Acta Biomaterialia 31 (2016) 134–143

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Hybrid hydrogel-aligned carbon nanotube scaffolds to enhance cardiac differentiation of embryoid bodies

Samad Ahadian [a], Shukuyo Yamada [b], Javier Ramón-Azcón [c,d], Mehdi Estili [e], Xiaobin Liang [a], Ken Nakajima [a], Hitoshi Shiku [b], Ali Khademhosseini [a,f,g,h,i,j,*], Tomokazu Matsue [a,b,*]

[a] WPI-Advanced Institute for Materials Research, Tohoku University, Sendai 980-8577, Japan
[b] Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[c] Nanobiotechnology for Diagnostics (Nb4D) Group, IQAC-CSIC, Jordi Girona 18-26, Barcelona 08034, Spain
[d] CIBER de Bioingenieria, Biomateriales y Nanomedicina (CIBER-BBN), Jordi Girona 18-26, Barcelona 08034, Spain
[e] Advanced Ceramics Group, Materials Processing Unit, National Institute for Materials Science (NIMS), Tsukuba 305-0047, Japan
[f] Department of Medicine, Center for Biomedical Engineering, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA
[g] Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[h] Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA
[i] College of Animal Bioscience and Technology, Department of Bioindustrial Technologies, Konkuk University, Hwayang-dong, Kwangjin-gu, Seoul 143-701, Republic of Korea
[j] Department of Physics, Faculty of Science, King Abdulaziz University, Jeddah 21569, Saudi Arabia

ARTICLE INFO

Article history:
Received 14 July 2015
Received in revised form 20 November 2015
Accepted 23 November 2015
Available online 24 November 2015

Keywords:
Carbon nanotubes
Cardiac differentiation
Electrical stimulation
Embryoid body
Hydrogel

ABSTRACT

Carbon nanotubes (CNTs) were aligned in gelatin methacryloyl (GelMA) hydrogels using dielectrophoresis approach. Mouse embryoid bodies (EBs) were cultured in the microwells fabricated on the aligned CNT-hydrogel scaffolds. The GelMA-dielectrophoretically aligned CNT hydrogels enhanced the cardiac differentiation of the EBs compared with the pure GelMA and GelMA-random CNT hydrogels. This result was confirmed by Troponin-T immunostaining, the expression of cardiac genes (i.e., Tnnt2, Nkx2-5, and Actc1), and beating analysis of the EBs. The effect on EB properties was significantly enhanced by applying an electrical pulse stimulation (frequency, 1 Hz; voltage, 3 V; duration, 10 ms) to the EBs for two continuous days. Taken together, the fabricated hybrid hydrogel-aligned CNT scaffolds with tunable mechanical and electrical characteristics offer an efficient and controllable platform for electrically induced differentiation and stimulation of stem cells for potential tissue regeneration and cell therapy applications.

Statement of significance

Dielectrophoresis approach was used to rapidly align carbon nanotubes (CNTs) in gelatin methacryloyl (GelMA) hydrogels resulting in hybrid GelMA-CNT hydrogels with tunable and anisotropic electrical and mechanical properties. The GelMA-aligned CNT hydrogels may be used to apply accurate and controllable electrical pulses to cell and tissue constructs and thereby regulating their behavior and function. In this work, it was demonstrated that the GelMA hydrogels containing the aligned CNTs had superior performance in cardiac differentiation of stem cells upon applying electrical stimulation in contrast with control gels. Due to broad use of electrical stimulation in tissue engineering and stem cell differentiation, it is envisioned that the GelMA-aligned CNT hydrogels would find wide applications in tissue regeneration and stem cell therapy.

1. Introduction

The capability of stem cells to undergo unlimited self-renewal and differentiate into multiple cell types has prompted their widespread application in tissue engineering (TE) and cell therapy fields [1]. The stem cell microenvironment has a major effect on stem cell

* Corresponding authors at: Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA (A. Khademhosseini). Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan (T. Matsue).
E-mail addresses: alik@rics.bwh.harvard.edu (A. Khademhosseini), matsue@bioinfo.che.tohoku.ac.jp (T. Matsue).

http://dx.doi.org/10.1016/j.actbio.2015.11.047
1742-7061/

renewal and differentiation. This microenvironment, termed the niche, was first described by Schofield [2]. The stem cell niche is composed of extracellular matrix (ECM) components, soluble factors, and supportive cells, which provide spatiotemporal signals to stem cells to direct their fate. Creating biomimetic stem cell niches *in vitro* is crucial for controlling stem cell behavior in therapeutic applications [3]. Therefore, the design and fabrication of biomaterials and tools to mimic various aspects of the stem cell niche to control stem cell behavior are an interesting area of research. In particular, the precise control of stem cell differentiation and fate needs to be further achieved [4].

Carbon nanotubes (CNTs) have recently attracted significant attention in biological applications ranging from bioimaging [5], drug delivery [6], biosensing [7], cancer therapy [8], and TE scaffolding [9]. This recent interest in CNTs arises from their outstanding chemical, mechanical, electrical, and optical properties [10]. Incorporating CNTs into TE scaffolds leads to enhanced scaffold flexibility, strength, and electrical conductivity. For example, adding CNTs to gelatin methacryloyl (GelMA) hydrogel scaffolds increases their Young's modulus and electrical conductivity without significantly affecting gel porosity [11]. GelMA is a biocompatible, biodegradable, and photocrosslinkable hydrogel that is suitable for culturing different cell types and fabricating various tissues [12]. However, the relatively poor electrical conductance of this gel limits its application in the regulation of electro-active cell behaviors and electrical stimulation (ES) of cell and tissue constructs [13]. Here, hybrid GelMA-CNT hydrogels with tunable electrical and mechanical characteristics were used as scaffolds to culture and electrically regulate the cardiac differentiation of mouse embryoid bodies (EBs).

