affinity for insulin greatly enhances adipogenic differentiation.

In another research, stem cell behavior on functionalized graphene was observed. A study of Wang *et al*.[26] with fluorinated graphene indicated that it can promote neuro-induction of hMSCs as the strong polarity imposed by carbon-fluorine bond induces biological responses.[27] In previous studies, the introduction of fluorine to pharmaceutical drugs improved metabolic stability and increased the strength of interaction with targeted proteins.[28] Due to fluorine's high electronegativity and small atom size, it affects the properties of surrounding molecules. Wang et al. showed that fluorinated graphene(FG) induced higher proliferation and stronger polarization of MSCs. Morphological changes in terms of cytoskeletal and nuclear alignment promote differentiation of MSCs toward the neuronal lineage. MSCs on FG are more spindly and elongated which leads to the higher density and tighter junction of the cells, unlike those on unfunctionalized graphene. The surface chemistry of the FG changes as the roughness increases by 3 fold and the water contact angle gets reduced from 83 degree to nearly one degree. It is caused by hydrogen bonding between fluorinated graphene and water molecules, decreasing the free surface energy of the solid/liquid interface.[29] Since cytoskeleton and nucleus elongation is known to induce neuronal differentiation,[30] significantly enhanced nucleus elongation of cells cultured on FG facilitates differentiation into neuron compared to the graphene group. The presented differences could be attributed to the polarization effect of the carbon-fluorine bond. Such electrostatic induction at the interface of cell-FG facilitates cell alignment and nucleus elongation. On day 21, MSCs cultured on FG demonstrated neuron-like morphology with visible neurite protrusion, indicating further neuronal maturation. In addition to morphological changes, neuronal protein such as Tuj1 and MAP2 are highly expressed in MSCs cultured on FG. Moreover, the glial specific marker, glial-fibrillary acidic protein (GFAP), is weakly detected, suggesting a tendency to induce neural differentiation over glial differentiation. Thus this study was able to show that fluorinated graphene can be used to enhance cell adhesion and proliferation of MSCs, and it exhibits a neuro-inductive effect via spontaneous cell polarization through the significant effect on cell morphology, cytoskeletal and nuclear elongation of MSCs.

4.2 Induced Pluripotent Stem Cells

Chen *et al*.[31] studied differentiation of induced puripotent stem cells(iPSC) differentiation on graphene and GO coated

