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Fabrication of poly(ethylene glycol) hydrogels containing vertically and horizontally aligned graphene using dielectrophoresis: An experimental and modeling study

Samad Ahadian [a, *], Ushio Naito [b], Velappa Jayaraman Surya [c, d, e], Sorour Darvishi [f], Mehdi Estili [g], Xiaobin Liang [h], Ken Nakajima [h, i], Hitoshi Shiku [b], Yoshiyuki Kawazoe [c, d], Tomokazu Matsue [b, i, **]

[a] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto M5S 3G9, Canada
[b] Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[c] New Industry Creation Hatchery Center, Tohoku University, Sendai 980-8579, Japan
[d] Department of Physics and Nanotechnology, SRM University, Kattankulathur, Tamil Nadu 603203, India
[e] SRM Research Institute, SRM University, Kattankulathur, Tamil Nadu 603203, India
[f] Department of Materials Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran
[g] Research Center for Functional Materials (RCFM), National Institute for Materials Science (NIMS), Tsukuba 305-0047, Japan
[h] School of Materials and Chemical Technology, Tokyo Institute of Technology, Tokyo 152-8552, Japan
[i] Advanced Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan

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ABSTRACT

We incorporated bovine serum albumin (BSA)-functionalized graphene in poly(ethylene glycol) diacrylate (PEG) hydrogels. The graphene alignment in PEG hydrogels was changed using dielectrophoresis (DEP) forces. Effect of graphene concentration (2.55 and 5.1 mg/mL) and alignment (vertical and horizontal) on electrical and mechanical properties of hybrid graphene-PEG gels was evaluated. In addition, the molecular interaction of BSA peptides with graphene in the presence and absence of an electric field was assessed. The incorporation of graphene in PEG improved mechanical properties of hybrid gels. Dielectrophoretically aligned graphene-PEG hydrogels showed an anisotropic electrical conductivity. Young's modulus of 5.1 mg/mL graphene-PEG was almost three times higher than that of pristine PEG. Viability and proliferation of mouse embryonic stem cells on graphene-PEG gels were comparable with control PEG hydrogels. *Ab initio* calculations showed longer peptides had higher binding affinity towards graphene and caused a change in dipole moment due to the presence of electric field. The electric field also expanded graphene and peptide chains, which confirms the graphene alignment using DEP forces. Hybrid aligned graphene-PEG hydrogels may serve as functional biomaterials to engineer biological tissues and fabricate biomedical devices.

1. Introduction

Stem cells constantly experience numerous biochemical and biophysical signals from their microenvironment in the body [1]. Such signals affect stem cell behavior and fate [2,3]. Scaffolds have been extensively used to provide biological cues and structural support for stem cells [4]. While several biomaterials have been investigated as scaffold for stem cell culture, hydrogels have been always an attractive candidate to do that [5]. In general, hydrogels have been used as scaffold in tissue engineering [6], as biological assays or sensors [7,8], and in contact lenses [9]. In general, hydrogels are hydrophilic, biocompatible, and non-cytotoxic materials that somehow resemble the physiological environment of cells. In particular, UV-crosslinkable poly(ethylene glycol) diacrylate (PEG) hydrogels have been intensely investigated for stem cell culture and tissue engineering applications due to their tunable

* Corresponding author.
** Corresponding author. Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan.
E-mail addresses: samad.ahadian@utoronto.ca (S. Ahadian), matsue@bioinfo.che.tohoku.ac.jp (T. Matsue).

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biological properties and facile and controllable synthesis and processing [10–12]. PEG degrades easily in biological environments. Moreover, the cell affinity of PEG can be enhanced by incorporating cell-adhesive moieties (*e.g.*, arginine-glycine-aspartic acid) in PEG [13,14] or co-crosslinking with extracellular matrix-derived components [15,16]. However, PEG hydrogel is not electrically conductive and mechanically strong for successful engineering electro-active tissues and making biomedical devices.

Carbon-based nanomaterials (*e.g.*, carbon nanotubes (CNTs) and graphene) have attracted much attention in biomedicine owing to their unique properties, such as high electrical conductivity, mechanical strength, and unique optical characteristics [17]. Recent reports have shown the use of carbon-based nanomaterials as scaffolds in tissue engineering, substrates for stem cell differentiation, and biomedical implants. For instance, Ryoo et al. reported that fibroblast cells proliferated well on thin films of graphene and CNTs [18]. We used gelatin methacryloyl (GelMA)-aligned CNT hydrogels to fabricate functional C2C12 muscle myofibers. Myofibers cultivated on these materials demonstrated high maturation due to high electrical conductivity of hybrid hydrogels [19]. In another study, we cultured mouse embryoid bodies (EBs) in microwells of GelMA-aligned CNT materials [6]. These microwells provided a controllable platform for electrically induced differentiation and stimulation of stem cells.

Graphene is a two-dimensional (2D) nanomaterial with exceptional electrical, mechanical, and optical characteristics [20], which has been utilized in various biomedical applications, such as biosensing [21], cancer therapy [22], gene and drug delivery [23], and tissue regeneration [24,25]. For instance, Nayak et al. reported that graphene-coating substrates remarkably accelerated differentiation of human mesenchymal stem cells (MSCs) [26]. Li et al. used three-dimensional (3D) graphene foams to support neural stem cell growth [27]. We reported that graphene enhanced mechanical properties and electrical conductivity of mouse EBs and induced cardiac differentiation of EBs [28].