Micro- and nanoscale technologies have widely been used in biomedicine [14]. They can be used to precisely fabricate biomaterials or cellular structures that mimic the complex architecture of native biological constructs. Dielectrophoresis (DEP) is one such useful and versatile technology. DEP is based on particle polarization and manipulation in a medium by applying a non-uniform electric field [15]. For example, we recently reported the use of DEP for the rapid formation of three-dimensional (3D) EBs in GelMA hydrogel [16]. It was possible to fabricate 3D EBs of varying sizes and shapes using a high-throughput approach. The DEP method was also employed to align CNTs in GelMA gels [17,18]. The hybrid GelMA-aligned CNT scaffolds showed better performance in the generation of functional and contractile skeletal muscle myofibers in contrast with pure GelMA and GelMA-random CNT scaffolds.

Here, DEP was utilized to fabricate hybrid GelMA-aligned CNT gels. The mechanical and electrical properties of these gels were measured and compared against pure GelMA and GelMA-randomly dispersed CNT hydrogels as the control samples. We then used the GelMA-aligned CNT hydrogels to support the cardiac differentiation of EBs in response to ES. The efficiency of the GelMA hydrogel containing the aligned CNTs and control hydrogels (i.e. pure GelMA and GelMA-randomly dispersed CNT gels) in supporting the cardiac differentiation of EBs was determined by using gene and protein expression analyses and beating activity of the differentiated cells.

2. Materials and methods

2.1. Materials

The following materials were used: developer (MF CD-26; Shipley Far East, Japan); photoresist (S1818; Rohm and Haas, USA); SU-8 3050 and SU-8 developers (MicroChem, USA); methacrylic anhydride, gelatin type A from porcine skin, trichloro (1H,2H,2H-perfluorooctyl)silane, 3-(trimethoxysilyl)propyl methacrylate (TMSPMA), and penicillin/streptomycin (P/S) (Sigma–Aldrich Chemical, USA); multi-walled CNTs (Hodogaya Chemical, Japan); 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propa none (Irgacure 2959; Ciba Chemicals, Japan); fetal bovine serum (FBS; Bioserum, Japan); and indium tin oxide (ITO) glass slides (Hiraoka Special Glass, Japan).

2.2. Fabrication of interdigitated array of ITO (IDA-ITO) electrodes and SU-8 microstamp

The IDA-ITO electrodes and the SU-8 microstamp were made on a glass slide (Matsunami, Japan) using photolithography and chemical etching techniques [13]. The glass slides were cleaned using plasma oxygen prior to the photolithography. To fabricate the IDA-ITO electrodes, S1818 and MF CD-26 were used as the positive photoresist and developer, respectively. SU-8 3050 (photoresist) and SU-8 (developer) were used to fabricate the SU-8 microstamp. The etchant solution was a mixture of HCl, HNO_3, and H_2O in a volume ratio of 4:1:5. The etching was performed for 120 min under stirring. The photoresist was removed with acetone.

2.3. Chemical functionalization of the CNTs

A controlled acid treatment process was used to functionalize the multi-walled CNTs. In brief, the CNTs were refluxed in 68 wt % HNO_3 and 98 wt% H_2SO_4 (volume ratio 1:3) at 110 °C for 20 min. After washing with pure water on a 1.2 μm membrane, an aqueous dispersion of the CNTs was prepared using probe sonication. As shown in our previous study [18], the zeta potential of the CNTs was ~−40 mV at a pH of ~4.1. In addition, the CNTs had a high purity, which was confirmed by Raman spectroscopy and microscopy.

2.4. Synthesis of the GelMA prepolymer

Gelatin (6 g) and methacrylic anhydride (12 mL) were dissolved in Dulbecco's phosphate-buffered saline (DPBS) (60 mL) at 50 °C for 1 h. The degree of gelatin modification was ~80%. The mixture was dialyzed against pure water using a 12–14 kDa dialysis membrane at 40 °C for 1 week. The mixture was then lyophilized for 1 week. Photoinitiator (1% (w/v); Irgacure 2959) was added to the 10% (w/v) GelMA prepolymer in pure water at 60 °C to obtain the GelMA prepolymer solution.

2.5. Dielectrophoretic alignment of the CNTs in GelMA gels

The interdigitated electrodes were subjected to plasma oxygen treatment followed by methacrylation using TMSPMA under vacuum for 2 h to obtain good adhesion between the electrodes and the GelMA hydrogels. The SU-8 microstamp was treated using tri chloro(1H,2H,2H-perfluorooctyl)silane to avoid attachment to the GelMA hydrogel. A chamber was created for the DEP experiments by mounting the SU-8 microstamp on the electrodes. The thickness of the chamber was 200 μm (Fig. 1B). The 10% (w/v) GelMA prepolymer solution was mixed with the CNT aqueous solution at a ratio of 1:1 to obtain a final GelMA concentration of 5% (w/v). The GelMA-CNT prepolymer was sonicated for 15 min to obtain a homogeneous mixture. The GelMA-CNT prepolymer was then injected into the chamber. A function generator (Hioki 7075, Hioki, Japan) applied an electric field (frequency, 1 MHz; voltage, 20 V) to horizontally align the CNTs in the GelMA prepolymer. The GelMA gels containing the aligned CNTs were then crosslinked with UV (Hayashi UL-410UV-1, Hayashi Electronic Shenzen, Japan) for 150 s. After crosslinking, the SU-8 microstamp was removed from