Biomaterials xxx (2014) 1–9

Contents lists available at ScienceDirect

Biomaterials

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

Cell reprogramming into the pluripotent state using graphene based substrates

Junsang Yoo [a], Jongmin Kim [b], Soonbong Baek [a], Youngsin Park [c], Hyunsik Im [b, *], Jongpil Kim [a, *]

[a] Laboratory of Stem Cells and Cell Reprogramming, Department of Biomedical Engineering, Dongguk University, Seoul 100-715, Republic of Korea
[b] Division of Physics and Semiconductor Science, Dongguk University, Seoul 100-715, Republic of Korea
[c] School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan 689-798, Republic of Korea

ARTICLE INFO

Article history:
Received 26 May 2014
Accepted 30 May 2014
Available online xxx

Keywords:
Graphene
Reprogramming
Induced pluripotent stem cells
Mesenchymal-to-epithelial transition

ABSTRACT

Graphene has been attracting considerable interest in the field of biomedical engineering because graphene and its derivatives are considered to be ideal platforms for supporting cell growth and differentiation. Here we report that graphene promotes the reprogramming of mouse somatic fibroblasts into induced pluripotent stem cells (iPSCs). We constructed a layer of graphene film on a glass substrate and characterized it as a monolayer using Raman spectroscopy. We found that the graphene substrate significantly improved cellular reprogramming efficiency by inducing mesenchymal-to-epithelial-transition (MET) which is known to affect H3K4me3 levels. Thus, our results reveal that a graphene substrate directly regulates dynamic epigenetic changes associated with reprogramming, providing an efficient tool for epigenetic pluripotent reprogramming.

1. Introduction

Graphene is a single atom thick sheet of carbon atoms arranged in two-dimensional (2D) honeycomb structures with unique physical, chemical and mechanical properties [1,2]. The capability of biofunctionalization has highlighted these nanomaterials for a plethora of applications in biotechnology [3–6]. Recent studies have shown that graphene and its derivatives are improved non-cytotoxic materials that enhance the growth and differentiation of mammalian cells [7]. For example, graphene-based substrates promote the cell adhesion and proliferation of neuronal stem cells, osteoblasts, and kidney cells [7–9]. Furthermore, graphene materials have been determined to enhance stem cells for promoting cell fate changes associated with differentiation and developmental processes [10]. These unique characteristics have motivated the use of these materials in cell fate conversion via epigenetic reprogramming.

The generation of embryonic stem cell (ESC)-like cells from somatic cells by ectopic expression of defined factors is an approach commonly known as cell reprogramming [11]. This technology is devoid of ethical concerns, because iPSCs can be produced from an individual to maintain compatibility his/her own immune system. Moreover, because iPSC generation is known to be a multiple-step process mediated by overexpression of the transcription factors, Oct4, Sox2, Klf4, and cMyc to progressively induce the expression of ESC-like genes and suppress somatic cell genetic characteristics, iPSCs offer a unique experimental system to investigate key questions regarding cell fate determination and epigenetic regulation [11]. However, the generation of iPSCs is very inefficient, and cell reprogramming is considered a stochastic process in which successive barriers must be overcome to reach a state of pluripotency [12]. In particular, one of the first noticeable changes during the reprogramming of somatic fibroblasts is their transformation into tightly packed clusters of rounded cells in a process that resembles a mesenchymal-to-epithelial-transition (MET) [13,14]. The importance of epithelialization has been demonstrated by inhibiting this process, which led to the suppression of epigenetic reprogramming [14]. More interestingly, recent studies found that microtopography substrate affects the MET, improving reprogramming efficiency [15].

In this study, we examine whether somatic fibroblasts would be efficiently reprogrammed into the pluripotent state on graphene-based substrate. To address this hypothesis, we prepare the monolayer graphene substrate on the plates and show that graphene substrate can effectively induce reprogramming of somatic cells into iPS cells. Finally, we investigate whether graphene

* Corresponding authors.
E-mail addresses: jk2316@gmail.com, jpkim153@dongguk.edu (J. Kim).

http://dx.doi.org/10.1016/j.biomaterials.2014.05.096
0142-9612/

substrate can induce the epigenetic changes associated with cell fate conversion of reprogramming.

2. Materials and methods

2.1. Preparation of graphene

Large-area graphene films were synthesized on polycrystalline copper foil (99.999%, Alfa Aesar) by using a cold-wall type chemical vapor deposition (CVD) system with a heating stage. Prior to synthesizing the graphene films, the Cu foil was annealed at 1000 °C for 30 min to remove the metal oxide layer on the Cu foil under a hydrogen flow (10 sccm). While maintaining this temperature, C_2H_2 gas was introduced at 20 sccm for 30 min at an operating pressure of 300 mTorr. After synthesis (methane flow) the sample was rapidly cooled to room temperature at a rate of 50 °C/min under the same hydrogen gas flow. The exposed Cu foil was dissolved in a 0.5 M $FeCl_3$ solution (100 ml D.I. water + 8.27 g $FeCl_3$) for several hours. A PMMA layer was placed on the whole floating sample as a marker because monolayer graphene is nearly transparent and can be difficult to locate after the copper substrate is etched away. After the copper was completely removed, the graphene sample was then ready for to be picked up by a target substrate. After cleaning in acetone for 20 min, the top PMMA layer was removed and the graphene sample was then rinsed in iso-propyl alcohol and D.I. water for 20 min in an attempt to remove residual Fe^{3+} ions.

