Microelectronic Engineering 198 (2018) 73–77

Contents lists available at ScienceDirect

Microelectronic Engineering

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

Research paper

# Fabrication of spaced monolayers of electrospun nanofibers for three-dimensional cell infiltration and proliferation

Bin Wang, Li Wang, Yadong Tang, Jian Shi, Jin Wei, Xiaolong Tu, Yong Chen*

*PASTEUR, Département de chimie, école normale supérieure, PSL University, Sorbonne Université, CNRS, 75005 Paris, France*

ARTICLE INFO

*Keywords:*
Scaffold
Nanofibers
3D culture

ABSTRACT

Biophysical and biochemical properties of the extracellular matrix (ECM) are important for regulation of cell behaviors and tissue functions. In this study, we fabricated monolayers of gelatin nanofibers on both sides of a honeycomb micro-frame by electrospinning and chemical crosslinking, resulting in tri-layer patches with adjustable thickness and pore size. Fibroblast cells were cultured on this tri-layer scaffold, showing an enhanced cell proliferation. More importantly, our results showed an efficient cell infiltration into the space between the two fiber layers and 3D cell distribution suitable for cell based assays.

## 1. Introduction

Micro- and nano-engineering techniques are used to manufacture sophisticated cell culture devices for both fundamental research and advanced applications [1,2]. Most of the previous studies based on two-dimensional (2D) surface engineering by creating topographic or biochemical patterns using different substrates or coating materials. More recently, attention has been paid to the three-dimensional (3D) engineering of natural or synthetic biopolymers, which is challenging in terms of both material processing and structure design. Of great importance is to recapitulate the biophysical and biochemical properties of in vivo, often three-dimensional, extracellular matrix (ECM), are not only a morphological resemblance but also integrated ligands and material stiffness regulating cell adhesion and signaling [3–5]. Nevertheless, the proposed methods should remain versatile, flexible and low cost for a broad range of cell-based assays.

Electrospun nanofibers are one of the most popular choices to create fibrous surface morphology using different materials. Previously, substrates coated by electrospun nanofibers were used in a variety of cell culture and engineering, including cardiac muscle, nerves and cartilage tissues [6–11]. The electrospun nanofibers are, however, often tightly packed and hardly applicable to the three-dimensional (3D) cell culture due to the difficulty of cell infiltration. Alternatively, electrospun nanofibers of large pores could be obtained by selective removal of sacrificial materials [12,13]. This approach resulted in an increased cell infiltration, but it is often not easy to avoid the effect of distortion, shrinkage and delamination. The present study is based on nanofiber monolayers deposited on both sides of a photolithography defined honeycomb microframe. Previous studies have shown similar approach with only one monolayer to culture primary hippocampal neurons [14,15] and to differentiate human induced pluripotent stem cells (hiPSCs) toward cardiomyocytes and motors neurons [8,16]. Here, we show that the device with two spaced nanofiber monolayers is relevant for 3D cell handling and proliferation, since it provides not only an ECM like morphology but also sufficient flexibility “plug-and-play” usage in a large variety of cell-based assays.

## 2. Materials and methods

### 2.1. Fabrication of tri-layer scaffolds

The honeycomb microframe was fabricated by conventional photolithography, soft lithography and micro-aspiration UV molding, as shown in Fig. 1. Firstly, a 50 μm or 100 μm thick resist (AZ40XT) layer was spun coated on the chrome mask with a honeycomb pattern (100 μm period and 10 μm line width) and exposed to a UV light (365 nm) at 12.7 mW $cm^{-2}$ for 90 s. After development, a pre-polymer mixture of polydimethylsiloxane (PDMS) components A and B at ratio 10:1 was poured on the top of the resist pattern and cured at 80 °C for 2 h. Then, the PDMS layer was peeled off and placed on a glass slide. After degassing in a dedicator, a solution of poly (ethylene glycol) diacrylate (PEGDA, Mw = 250, Sigma) with 1 v/v% photo-initiator (Irgacure 2959, Sigma) was introduced, following a UV exposure for 30 s (Fig. 1e). After PDMS removal, a PEGDA honeycomb microframe of thickness 50 μm (P50) or 100 μm (P100) was obtained (Fig. 1f). In order to facilitate handling, a PEGDA ring was mounted (Fig. 1h).