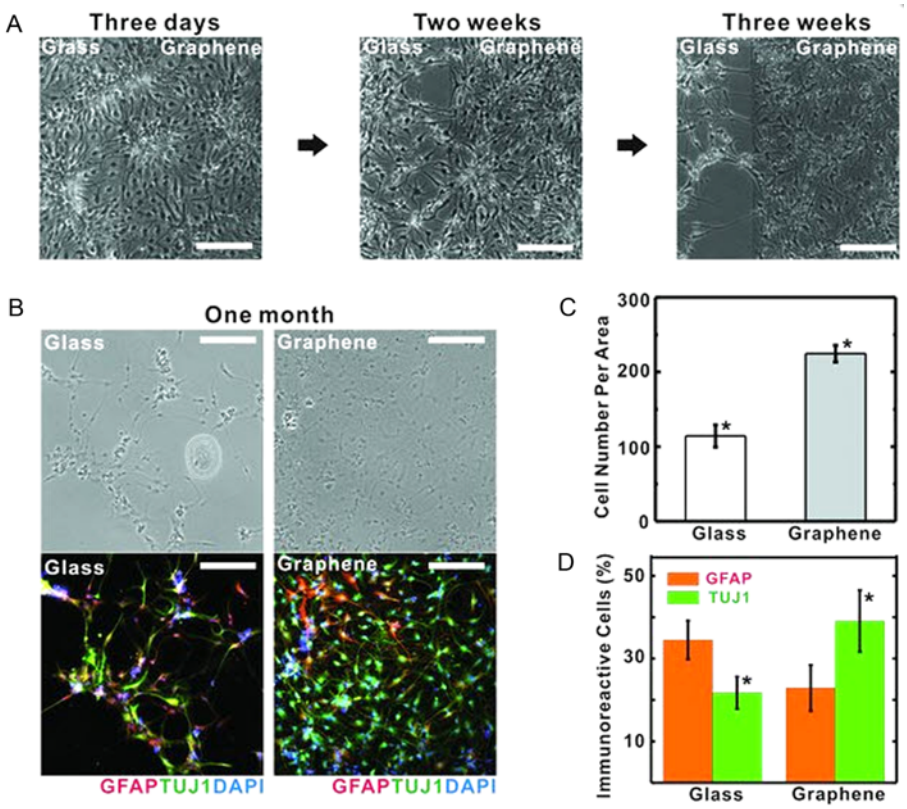

Figure 2. Enhanced neural differentiation of hNSCs on graphene films. (A) Bright-field images of the hNSCs differentiated for three days (left), two weeks (middle), and three weeks (right). (B) Bright-field (top row) and fluorescence (bottom row) images of hNSCs differentiated on glass (left) and graphene (right) after one month differentiation. Cells are stained with DAPI(blue), GFAP(red) and TUJ1(green). (C) Cell counting per area (0.64 mm^2) on graphene and glass substrate after one-month differentiation. (D) Percentage of immunoreactive cells for GFAP(red) and TUJ1(green) on glass and graphene. Scale bars are 200 μm.[33]

glass substrate. The collected data demonstrated that graphene and GO were biocompatible with iPSCs and supported iPSCs attachment and proliferation. The mRNA expression levels and immunohistochemical staining results suggested that, while the cells on the glass and GO spontaneously lost the pluripotency in a culture environment without leukemia inhibitory factor (LIF), graphene appeared to hamper spontaneous differentiation, especially toward the endodermal lineage. For the reasons of such phenomena, it is suggested that as certain functional groups (i.e. carboxylic group) can affect the embryonic stem cell differentiation,[32] the types of iPSCs surface receptors are influenced by different surface groups on graphene and GO. This leads to disparities in the differentiation propensity. However mechanism contributing to this discrepancy remains to be investigated, as the surface molecules and signal transduction pathways of iPSCs are poorly understood.

4.3 Neural Stem Cells

In order to apply hNSCs on brain repair and neural regeneration, it is crucial to induce human neural stem cell (hNSC) differentiation that is directed more towards neurons than glial cells. Park *et al*.[33] demonstrated enhanced differentiation of hNSCs into neurons on graphene coated glass substrate. The hNSCs were observed to attach rapidly onto graphene when compared to glass control group. After two weeks, the hNSCs detached and retracted during the differentiation process. After three weeks period of differentiation, a clear difference in morphology between the cells on graphene and on glass was shown (Fig 2A). Neurite outgrowths were observed on hNSCs cultured on graphene while hNSCs on the glass substrate were detached. After one month, the cells on both graphene and glass showed typical characteristics of neuronal differentiation of hNSCs such as elongated cell shapes and neurite outgrowths. On the contrary with the glass group, the majority of the differentiated cells on graphene attached quite stably. Moreover, graphene was found to provide more favorable microenvironments for hNSC differentiation and promote better cell adhesion and neurite outgrowths than conventional substrates such as glass (Fig 2B). The percentage of glia (GFAP-positive cells) and neurons (TUJ1-positive cells) on graphene and glass are shown in Fig

2B and 2D. The results show that the graphene substrate, compared to glass substrates, enhances neuronal differentiation of hNSCs differentiation to neurons. The laminin-related receptors in hNSCs on graphene were significantly upregulated compared to the control group. This indicated the adhesion of hNSCs on graphene was enhanced. A previous study showed that hNSCs differentiation into neurons could be enhanced when they were surrounded by a larger number of glial cells.[34] A plausible explanation could be that graphene better and stably retains this larger cell number during the differentiation process in comparison with the glass substrate. In addition, genes related to the calcium signaling pathway, such as NCX (Na/Ca exchangers) and G protein-coupled receptors, were significantly upregulated when hNSCs were differentiated on graphene. This implies that the differentiated neurons on graphene had key functional components such as ion channels or neurotransmitter-related proteins. Thus, through this study, the notion that graphene be utilized to enhanced neuronal differentiation of hNSCs was verified.