In our earlier work, the exfoliation of graphite into graphene sheets in bovine serum albumin (BSA) medium was reported [29]. BSA molecules were physically adsorbed on graphene and made a stable dispersion of graphene in water. The proposed approach to make aqueous graphene dispersion is simple, green, and scalable to fabricate high quality graphene for biomedical applications. In this paper, we used dielectrophoresis (DEP) technique to vertically and horizontally align BSA-functionalized graphene (simply *graphene* afterwards) in PEG hydrogels. Anisotropic electrical conductivity and mechanical characteristics of hybrid PEG hydrogels containing vertically and horizontally aligned graphene were measured and compared with those of pristine PEG and randomly dispersed graphene-PEG gels. Viability and proliferation of mouse embryonic stem cells on graphene-PEG hydrogels were assessed. Moreover, *ab initio* calculations were employed to reveal molecular interactions of BSA with graphene and effect of electric field on BSA-graphene.

2. Materials and methods

2.1. Materials

Following materials were used: hexamethyldisilazane (Tokyo Ohka Kogyo Co., Ltd., Kanagawa, Japan); developer (MF CD-26) and positive g-line photoresist (S1818) (Shipley Far East Ltd., Tokyo, Japan); graphite (200 mesh, 99.9995% purity) (Alfa Aesar, USA), PEG with average molecular weight of 1000 (Sigma–Aldrich, USA); 2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone (Irgacure 2959; Ciba Chemicals, Japan); indium tin oxide (ITO) glass slide (Hiraoka Special Glass, Japan); BSA (Sigma–Aldrich, USA).

2.2. Chemical preparation of graphene-PEG

Aqueous dispersion of graphene was synthesized as reported previously [29]. In summary, BSA (100 mg) was mixed with DI water (1800 mL) at ~50 °C for ~12 h. The pH of aqueous BSA solution was adjusted to ~3.6 with HCl. Then, 1 g of graphite was mixed with 200 mL of aqueous BSA solution. The mixture was sonicated for 3 h while being mixed with a magnetic stirrer. After removing large graphene aggregates and graphite particles from the sediment, the mixture was centrifuged at 3000 rpm for 30 min. Irgacure 2959 (previously dissolved in warm DI) water and PEG were added to the graphene suspension. The solution was ultrasonicated for 30 min. Final concentrations of PEG and Irgacure 2959 were 10% (v/v) and 0.5% (v/v), respectively. The graphene concentration was adjusted to 2.55 and 5.1 mg/mL for further characterization. 20 μL of mixed solution was pipetted in DEP chamber. The entire assembly was exposed to 7 mW/cm^2 UV light (Hayashi UL-410UV-1; Hayashi Electronic Shenzen Co., Ltd., Japan) for 150 s to polymerize PEG hydrogel.

2.3. Design and fabrication of IDA-Pt electrodes

Effective electrode dimensions were 8 by 12 mm^2. The band electrodes were 15 μm in width and the length between two neighboring band electrodes was 15 μm. Band electrodes were fabricated on a glass slide (thickness, 1 mm; Matsunami Glass IND. LTD., Japan) by conventional photolithography technique. In summary, hexamethyldisilazane and S1818 were poured on a glass slide following by baking at 90 °C for 10 min. The glass slide was then irradiated with UV light through a mask aligner (MA-20; Mikasa Co. Ltd., Japan) and developed with the MF CD-26. A Ti adhesive layer and then a Pt film (thickness, 100 nm) were then deposited on the glass slide. The electrode design was revealed using a lift-off technique. The glass slide was then immersed in acetone solution and ultrasonicated at room temperature for 15 min to clean up the glass surface and further reveal the microelectrodes.

2.4. Dielectrophoretic alignment of graphene in PEG gels

IDA-Pt electrodes were treated with plasma oxygen and then with 3-(trimethoxysilyl)propyl methacrylate under vacuum for 1 h to improve the adhesion between the electrodes and PEG hydrogels. 62 or 15 μm spacers made of polyethylene terephthalate (Lintec Co., Japan) were used to create a DEP chamber between a normal glass slide (or ITO glass slide) and IDA-Pt electrodes. 20 μL of graphene-hydrogel mixture was pipetted in the chamber. To vertically align the graphene, a sinusoidal AC signal (frequency 1 MHz and voltage 20 V) was applied to the IDA-Pt electrodes, and another independent AC signal with an opposite phase was applied to the top ITO glass slide to create a nonuniform electric field. The AC signals were applied using a waveform generator (WF 1946B Multifunction Synthesizer; NF Co., Japan). To obtain horizontally aligned graphene in PEG hydrogels, a sinusoidal AC signal (frequency 1 MHz and voltage 20 V) was applied to one electrode band of IDA-Pt electrodes, and another independent AC signal with an opposite phase was applied to other electrode band of IDA-Pt electrodes. After 1 min of applying the electric field, the PEG prepolymer was polymerized with 7 mW/cm^2 UV light for 150 s. After the polymerization, hybrid hydrogels were detached from the DEP device and used in further experiments.

2.5. Cell culture

Mouse stem cells (DS Pharma Biomedical, Japan) at a passage number <30 were used in this work. The cells were kept in