* Corresponding author.
*E-mail address:* yong.chen@ens.fr (Y. Chen).

https://doi.org/10.1016/j.mee.2018.07.005
Received 21 March 2018; Received in revised form 10 July 2018; Accepted 13 July 2018

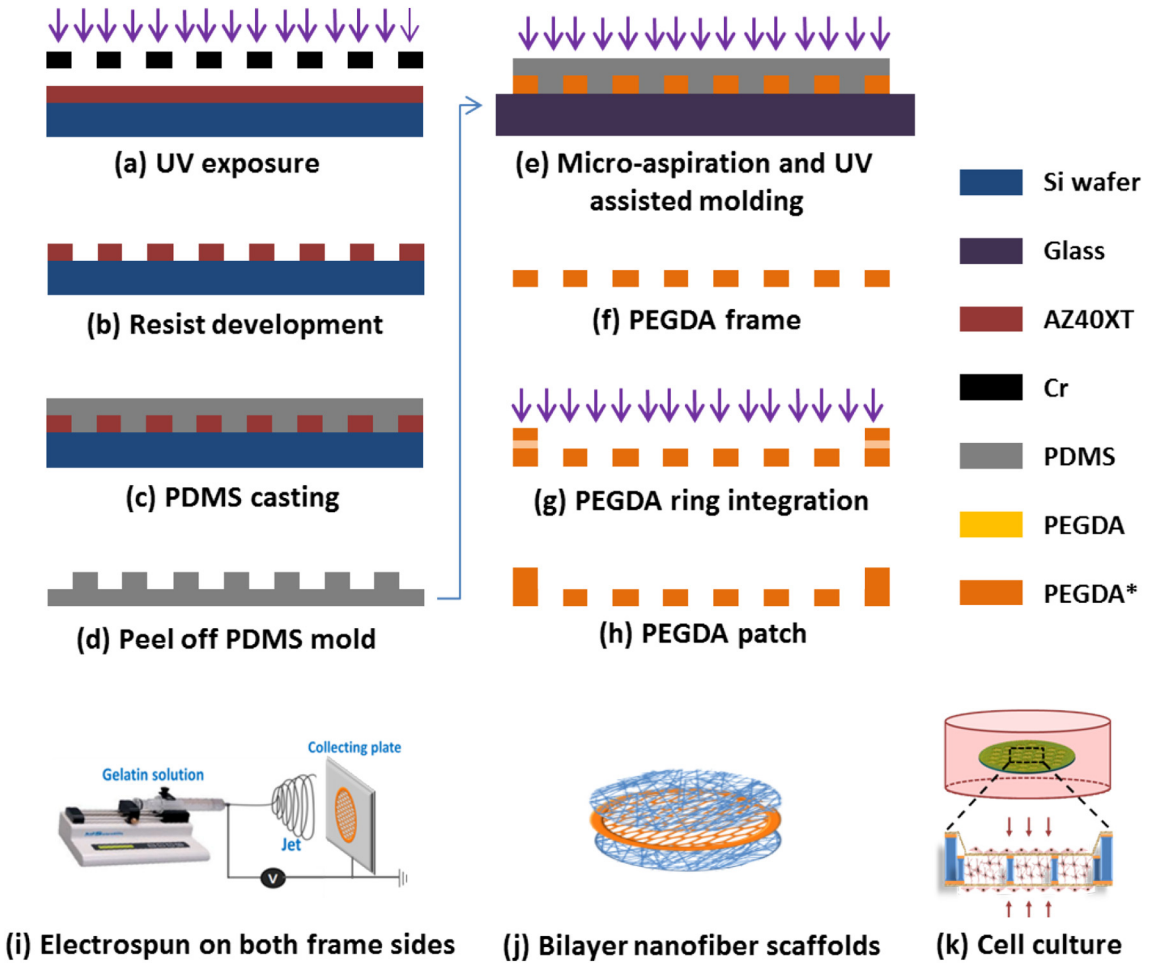


**Fig. 1.** Schematic diagram of the process flow for the fabrication of PEGDA honeycomb microframe (a–h), the electrospinning of nanofibers (i), a bilayer nanofiber scaffold (j) and 3D cell culture (k).

Gelatin nanofibers were electrospun on both sides of the PEGDA layer, 10 wt% gelatin powder was dissolved in the mixed solution of acetic acid, ethyl acetate and DI water at a volume ratio of 21:14:10. A silicon wafer placed 10 cm far away from the spinneret was grounded on which a PEGDA layer was taped for the nanofiber connection, collecting fibers 10 min for each layer side (Fig. 1i). The electrospinning was performed for 5, 10 or 20 min at a voltage of 12 kV with a feeding rate of 0.2 mL/h controlled by a syringe-pump. After electrospinning, gelatin nanofibers were dried in a desiccator overnight and cross-linked in a 0.2 M mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma) and N-hydroxysuccinimide (NHS, Sigma) in ethanol for 4 h. After crosslinking, samples were rinsed with 99.5% ethanol twice and dried in vacuum overnight to remove the remaining chemicals. For clarity, scaffolds of fibers integration on PEGDA with thickness 50 μm or 100 μm were labeled as S50 or S100.

### 2.2. Characterization of nanofibers and cell culture

The morphology of the microframe and nanofibers was observed with scanning electron microscope (SEM, TM-3030, Hitachi, Japan). Pore size distributions were calculated with ImageJ software. Prior to cell seeding, the fabricated devices were subjected to ultraviolet light for 30 min to sterilize the system. Then, NIH-3T3 cells (Sigma-Aldrich) at