5. Cytotoxicity of Graphene-Based Nanomaterials

The toxicological profile of graphene-based nanomaterials is not yet well elucidated. Kalbacova *et al*.[35] demonstrated for the first time that CVD grown graphene is not toxic for human osteoblasts and MSCs, The data illustrates that the cells cultivated on graphene films maintained a common morphology and proliferated well on CVD graphene. The literature reports to date clearly indicate that stably functionalized graphene-based nanomaterials are much less toxic than the unfunctionalized counterparts.[36] Also, studies indicated that several factors such as concentration, size, shape, type of dispersants can influence the cytotoxicity of graphene and GO.[37] MTT colorimetric assays revealed that graphene-chitosan composites were biocompatible to L929 cells.[38] Furthermore, most studies have indicated reduced or no cytotoxicity of GO in a variety of cells such as L929 cells,[39] HeLa cells,[40] human fibroblasts,[37] A549 human lung cancer cells,[41] and human hepatoma HepG2 cells.[42]

Recently, graphene was reported to elicit concentration-dependent cytotoxicity in cell-based studies,[36,43] decreasing cell adhesion, inducing cell apoptosis, and entering various cellular compartments. Single-layer GO internalized in cytoplasmic, membrane bound vacuoles of human lung epithelial cells[44] and fibroblasts[37] were observed to exert toxicity to cells when applied with high dosage (above 20 μg/mL) after 24 hr. In contrast, when the GO is applied extracellularly, Chang et al. found that GO presented minimal toxicity even at dose higher than 50 μg/mL, but there was no indication of cellular uptake. Instead, extracellular generation of reactive oxygen species (ROS) was present.[45] Chen et al. also unveiled that graphene and GO coated substrates are biocompatible with iPSCs and enable cell adherence and proliferation, which supports the notions that they exerts low cytotoxicity to mammalian cells.[31] Graphene was also reported to possess no harmful effect as a substrate for hNSCs *in vitro*.[33] When Nayak *et al*.[22] analyzed the influence of graphene on hMSCs coated on 4 different substrates (i.e. PDMS, PET, glass slide and Si/SiO2 substrate), they observed no significant difference in cell viability between graphene-coated and uncoated substrates. Moreover, the MTT assays and immunofluorescence images confirmed the good cell viability and morphology. Regardless of the substrate, cell growth and differentiation was not affected by the presence of graphene (Fig 3). In conclusion, unless cells are treated with high dosage of the graphene-based nanomaterials, it could be deduced that they will not impose harmful effect on mammalian cells.

6. Conclusion

The exploration of graphene has witnessed great advances over the last few years, even though this subject is still in its

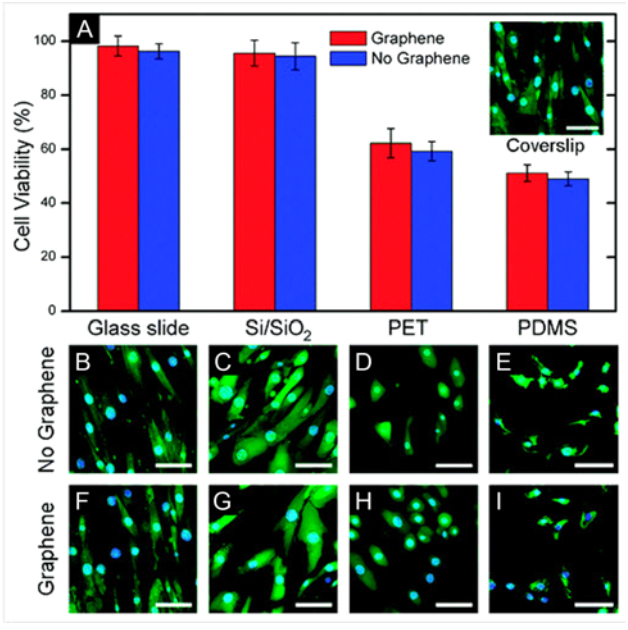

Figure 3. Cell viability and morphology of hMSCs grown on different substrates. (A) Cell viability is normalized to coverslips used as reference. (Inset) Morphology of hMSC grown on conventional coverslips. (B-E) immunohistological staining showing cell morphology on glass slide, Si/SiO2, PET, and PDMS without graphene. Cells are stained with DAPI(blue) and Calcein AM(green). (F-I) cells on glass slide, Si/SiO2, PET, and PDMS coated with graphene. Scale bars are 100 μm.[22]

infancy. Its unique characteristics such as excellent electrical conductivity, high elasticity and good molecule adsorption have been interesting subjects for tissue engineering applications. So in recent studies, both graphene and its derivatives have demonstrated as biocompatible substrates for the promotion of growth and spontaneous differentiation of various stem cells such as hMSCs, iPSCs, hNSCs and neural cells. A few major obstacles still remain to be investigated before active application of graphene in tissue engineering. To name a couple, graphene is non-biodegradable and its long-term toxicity has not been studied yet. Since behaviors of the graphene-based nanomaterials are altered depending on their concentration, size, shape, type of dispersants and many more, further studies must be carried out extensively. However with sustained research on this field, the future potential of graphene in biomedical applications is indeed worth to hope for.