2.2. Cell culture

HEK293 cells were used for packaging the virus. These cells were grown in fibroblast media (high glucose DMEM [Invitrogen], 10% FBS[Hyclone] and 5%penicillin/streptomycin[Invitrogen]). These cells were co-transfected with the lentivirus constructs, psPAX2, pMD2.G and tetO-OSKM/FUW-M2rtTA and OKSM, vectors using calcium phosphate co-precipitation. Cell culture medium was replaced 24 h after transfection and virus was harvested 72 h later. Mouse fibroblasts were transduced (40,000 cells) at passages 2 or 3 in 6-well culture dishes with lentivirus. Infected mouse fibroblasts were cultured in mESC media with Dox (2 ug/ml). To see the mesenchymal-to-epithelial transition (MET) inhibition effect, we treated with 1 mM noggin [Sigma] in 6-well culture dishes with lentivirus.

2.3. Alkaline phosphatase staining

Alkaline phosphatase staining was performed using the Alkaline Phosphatase-Substrate-Kit (Millipore) according to the manufacturer's recommendations. To count AP + colonies, equal numbers of cells were plated on 100 mm dishes coated with gelatin and induced reprogramming for 10 days. The experiments were repeated three times, and data represented the mean of triplicate wells ± SEM.

2.4. Immunofluorescence analysis

iPS cells were cultured on pretreated coverslips and, fixed with 4% PFA. The cells were then stained with primary antibodies against human/mouse Oct4 (Santa Cruz), mouse Nanog (Bethyl Lab), H3K4me3 (Abcam), human/mouse Sox2 (R&D) and mouse SSEA1(DSHB). Respective secondary antibodies were conjugated to Alexa Fluor (Invitrogen). Nuclei were counterstained with 4,6-diamidino-2-phenylindole(DAPI; Invitrogen). Cells were imaged with a Nikon Eclipse Ti Images were processed and analyzed with Adobe Photoshop software.

2.5. Flow cytometry

All flow cytometry was performed on a C6 cytometer (Accuri). Data were analyzed with FlowJo software (TreeStar). Briefly, cells were dissociated with trypsin for 5 min and single cells were then pelleted, resuspended in ice-cold 4% paraformaldehyde, and incubated for 10 min at 4 °C. The cells were washed twice, and resuspended in FACS buffer for FACS analysis.

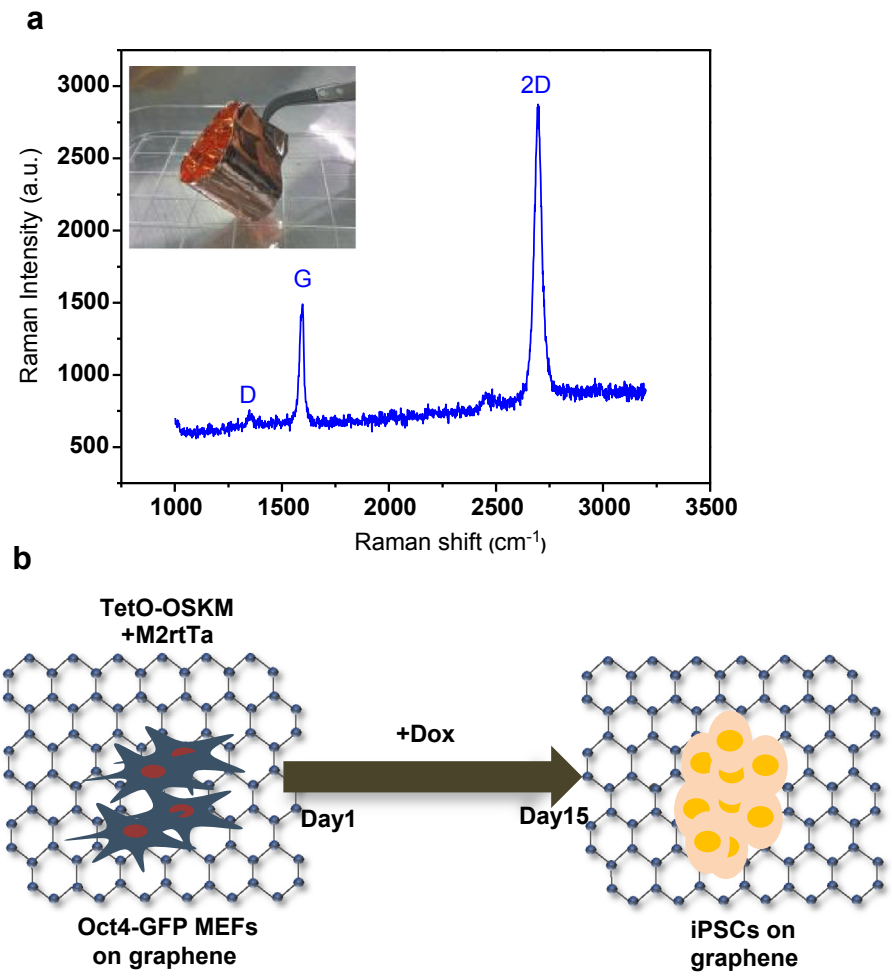

Fig. 1. Characteristics of graphene and experimental design for the efficient generation of iPS cells from mouse somatic cells plated on the graphene-coated substrate. (a) Micro-Raman spectrum of graphene prepared on a 280 nm-thick SiO_2 substrate. The intensity ratio between the 2D and G peaks is two peaks, confirming that the graphene layer is a monolayer. The full width half maximum (FWHM) of the 2D peak is 42 cm, validating the high quality of the graphene layer. The inset shows a photograph of the PMMA/graphene/Cu foil film. (b) Schematic drawing of cell reprogramming on the graphene-based substrate.

Please cite this article in press as: Yoo J, et al., Cell reprogramming into the pluripotent state using graphene based substrates, Biomaterials (2014), http://dx.doi.org/10.1016/j.biomaterials.2014.05.096