Manipulation of Human Mesenchymal Stem Cells by Multifunctional Graphene-PEDOT Microelectrode Arrays

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ABSTRACT

In this study, we report the development of all-solution-processed multifunctional organic bioelectronics, comprising reduced graphene oxide (rGO) and dexamethasone 21-phosphate disodium salt (DEX) drug loaded poly(3,4-ethylenedioxythiophene) (PEDOT) microelectrode arrays on indium tin oxide glass, that can be used to manipulate human mesenchymal stem cell (hMSC) spreading morphology on rGO micropattern arrays and trigger the drug releasing from the PEDOT micropatterns. In our devices, the rGO material were used as the adhesive coating to attract the adhesion of hMSC cells whereas PLL-g-PEG coated PEDOT electrodes served as the anti-adhesive coating where no hMSC cells can attach. In addition, PEDOT microelectrodes also work as drug releasing components where spatial-temporal control of DEX releasing from PEDOT matrix can be achieved for long-term cell culture for inducing the osteogenetic differentiation of hMSCs via cyclic potential stimulation (CPS).

KEYWORDS

Organic bioelectronics, graphene, poly(3,4-ethylenedioxythiophene) (PEDOT), human mesenchymal stem cell (hMSC), drug releasing.

1. INTRODUCTION

Electroactive biointerfaces, potentially bridging the communication gap between the biology and electronics, which have been widely operated to enhance the readout performance of the biological events in the form of the electronic signals and/or promote the gene expression and biological phenotypes through the electrical stimulation. In the development of electroactive biointerfaces, most studies have shown that conducting polymers (CPs) [poly(3,4-ethylenedioxythiophene) (PEDOT), etc.] [1, 2] and carbon materials (CMs) (graphene, etc.) [3, 4] feature high biocompatible as the tissue–electrode or cell–electrode interfaces for developmental biology and cell therapies. Several organic bioelectronic devices built with PEDOT and graphene materials are capable of manipulating the behavior of cells on the devices. Hence, this study describes a multifunctional electroactive biointerfacing platform to extend the individual advantage of CMs and CPs for promoting the osteogenetic differentiation rate and osteogenetic drug releasing performance, respectively. Moreover, this cell-based electronic device is to be able to manipulate the cell morphology in different growth phases of the culture, including the seeding location, proliferation, and differentiation. The image of our device and schematic are shown in figure 1 where the hMSC cells are found to adhere on the graphene micropatterns. This result confirms that our approach of using adhesive and anti-adhesive coating can effectively confine the location of hMSC cells on chip, which is also investigated as an electrically responsive drug releasing platform for inducing hMSCs to osteoblast-like cells.

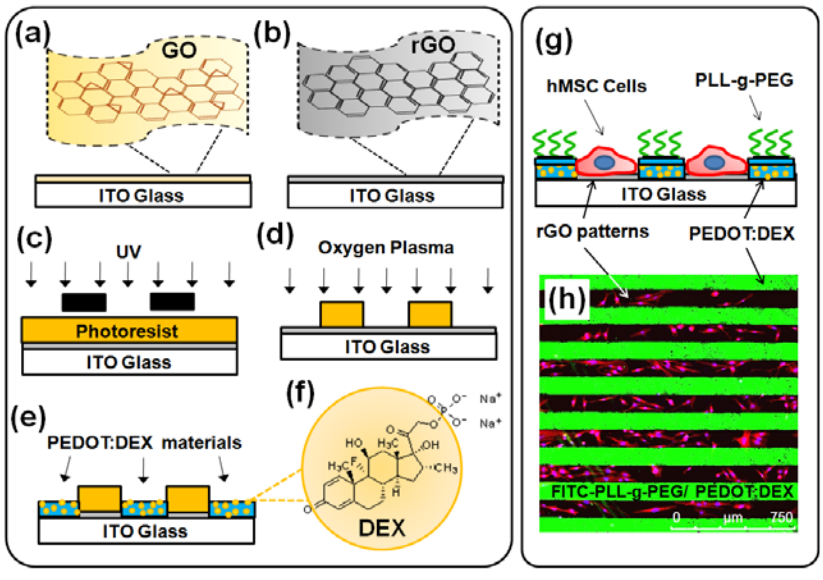

Figure 1. (a-e) Fabrication processes of rGO-PEDOT microelectrode array devices loaded with dexamethasone 21-phosphate disodium salt (DEX) drug. (f) Chemical structure of DEX. (g) Schematic illustration of hMSC cells adhered on rGO-PEDOT microelectrode arrays. (h) Merged image of hMSC cells grown on the rGO-PEDOT

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microelectrode arrays with F-actin (red) for cytoskeleton, DAPI (blue) for nucleus and FITC (green) for PLL-g-PEG.