Acknowledgments: This study was supported by a grant (A100443) from the Korean Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea.

References

1. KS Novoselov, AK Geim, SV Morozov, *et al*., Electric field effect in atomically thin carbon films, *Science*, **306**, 666 (2004).
2. L Zhang, TJ Webster, Nanotechnology and nanomaterials: promises for improved tissue regeneration, *Nano Today*, **4**, 66 (2009).
3. WC Lee, CH Lim, H Shi, *et al*., Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide, *ACS Nano*, **5**, 7334 (2011).
4. T Dvir, BP Timko, DS Kohane, *et al*., Nanotechnological strategies for engineering complex tissues, *Nat Nanotechnol*, **6**, 13 (2011).
5. OC Farokhzad, R Langer, Impact of nanotechnology on drug delivery, *ACS Nano*, **3**, 16 (2009).
6. AH Castro Neto, F Guinea, NMR Peres, *et al*., The electronic properties of graphene, *Reviews of Modern Physics*, **81**, 109 (2009).
7. KI Bolotin, KJ Sikes, J Hone, *et al*., Temperature-dependent transport in suspended graphene, *Phys Rev Lett*, **101**, 096802 (2008).
8. Z-S Wu, W Ren, L Gao, *et al*., Synthesis of graphene sheets with high electrical conductivity and good thermal stability by hydrogen arc discharge exfoliation, *ACS Nano*, **3**, 411 (2009).
9. C Lee, X Wei, JW Kysar, *et al*., Measurement of the elastic properties and intrinsic strength of monolayer graphene, *Science*, **321**, 385 (2008).
10. Y Lee, S Bae, H Jang, *et al*., Wafer-scale synthesis and transfer of graphene films, *Nano Lett*, **10**, 490 (2010).
11. SE Wheeler, Understanding substituent effects in noncovalent interactions involving aromatic rings, *Acc Chem Res*, DOI: 10.1021/ar300109n (2012).
12. T Utesch, G Daminelli, MA Mroginski, Molecular dynamics simulations of the adsorption of bone morphogenetic protein-2 on surfaces with medical relevance, *Langmuir*, **27**, 13144 (2011).
13. C Mücksch, HM Urbassek, Adsorption of BMP-2 on a hydrophobic graphite surface: a molecular dynamics study, *Chemical Physics Letters*, **510**, 252 (2011).
14. G Xu, X Chen, J Hu, *et al*., Immobilization of trypsin on graphene oxide for microwave-assisted on-plate proteolysis combined with MALDI-MS analysis, *Analyst*, **137**, 2757 (2012).
15. DY Lee, Z Khatun, J-H Lee, *et al*., Blood compatible graphene/heparin conjugate through noncovalent chemistry, *Biomacromolecules*, **12**, 336 (2011).
16. G Raffaini, F Ganazzoli, Protein adsorption on a hydrophobic surface: a molecular dynamics study of lysozyme on graphite, *Langmuir*, **26**, 5679 (2010).
17. Y Sheng, W Wang, P Chen, Interaction of an ionic complementary peptide with a hydrophobic graphite surface, *Protein Sci*, **19**, 1639 (2010).
18. C Heo, J Yoo, S Lee, *et al*., The control of neural cell-to-cell interactions through non-contact electrical field stimulation using graphene electrodes, *Biomaterials*, **32**, 19 (2011).
19. N Li, X Zhang, Q Song, *et al*., The promotion of neurite sprouting and outgrowth of mouse hippocampal cells in culture by graphene substrates, *Biomaterials*, **32**, 9374 (2011).
20. H Hu, Y Ni, V Montana, *et al*., Chemically functionalized carbon nanotubes as substrates for neuronal growth, *Nano Lett*, **4**, 507 (2004).
21. EB Malarkey, KA Fisher, E Bekyarova, *et al*., Conductive single-walled carbon nanotube substrates modulate neuronal growth, *Nano Lett*, **9**, 264 (2009).
22. TR Nayak, H Andersen, VS Makam, *et al*., Graphene for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells, *ACS Nano*, **5**, 4670 (2011).
23. SJ Kim, JK Lee, JW Kim, *et al*., Surface modification of polydimethylsiloxane (PDMS) induced proliferation and neural-like cells differentiation of umbilical cord blood-derived mesenchymal stem cells, *J Mater Sci Mater Med*, **19**, 2953 (2008).
24. S Oh, KS Brammer, YS Li, *et al*., Stem cell fate dictated solely by altered nanotube dimension, *Proc Natl Acad Sci U S A*, **106**, 2130 (2009).
25. N Jaiswal, SE Haynesworth, AI Caplan, *et al*., Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro, *J Cell Biochem*, **64**, 295 (1997).
26. Y Wang, WC Lee, KK Manga, *et al*., Fluorinated graphene for promoting neuro-induction of stem cells, *Adv Mater*, **24**, 4285 (2012).
27. W Cai, X Chen, Nanoplatforms for targeted molecular imaging in living subjects, *Small*, **3**, 1840 (2007).
28. Y Zhang, J Zhang, X Huang, *et al*., Assembly of graphene oxide-enzyme conjugates through hydrophobic interaction, *Small*, **8**, 154 (2012).
29. X Li, X Wang, L Zhang, *et al*., Chemically derived, ultrasmooth graphene nanoribbon semiconductors, *Science*, **319**, 1229 (2008).
30. Y Wang, Z Li, J Wang, *et al*., Graphene and graphene oxide:

