Materials Letters 131 (2014) 328–331

Contents lists available at ScienceDirect

Materials Letters

journal homepage: www.elsevier.com/locate/matlet

Neuroblastoma cells grown on fluorine or oxygen treated graphene sheets

Hong-Gi Oh [a], Hyo-Geun Nam [a], Dae-Hoon Kim [a], Min-Hye Kim [a], Kwang-Hwan Jhee [b], Kwang Soup Song [a,*]

[a] Department of Medical IT Convergence Engineering, Kumoh National Institute of Technology, Gumi 730-701, South Korea
[b] Department of Applied Chemistry, Kumoh National Institute of Technology, Gumi 730-701, South Korea

ARTICLE INFO

Article history:
Received 6 December 2013
Accepted 5 June 2014
Available online 13 June 2014

Keywords:
Graphene
Oxygen
Fluorine
Cell
SH-SY5Y

ABSTRACT

We investigate the effect of cell culture conditions, using functionalized graphene sheets with oxygen (–O) or fluorine (–F) as growth surfaces, on the human nerve cell line (SH-SY5Y). We applied the fluorescence microscopy of cells stained with Hochest 33342, Calcein AM, and MTT assay to evaluate cell viability and morphology. The effect of the graphene on the cells in culture was dependent on the atomic state of the graphene sheet. SH-SY5Y cells exhibited approximately 138% viability (MTT assay) on the oxygenated graphene sheets and 50% viability on the fluorinated graphene sheets, as compared to the data from culturing on pristine graphene.

1. Introduction

Graphene is a single atom sheet of sp^2 bonded carbon atoms in a closely packed honeycomb structure. It is one of the most attractive nanostructured materials, with physical, chemical, and biological applications [1–3]. In order to assess the potential for biomedical applications of graphene sheets, the nervous system would be an ideal breakthrough model due to the property of electrical conductivity exhibited by graphene sheets [4,5]. Functions of the nervous system are based on electrical responses, and our chosen model cell line, human nerve SH-SY5Y cells, is electrically active [6,7].

Recently, a number of techniques have been introduced to functionalize, or etch sp^2 graphene sheets using physical or chemical methods [8–10]. And previous studies provided quantitative and morphological evidence of cell interactions on graphene oxide, reduced graphene, and pristine graphene [3,4,8]. However, the biological responses of cells remain unclear on the functionalized graphene sheet.

In this work, we directly functionalize graphene sheets with oxygen (–O) or fluorine (–F) using treatment with plasma in O_2 or C_3F_8 gas environments. The coverage of fluorine or oxygen on graphene sheets can be controlled by the exposure time to the plasma. We then characterize the morphology and cell viability of SH-SY5Y cells grown on partially functionalized graphene sheets with oxygen or fluorine, and compare these factors to those exhibited by cells grown on pristine graphene.

2. Experimental

Graphene sheets were purchased from Samsung Co., Korea [11,12]. We used SiO_2/Si (100, p-typed), and polyethylene terephthalate (PET) as the target substrates for the released monolayer graphene sheets. The application of graphene sheets onto PET substrates has a potential for use in bioscience, owing to the flexibility of these substrates.

The characteristics of the carbon in the graphene sheets were analyzed with Raman spectroscopy (Renishaw, System 1000), using an Ar-ion laser at a wavelength of 514 nm, and spatially resolved X-ray photoelectron spectroscopy (XPS; monochromatic Al Kα X-ray source, 1486.6 eV, beam diameter 400 μm) was used for determining the atomic functional sites on the graphene sheets. The surface morphology and roughness of the graphene sheet were evaluated using atomic force microscopy (AFM, ParkSYSTEMS, XE-100) operated in noncontact mode in air at room temperature and cold field emission-scanning electron microscopy (FE-SEM, JEOL, JSM6701F, working distance of 8 mm, accelerating voltage at 15.0 kV).

Fluorinated graphene sheets were obtained by plasma treatment in a C_3F_8 gas environment, employing a 50 kHz radio-frequency plasma source at 30 W. The plasma treatment was carried out at room temperature with the C_3F_8 gas pressure fixed at 1×10^{-2} Torr for 10 or 20 min. The C_3F_8 gas flow rate was kept

* Corresponding author. Tel.: +82 54 478 7435; fax: +82 54 478 7445.
E-mail address: kssong10@kumoh.ac.kr (K.S. Song).

