Ecotoxicology and Environmental Safety 160 (2018) 273–281

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Directly repurposing waste optical discs with prefabricated nanogrooves as a platform for investigation of cell-substrate interactions and guiding neuronal growth

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ARTICLE INFO

Keywords:
Electronic waste
Nanopatterns
Neuronal guidance
Neuronal tissue engineering
PC12 cells

ABSTRACT

Due to rapid change in information technology, many consumer electronics become electronic waste which is the fastest-growing pollution problems worldwide. In fact, many discarded electronics with prefabricated micro/nanostructures may provide a good basis to fulfill special needs of other fields, such as tissue engineering, biosensors, and energy. Herein, to take waste optical discs as an example, we demonstrate that discarded electronics can be directly repurposed as highly anisotropic platforms for in vitro investigation of cell behaviors, such as cell adhesion, cell alignment, and cell-cell interactions. The PC12 cells cultured on biocompatible DVD polycarbonate layers with flat and grooved morphology show a distinct cell morphology, indicating the topographical cue of nanogrooves plays a key role in guidance of neurites growth. By further monitoring cell morphology and alignment of PC12 cells cultured on the DVD nanogrooves at different differentiation times, we find that cell contact interaction with nanotopographies is dynamically adjustable with differentiation time from initial disorder to final order. This study adds a new dimension to not only solving the problems of supply of materials and fabrication of nanopatterns in neural tissue engineering, but may also offering a new promising way of waste minimization or reuse for environmental protection.

1. Introduction

Nowadays, a wide range of consumer electronics (such as storage, display, and smart devices) are constantly and quickly being upgraded and changed, and advance toward higher density (more micro/nanostructures per unit area) and miniaturization (Sasajima et al., 2017). However, rapid changes in technology and falling prices have led to a fast-growing surplus of electronic waste (EW) around the globe, which cause adverse effects on human health and the environment (Robinson, 2009). Among EW, optical discs (CDs and DVDs) store digitally encoded data, video and audio information in pits — spiral micro/nano-grooves that run from the center of the disc to its edges, and have contributed significantly to the total EW (0.1 million tons optical media per year worldwide, excluding their packaging) (Rajarao et al., 2014). The current recycling processes are not efficient and have many drawbacks, including the use of toxic chemicals, exceptionally high temperatures and energy, although some interesting studies have tried to recycle waste optical discs into useful materials, such as activated carbons (Choma et al., 2015), silicon carbide nanoparticles (Rajarao et al., 2014), and nano-fibers (Zander et al., 2015). In fact, besides optical discs, numerous discarded consumer electronics (like light-guide plates in liquid-crystal display systems and finely-patterned chips) with prefabricated particular micro/nanopatterns may provide a good basis to fulfill the special needs of some fields, such as tissue engineering, biomedicine, biosensors, and energy (Birkholz et al., 2016; Kang et al., 2015; Pan and Wang, 2010).

In neural tissue engineering, substantial attention has been devoted recently to develop new materials and technologies for providing appropriate physical (i.e., micro/nanostructures) and chemical cues to direct the neurite outgrowth toward a targeted function (Green and Abidian, 2015; Hoffman-Kim et al., 2010), which is a very promising approach for the treatment of nerve injury caused by accidents or diseases (Huang et al., 2015). An ideal material onto which neuronal cells attach, proliferate, migrate, and differentiate plays a key role in neural

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https://doi.org/10.1016/j.ecoenv.2018.05.067
Received 23 February 2018; Received in revised form 8 May 2018; Accepted 24 May 2018
0147-6513/

tissue engineering. Various natural-based and synthetic materials have been exploited for the scaffold to control cell behaviors (Li et al., 2017), such as peptides (Berns et al., 2016, 2014; Manchineella et al., 2016), protein (Ham et al., 2017; Li et al., 2016; Wylie et al., 2011), chiral materials (Gao et al., 2017a; Sun et al., 2017; Zhao et al., 2017), nanomaterials (Bodelón et al., 2017; Defteralı et al., 2016; Shin et al., 2016; Yang et al., 2016). Unfortunately, there are several disadvantages of natural materials (including complex structures, environmental sensitivity, and difficulty in purifying) (Ham et al., 2017; Li et al., 2016; Wylie et al., 2011) and for most existing artificial materials (including time-consuming synthesis (Hackett et al., 2017; Recknor et al., 2006; Yeh et al., 2017; Zou et al., 2016), harsh reaction conditions (Yeh et al., 2017) or hazardous reagents (Defteralı et al., 2016; Fabbro et al., 2013; Yang et al., 2016)), which greatly impede their applications and popularization.

Moreover, most of the published studies examined have emphasized the use of artificial material surface topography (i.e., different surface features such as grooves, ridges, and pillars) to mimic native topographical cues of extracellular matrix for effectively promoting of the neurite outgrowth and guiding its direction (Flemming et al., 1999; Hoffman-Kim et al., 2010). Current technologies used to fabricate micro/nanostructured substrates with anisotropic geometries and properties for cell culture (Lu et al., 2018; Tawfick et al., 2012) include photolithography (Marino et al., 2013), soft lithography, nanoimprinting, and so on (Zou et al., 2016). Regrettably, these techniques have some disadvantages, such as the high cost of manufacture, lengthy procedures, the need of specialized equipment, and highly skilled operatives, which also greatly limit the practical and routine cell culture applications. Therefore, how to overcome these disadvantages of preparation or supply of materials and the fabrication technologies of micro/nanostructures on material surfaces is a big challenge for promoting tissue engineering from lab to clinic. Directly repurposing EW for applications in neural tissue engineering may not only provide an amount of ready-made discarded materials with various micro/nanostructures on their surfaces for solving the problems of supply of materials and fabrication of micro/nanopatterns, but may also offer a new promising way of protecting environment.