2. EXPERIMENTAL

Graphene oxide (GO) was synthesized based on the Hummers method [5]. The thin GO layer was spin-coated on the indium tin oxide (ITO) glass, which was chemical reduced in hydrazine vapor at 60 ^{o}C for 6 hr to obtain the large-scale reduced GO (rGO) layers on ITO glass. A standard photolithography process, with positive resist (S-1813, Shipley) and development (MF-319, Shipley), was used to fabricate S-1813 micro-array patterns of three different sizes (flat; 100 and 20 μm) on the rGO coated ITO glass and subjected to oxygen plasma treatment to remove the opening area of rGO from the ITO surface. Thin layers of PEDOT were then deposited electrochemically on ITO—from a bath solution containing 0.01 M 3,4-ethylene dioxythiophene (EDOT) (Sigma–Aldrich) and 0.01 M Dexamethasone 21-phosphate disodium salt (DEX; Sigma–Aldrich)—using the three-electrode system of an Autolab PGSTAT-12 (EcoChemie, Utrecht, Netherlands) electrochemical analyzer. A constant potential of 1 V (vs. Ag/Ag^{+}) was used to produce PEDOT layers. The S-1813 photoresist was removed by washing with acetone, leaving the surfaces of the ITO glasses covered with rGO-PEDOT microelectrode arrays (rGO-PEDOT) [Figure 2(a-b)]. The PLL-g-PEG was used to modify the PEDOT surface of rGO-PEDOT [Figure 2(c)]. For drug releasing studies, the cyclic potential stimulation (CPS) was done with the sweeping voltage from -1V to +1V at a scan rate of 100 mV/s for nth cycles in PBS (1x).

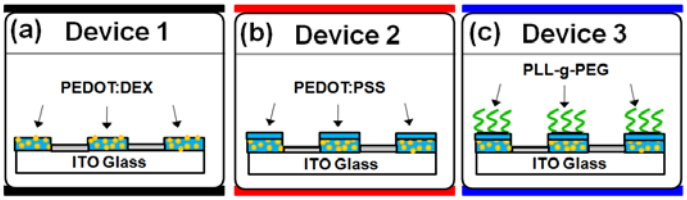

Figure 2. rGO-PEDOT microelectrode array devices loaded with dexamethasone 21-phosphate disodium salt (DEX) drug. (a) rGO-PEDOT:DEX microelectrode array device (**Device 1**). (b) Device 1 covered with PEDOT:PSS (**Device 2**). (c) Device 2 coated with PLL-g-PEG (**Device 3**)

3. RESULTS AND DISCUSSION

To spatially control the location of hMSCs on the devices, the concepts of contact attraction and contact repulsion were introduced in this study. Contact attraction coating with rGO is used to promote hMSC adhesion while contact repulsion coating with poly(L-lysine-graft-ethylene glycol) (PLL-g-PEG) prevents the adsorption of proteins, resulting in a cell-free region [Figure 3(a, d, g)]. The PEG rich surface of PLL-g-PEG coated PEDOT:PSS served as a contact repulsion coating, preventing cell seeding in the first growth phase. Moreover, upon CPS for triggering DEX drug release, the PLL-g-PEG coating on the PEDOT:PSS was not removed, keeping the surface property of contact repulsive efficiency.

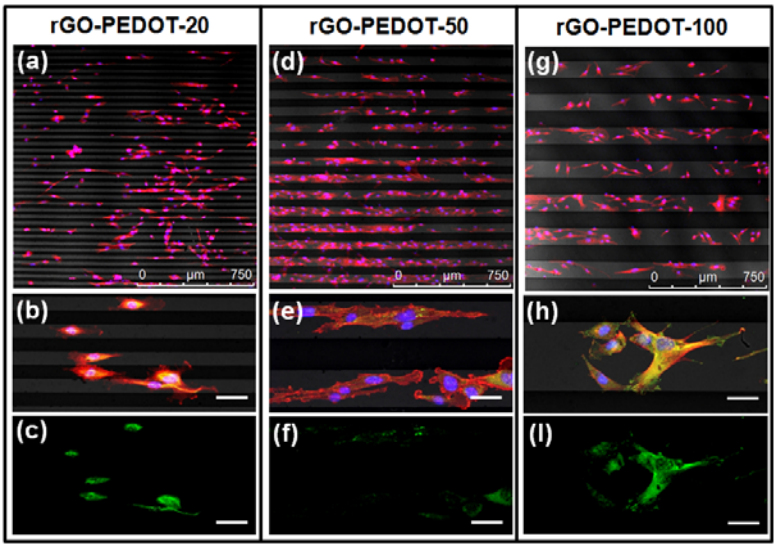

Figure 3. (a, d and g) Merged image of hMSC cells grown on different size (20, 50 and 100 μm) of the rGO-PEDOT microelectrode arrays with F-actin (red) for cytoskeleton, DAPI (blue). (b, e and h) Analysis of hMSCs adhesion on on different size (20, 50 and 100 μm) of the rGO-PEDOT microelectrode arrays after the CPS with F-actin (red) for cytoskeleton, DAPI (blue) and vinculin (green). (c, f, i) hMSCs were stained for vinculin (green) as marker for adhesion.