http://dx.doi.org/10.1016/j.matlet.2014.06.013
0167-577X/

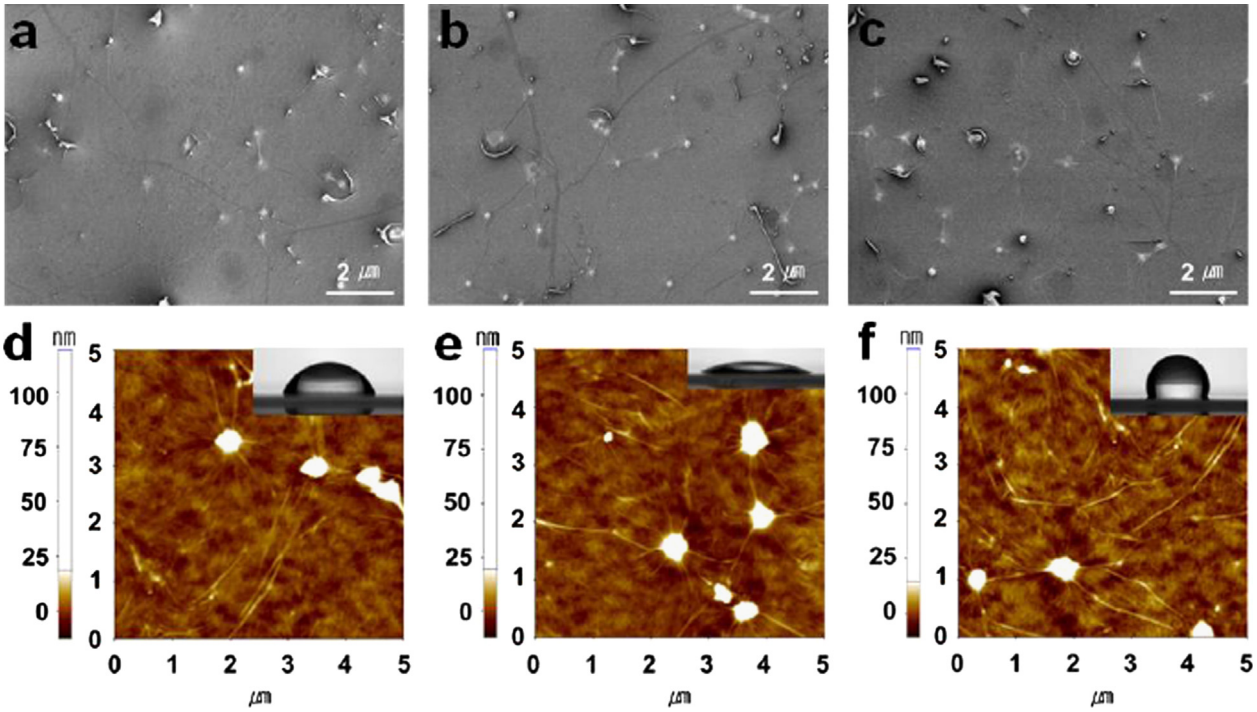

Fig. 1. Surface morphologies of graphene sheets transferred onto a PET substrates, according to imaging by FE-SEM ((a)–(c)) and AFM ((d)–(f)). The data for pristine graphene were shown in (a) and (d), whereas the data for oxygen-treated graphene and fluorine-treated graphene were shown in (b) and (e), and (c) and (f), respectively.

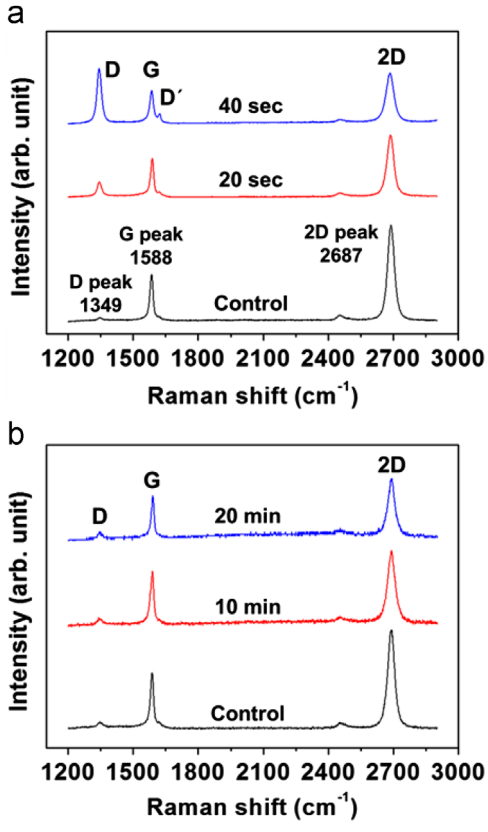

Fig. 2. Raman spectra of a graphene sheet transferred onto a SiO_2/Si substrate. Oxygen-treated graphene (a) and fluorine-treated graphene (b) were shown, respectively. Each peak for oxygen and fluorine-treated time were indicated inset in (a) and (b).

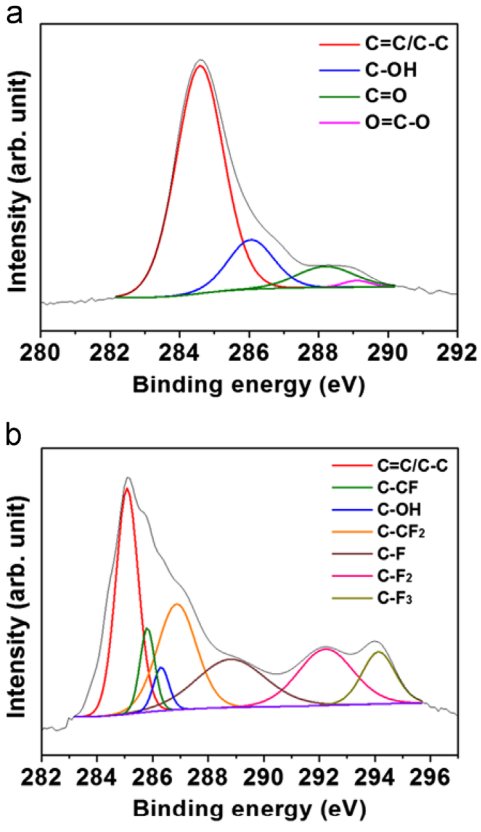

Fig. 3. Carbon (C 1s) spectrum from XPS on functionalized graphene sheets with oxygen (a) or fluorine (b).

at 10 sccm. Graphene sheets were functionalized with oxygen by plasma treatment as above condition at 20 W with O_2 gas pressure fixed at 1×10^{-2} Torr for 20 or 40 s.

The human neuroblastoma cell line SH-SY5Y (KCLB no. 22266, Korea) was incubated in Dulbecco's Modified Eagle's Medium (DMEM) (Welgene, Korea) supplemented with 10% fetal bovine