Highly enhanced compatibility of human brain vascular pericyte cells on monolayer graphene

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ABSTRACT
We introduce a method for increasing the compatibility of human brain vascular pericyte (HBVP) cells on a glass substrate, based on wet transferred monolayer graphene without any treatment. As a novel material, graphene has key properties for incubating cells, such as chemical stability, transparency, appropriate roughness, hydrophobicity and high electrical conductivity. These outstanding properties of graphene were examined by Raman spectroscopy, water contact angle measurements and atomic force microscopy. The performance of this graphene-based implant was investigated by a cell compatibility test, comparing the growth rate of cells on the graphene surface and that on a bare glass substrate. After an incubation period of 72 h, the number of live HBVP cells on a graphene surface with an area of $1 \times 1 \text{ mm}^2$ was 1.83 times greater than that on the glass substrate.

KEYWORDS
cell compatibility; graphene; graphene biocompatibility; graphene bio-implant; human brain vascular pericyte cells

Introduction

In recent decades, many studies have been performed to detect diseases or changes in cell movement and growth by using a cell-based implant system combined with novel materials. These novel materials, which play an important role in realizing a cell-based implant system to imitate actual human organ systems, have been a key factor in cell research.1-3 In the bio-implant field, surface treatment of the novel material is an important task because cells are in direct contact with the material surface. In particular, there are several important factors to consider among the various surface treatments, such as the cell’s adhesion, chemical stability, roughness and biocompatibility, in establishing an ideal surface coating material for bioengineering. Additionally, the conductivity and transparency of the coating material must be considered as important factors in neural implant systems, to allow for the sensing of electrical signals, particularly as neural signal analysis has become a pressing issue in the bio-implant field.4,6 However, thus far, various experiments have reported that for different materials, such as diamond, carbon, SiC, TiN, TiO2, and polymer series,6-18 to be utilized as a surface coating layer, improvements are required in terms of biocompatibility, transparency and adhesion between cells and the surface of the coated material. Therefore, solving these problems has become one of the main goals in the medical implant research field.2,19,20

Over the last decade, graphene has been extensively investigated to replace the existing materials based on its outstanding properties, such as chemical stability and high biocompatibility.21-30 Additionally, graphene has excellent electrical conductivity, which allows electrical signals from cells to be easily measured and permits electrical signals to be transferred to cells through this material in a nerve system.21 Moreover, the high transmittance and flexibility of graphene render a more stable implant structure with respect to mechanical deformation, and the cell system allows for observations of morphological changes in the cells via microscope measurements. In addition, graphene can be applied to any type of substrate because it conformally attaches, following the surface roughness of the target substrate.31,32,33
Among various types of cells in the bio-implant system, human brain vascular pericyte (HBVP) cells have an important position, which play a critical role in the formation of the blood-brain barrier, the regulation of angiogenesis, the phagocytosis of endothelial cells and the clearing of cellular debris. The degree of vascular pericyte activity implies various pathological conditions such as Alzheimer disease, diabetic retinopathy, hypertension, multiple sclerosis, and central nervous system tumor formation because endothelial cell structure and blood flow are closely related. Therefore, to understand and research these cells, the cell culturing in an implant system is to be first addressed. However, until now, there has been no research on the compatibility of HBVP cells on a graphene surface, despite graphene’s outstanding properties to build the bio-implant system.

In this paper, the compatibility of HBVP cells on monolayer graphene transferred to a glass substrate was investigated. The electromechanical properties of monolayer graphene with respect to cell compatibility were examined by Raman spectroscopy, water contact angle measurements, and atomic force microscopy (AFM). Finally, we compared the number of growing HBVP cells on monolayer graphene to that on the original glass substrate.

**Experimental**

To fabricate an HBVP cell-based implant system on monolayer graphene, graphene should first be transferred onto a glass substrate. To synthesize monolayer graphene, 25-μm-thick copper foil (Alfa Aesar, No.13382, ≥99.8 %) was loaded into a thermal chemical vapor deposition (TCVD) chamber and annealed at 1000 °C under 0.5 mTorr for 40 min with gas flow (H2 20 sccm, Ar 50 sccm). Next, monolayer graphene was synthesized under gas flow (30 sccm CH4 and H2) at 1000 °C for 30 min. Before placing the graphene specimen on copper, the sheet was cooled to room temperature under an Ar gas flow. This synthesized monolayer graphene was transferred onto a glass substrate using a wet transfer method, as shown in Fig. 1A. First, the back side of the graphene was etched by O2 plasma at 30 sccm for 20 sec to increase the contact area between the copper and the copper etchant solution. Next, PMMA (poly (methyl methacrylate)) was spin-coated onto the front side of the graphene layer at 1000 rpm for 60 sec, followed by 2000 rpm for 45 sec as a mechanical support during the wet process. This PMMA/graphene/copper specimen was floated on a copper etchant solution, ammonium persulfate (APS-100, 0.1 M), for 4 h to etch the copper layer. After the etching process, the PMMA/graphene layers were transferred to the target glass substrate and heated at 60 °C in an oven to remove any moisture. Finally, the PMMA on the graphene layer was dissolved by acetone, as illustrated in Fig. 1A.