The drug releasing mechanism of our devices is based on the swelling and deswelling process of PEDOT polymers upon electrical stimulation. During the CPS, the PEDOT-DEX film undergoes a fast swelling and deswelling process caused by ion and water movement in and out of the film, which may act as a pump to push the anions out and dramatically speed up the drug release (seconds or minutes). The architectures of the drug releasing

devices are shown in figure 2. The DEX release profiles from various rGO-PEDOT devices (**Device 1**; **Device 2**; **Device 3**) in PBS (1x) solution were measured by UV spectrometry at 242 nm as shown in figure 4(a). As expected, without the poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) blocking layer on the PEDOT:DEX surfaces (**Device 1**), a significant release of DEX from **rGO-PEDOT** microelectrode arrays was detected without electrical stimulation due to diffusion. However, when a layer of PEDOT:PSS was used, DEX could be sealed very well in **Device 2** and **Device 3** without electrical stimulation. Upon electrical stimulation, the DEX was released linearly with time in **Device 2** and **Device 3.** Figure 4(b) illustrates the long-term DEX release profile of **Device 3** in PBS solution where almost no DEX was released without electrical stimulation. Upon electrical stimulation, μg of DEX can be released in our device after staying in incubator for six days. To apply this electrically response drug release system for investigating the osteogenetic differentiation of hMSCs, we further optimize the DEX loaded rGO-PEDOT micropattern arrays into three active areas, which can support the drug release for three times of osteogenetic induction periods (CPS1; CPS2; CPS3).

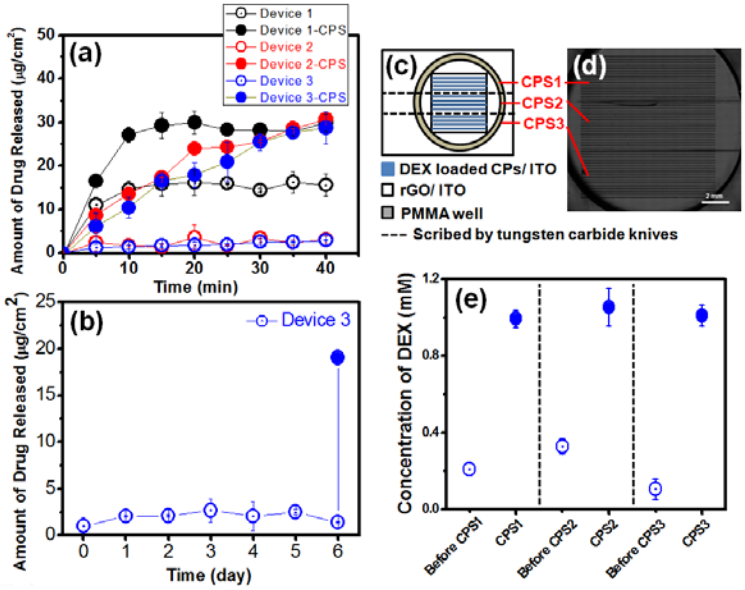

Figure 4. (a) DEX release profile of the rGO-PEDOT microelectrode array devices. The release of DEX (n=5) was detected using UV-vis spectroscopy at 242 nm in PBS (1x) w/ and w/o CPS. (b) Long-term stability and DEX release by CPS in the incubator at 37 ^{o}C. (c-d) Schematic representation and photograph of rGO-PEDOT microelectrode array devices for three times DEX releasing. (e) Individual control of DEX concentration in PBS (1x) by three times of drug release. Before the CPS, PBS (1x) would be replaced with fresh one and measured the DEX concentration (after 40 min of changing each PBS).

4. CONCLUSIONS

We have proposed a multifunctional rGO-PEDOT device for manipulating hMSCs spreading morphology and performed the long-term drug releasing model for inducing the osteoblast-like cell differentiation of hMSCs in the present of osteogenetic inducer, DEX. Since the DEX loaded rGO-PEDOT device could support the replacement with fresh DEX releasing every 3 days for 9 days, our devices may be used to manipulate the differentiation of hMSC with spatial-temporal control of DEX releasing by electrical stimulation.

ACKNOWLEDGEMENT

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