biofunctionalization and applications in biotechnology, *Trends in Biotech*, **29**, 205 (2011).
31. GY Chen, DWP Pang, SM Hwang, *et al*., A graphene-based platform for induced pluripotent stem cells culture and differentiation, *Biomaterials*, **33**, 418 (2012).
32. T-I Chao, S Xiang, C-S Chen, *et al*., Carbon nanotubes promote neuron differentiation from human embryonic stem cells, *Biochem Biophy Res Comm*, **384**, 426 (2009).
33. SY Park, J Park, SH Sim, *et al*., Enhanced differentiation of human neural stem cells into neurons on graphene, *Adv Mater*, **23**, H263 (2011).
34. H Song, CF Stevens, FH Gage, Astroglia induce neurogenesis from adult neural stem cells, *Nature*, **417**, 39 (2002).
35. M Kalbacova, A Broz, J Kong, *et al*., Graphene substrates promote adherence of human osteoblasts and mesenchymal stromal cells, *Carbon*, **48**, 4323 (2010).
36. A Sasidharan, LS Panchakarla, P Chandran, *et al*., Differential nano-bio interactions and toxicity effects of pristine versus functionalized graphene, *Nanoscale,* **3**, 2461 (2011).
37. K-H Liao, Y-S Lin, CW Macosko, *et al*., Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts, *ACS Applied Mater Int*, **3**, 2607 (2011).
38. H Fan, L Wang, K Zhao, *et al*., Fabrication, mechanical properties, and biocompatibility of graphene-reinforced chitosan composites, *Biomacromolecules*, **11**, 2345 (2010).
39. M Wojtoniszak, X Chen, RJ Kalenczuk, *et al*., Synthesis, dispersion, and cytocompatibility of graphene oxide and reduced graphene oxide, *Colloids Surf B: Biointerfaces*, **89**, 79 (2012).
40. C-H Lu, C-L Zhu, J Li, *et al*., Using graphene to protect DNA from cleavage during cellular delivery, *Chemi Comm*, **46**, 3116 (2010).
41. Y Chang, S-T Yang, J-H Liu, *et al*., In vitro toxicity evaluation of graphene oxide on A549 cells, *Toxicol Lett*, **200**, 201 (2011).
42. J Yuan, H Gao, J Sui, *et al*., Cytotoxicity evaluation of oxidized single-walled carbon nanotubes and graphene oxide on human hepatoma HepG2 cells: an iTRAQ-coupled 2D LC-MS/MS proteome analysis, *Toxicol Sci*, **126**, 149 (2012).
43. Y Zhang, SF Ali, E Dervishi, *et al*., Cytotoxicity effects of graphene and single-wall carbon nanotubes in neural phaeochromocytoma-derived PC12 cells, *ACS Nano*, **4**, 3181 (2010).
44. K Wang, J Ruan, H Song, *et al*., Biocompatibility of graphene oxide, *Nanoscale Res Lett*, **6**, 1 (2011).
45. P Huang, C Xu, J Lin, *et al*., Folic acid-conjugated graphene oxide loaded with photosensitizers for targeting photodynamic therapy, *Theranostics*, **1**, 240 (2011).
46. VC Sanchez, A Jachak, RH Hurt, *et al*., Biological interactions of graphene-family nanomaterials: an interdisciplinary review, *Chem Res Toxicol*, **25**, 15 (2011).