the density of $1 \times 10^6$/mL and 50 μL cell suspension was put onto the upper surface of the devices. To visualize cell attachment and infiltration, cells were fixed with 4% formaldehyde PBS solution for 15 min, permeabilized in 0.5% Triton-X-100 PBS solution for 10 min and then blocked in PBS containing 0.1% TX and 3% BSA at room temperature for 30 min, followed by incubating with Phalloidin-FITC (1 μg/mL) and DAPI (100 ng/mL). Colonization of the scaffolds was also evaluated using a LSM Zeiss 710 confocal microscope. 5 samples in each group were analyzed and quantitative findings are presented as mean ± standard deviation. A live/dead staining was performed to evaluate cell viability, samples were incubated in a DPBS solution containing 2 μM Calcein AM and 4 μM EthD-1 for 30 min. After washing with DPBS, the fluorescence images were taken with confocal microscope. The cell proliferation was evaluated by 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) test. The specimens were incubated with 1 mL culture medium and 100 μL 5 mg/mL MTT for 4 h. Thereafter, the culture media were extracted and 200 μL dimethylsulfoxide (DMSO) was added and when the crystal was sufficiently dissolved, 100 μL of the solution was pipetted into the well of a 96-well plate and tested by a microplate reader at an absorbance of 490 nm.

### 2.3. Statistical analysis

All data are expressed as mean ± standard deviation (Mean ± SD) unless otherwise stated. A minimum sample number of 4 was maintained throughout the study. All quantitative analyses were examined using one-way analyses of variance (ANOVA) with the addition of Tukey's correction for multiple comparisons testing.

## 3. Results and discussion

Fig. 2A shows the SEM photos of fabricated PEGDA frame P50 and P100 with the same the honeycomb network (period 100 μm and line