Herein, to take waste optical discs as an example, we demonstrate that discarded consumer electronics can be directly reused as highly anisotropic substrates for the in vitro investigation of cell behaviors, such as cell adhesion, cell alignment, and cell-cell interactions (Scheme 1). Both sides of the waste DVD polycarbonate (PC) layer with very different topography are flat and grooved, respectively. The PC12 cells cultured on the two different substrates show a distinct cell morphology, only the DVD-groove substrate could well align the PC12 cells (Scheme 1c). This study adds a new dimension to expanding application of information technology and solving the environmental problem of EW. The present report will provide more opportunities in combining both theories and advantages of information technology and other fields and using a combination of biochemical building blocks, engineering and materials methods to construct and design novel biointerfaces for applications in biomedicine, biosensors, and energy.

2. Material and methods

2.1. Characterization of the waste optical discs

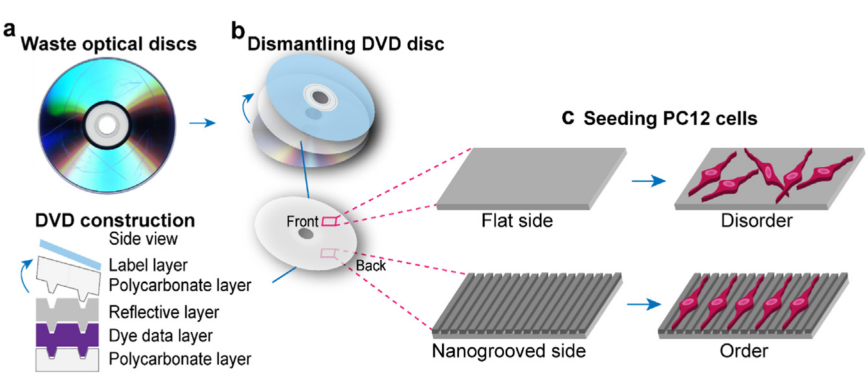
The waste DVD medium consists of five layers, including a label layer, two PC layers, a reflective layer, and a dye data layer (Scheme 1a). To facilitate studying cell behavior on the waste DVD substrates, we used a boxcutter to carefully peel off the waste DVD into two parts to obtain the label layer and transparent PC layer on the top and the reflective layer and PC layer base on the bottom, then soaked them in ethanol for 10 min to remove the label layer and other impurities (Scheme 1b). Both sides of the PC layer with different topography are flat (represented as the DVD-flat side) and grooved (represented as the DVD-groove side), respectively. For further characterizing and cell culture, the PC layer was cut into some pieces. The morphology of the PC layer was investigated with a JSM-7100F field-emission scanning electron microscope (SEM, JEOL Ltd., Japan) at 15 kV. Contact angle measurement was performed by a TX 500 H spinning drop interfacial tensiometer (KINO Ltd., USA). DVD nanogrooves imaging was performed with a SPA-400 atomic force microscope (AFM, Seiko Instruments Inc., Japan) in tapping mode. All AFM images were processed with Gwyddion 2.30 (http://gwyddion.net/). The X-ray photoelectron spectroscopic (XPS) characterizations were captured by a Thermo EscaLab 250 high performance photoelectron spectrometer with an Al Kα (1486.6 eV) radiation and analyzed using XPSPEAK41.

2.2. Cell culture

The highly differentiated PC12 cells were obtained from Cell Bank of Chinese Academy of Sciences and maintained in high-glucose Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% heat-inactivated newborn calf serum (Sijiqing Biotech, China), penicillin (100 units/mL), and streptomycin (100 μg/mL, Gibco) in a water-saturated atmosphere of 5% CO_2 at 37 °C. Before the cell seeding, DVD-flat and DVD-groove substrates with the identical sizes were sterilized in 75% ethanol for 30 min, UV irradiation for 30 min, then rinsed with PBS solution three times, and added to individual wells.

2.3. Cytotoxicity assay

Cells were divided into three groups: control (cell culture plate), DVD-flat, and DVD-groove. To better evaluate cytotoxicity of the polycarbonate layer to PC12 cells, the PC12 cells (2×10^4 cells/well) were seeded on three substrates and exposed to longer time points (1, 2, 3, 4 days), and then analyzed by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and lactate dehydrogenase (LDH) assay on a Bio-Tek ELx800 plate reader according to previous study (Lu et al., 2018) and Nanjing Jiancheng Bioengineering's kit protocol.



Scheme 1. An illustration of directly repurposing waste optical discs with prefabricated nanogrooves as a platform for investigation of cell-substrate interactions and guiding neuronal growth. (a) The photo and construction of a waste optical disc. (b) An illustration of dismantling DVD disc. (c) The effect of both sides of waste DVD PC layer on cell behaviors.