After these wet transfer processes, we coated 25 ug/ml fibronectin diluted in DI water onto the graphene surface to allow for the attachment of HBVP cells.
Therefore, we performed an individualization of cells, as shown in Fig. 1B. We inserted medium into the cell flask to provide the necessary ingredients for growing cells. Next, the cells with the medium were rinsed with PBS (phosphate-buffered saline) solution. Trypsin was then added to the flask to detach cells from the flask walls. After the cells were grown via incubation, we added new medium to the trypsin and cell solution and performed centrifugation to sink the cells in the solution. The trypsin-medium solution was removed, and only the HBVP cells remained in the centrifuge flask. Afterwards, medium was repetitively inserted to feed the cells. Finally, we coated these HBVP cells onto the wet transferred monolayer graphene surface, as shown in Fig. 1C.

**Result and discussion**

To investigate the properties of the wet transferred graphene on the glass substrate, such as defects,38 doping,39 and the number of layers,38,40 we obtained Raman spectra using a high-resolution dispersive Raman microscope (NRS 3000, Jasco). Figure 2A shows the Raman spectra of the monolayer graphene on a glass substrate. The G peak at 1584 cm\(^{-1}\) and the 2D peak at 2687 cm\(^{-1}\) are distinct. In contrast, the D peak at 1345 cm\(^{-1}\), indicating defects in the graphene, is not distinguishable compared to the G and 2D peaks. Additionally, the ratio of the 2D peak to the G peak is 5.11, demonstrating that the wet transferred graphene is a uniform monolayer of high quality without defects over the entire area.33,41 This defect-free monolayer graphene transferred onto a glass substrate provides uniform conditions for the HBVP cells.

Additionally, we measured the water contact angle of the monolayer graphene and the bare glass substrate using a 50-μl DI water droplet, as shown in Fig. 2B. The hydrophobicity of the surface on which cells are adhered is an important factor in cell compatibility.1,42-44 The measured contact angle of the graphene was 81.13 deg. However, the bare glass area showed a lower contact angle of 47.83 deg. Based on this result, the monolayer graphene, with its hydrophobic surface, provides more suitable conditions for

![Figure 2.](image-url)
cell growth compared to the bare glass substrate because release of water molecules bound to the proteins, which leads to an increase in entropy.45

Several studies have shown that a surface with high roughness and high hydrophobicity will exhibit increased cell adhesion and compatibility.44 We observed the roughness of the graphene and the bare glass substrate using tapping mode AFM, as shown in Fig. 2C and D. Figure 2C and D present a topographic image and the surface roughness of the graphene layer and the glass substrate. The results indicate that there are no defects such as tearing or PMMA residue on the monolayer graphene. The highest height difference on the graphene surface is 4.23 nm, and the root mean square (RMS) value is 2.5506 nm. For the bare glass substrate, the highest height difference is 0.31 nm, and the RMS value is 0.0402 nm. These findings imply that the surface of the wet transferred monolayer graphene is more appropriate for HBVP cell growth compared to the bare glass substrate. These uneven surface of graphene can provide appropriate area to HBVP cells to strongly attach and adhere to the substrate.

Finally, we investigated the compatibility of the graphene surface for HBVP cells by observing the number of growing cells after several hours. We observed the cells using an optical microscope, as shown in Fig. 3. Figure 3A–C present the status of cell growth on the glass substrate after 24 hours, 48 hours, and 72 hours, respectively. For the graphene surface, the change in the number of growing cells is shown in Fig. 3D–F. As the incubation period increased, the number of cells increased dramatically on the graphene surface. This result shows that HBVP cells can stably attach to and grow on the graphene surface. In contrast, for the bare glass substrate, Fig. 3C shows that the HBVP cell growth was lower than that on the graphene surface. A comparison of the number of cells on the bare glass substrate and the graphene surface is presented in Fig. 4. For the first 24-h period, there were more cells on the glass substrate than the graphene surface. However, the number of cells on the graphene surface increased drastically after another 24 h. On the graphene surface, the number of live cells was $31 \pm 11$ after 24 h, $115 \pm 11$ after 48 h, and $141 \pm 9$ after 72 h in 1 mm$^2$ area. In comparison, the

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Optical microscope images of HBVP cell growth on the (A)-(C) glass substrate and (D)-(F) graphene surface for time periods of 24 h, 48 h and 72 h, respectively.
The number of live cells on the glass substrate was 37 ± 12 after 24 h, 53 ± 9 after 48 h, and 77 ± 14 after 72 h, as shown in Fig. 4. Although there were fewer live cells on the graphene surface at 24 h, the number of cells on the graphene surface increased nearly 3.7-fold compared to the initial state after 48 h. The number of live cells on the graphene was 1.83 times larger than that of the glass substrate after 48 h. These results indicate that the uniform graphene surface, with its more suitable surface roughness and hydrophobicity, exhibits a higher adhesion and compatibility for HBVP cells compared to the bare glass substrate.

Conclusion

This paper reports that monolayer graphene has outstanding compatibility for the growth of HBVP cells. The excellent performance of this graphene-based HBVP cell implant system is based on the electromechanical properties of graphene, such as a defect-free uniform hydrophobic surface with appropriate roughness, transparency, and conductivity. The graphene-based HBVP cell implant was more effective and exhibited 1.83-fold more cell growth on the surface than the commonly used bare glass substrate. These results reveal that this graphene-based HBVP cell implant system can be beneficial in developing wide-ranging applications such as graphene-based bioengineering devices.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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