Multifunctional Micropatterned Nanofiber Scaffolds Capable of Cell Patterning, Metabolite Detection, and Growth Factor Delivery

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ABSTRACT

This study describes the development of multifunctional micropatterned nanofiber scaffolds consisting of multiple layers of nanofiber. We fabricated double-layered nanofiber scaffolds that were micropatterned with PEG hydrogels by combining two-step sequential electrospinning and hydrogel lithography. We successfully demonstrated that the upper layer could be used to create micropatterns of HepG2 spheroids with uniform size, while the bottom layer could be used for in situ detection of albumin secreted by HepG2 spheroids in the upper layer. Finally, we immobilized growth factors and showed possibility of controlled release of growth factors from micropatterned nanofiber scaffolds

Keywords: cell patterning, nanofiber, hydrogel,

1 INTRODUCTION

The ability to generate cell or protein micropatterns is important for biosensors, tissue engineering, and fundamental studies of cell biology [1-2]. A number of microfabrication techniques combined with surface chemistry have been widely utilized for the spatial control of cells and proteins on various surfaces [3-6]. In most of these applications, however, cells and proteins are immobilized on a flat two-dimensional substrate, resulting in several limitations. Specifically, cells are maintained in an unnatural environment and often exhibit unnatural behaviors because most cells exist in three-dimensional (3D) fibrous extracellular matrix in native tissue [7-8]. Further, the amount of proteins that can attach to a planar surface is limited, generating weak analytical signals when micropatterned proteins are used for bioassays [9-10]. Therefore, there has been strong demands to develop cell pattern within 3D environments and biosensor with higher sensitivity. Electrospun nanofiber can be one solution that can solve those problems due to its 3D-fibrous structures with great surface area.

2 EXPERIMENTAL

2.1 Preparation of multi-layered nanofiber

Nanofiber scaffolds that can be vertically divided into two different regions were prepared by a two-step sequential electrospinning technique. The first electrospun PS nanofibers were collected for 30 minutes on clean aluminum foil. After treatment with oxygen plasma, the PS nanofibers were then soaked with 10 μg/mL anti-albumin solution in 50 mM carbonate-bicarbonate buffer for 5 hours at 37°C. After water was removed from the first nanofiber layers by patting them dry, the second layer of nanofiber scaffolds was directly deposited on the anti-albumin-immobilized first nanofiber layer by the same electrospinning process that was used for the first layer. The second layer was responsible for cell attachment and growth.

2.2 Micropatterning with hydrogel

PEG hydrogel micropatterns incorporated with nanofiber scaffolds were fabricated by pthotolithograpy. After hydrogel precursor solution permeated through the nanofiber scaffold, the photomask was placed onto the resultant nanofibers and exposed to 365 nm, 300 mW/cm^2 UV light. After development with water to remove unreacted precursor solution, the micropatterned scaffolds were sterilized in 70 % v/v ethanol solution for 30 minutes and washed five times in phosphate buffered saline (PBS) to remove traces of ethanol.

2.3 Cell and protein asssay

A live/dead viability/cytotoxicity fluorescence assay was used to investigate the viability of adherent cells on the micropatterned substrate. MTT assays were performed to measure cellular activity and 10% v/v MTT solution (5 mg/ml) in culture medium was added to the cell. The feasibility of micropatterned nanofibers to detect albumin was demonstrated using sandwich immunoassays with fluorescence-labeled antibodies.

3 RESULTS

Micropatterned polymeric nanofiber was prepared by combining electrospinning process and hydrogel lithography. As shown in Fig. 1, the micropatterned polystyrene nanofiber was successfully fabricated as a form of free-standing sheet, the size of patterned domain and thickness of sheet can be easily controlled.

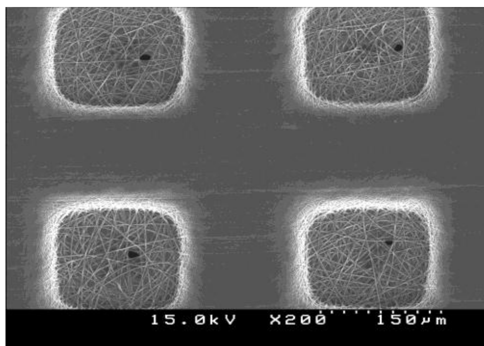

Fig. 1 SEM image of micropatterned nanofiber

After fabrication of micropatterned nanofibers, we examined the their capability of to create a microarray of HepG2 spheroids with uniform size. We hypothesized that our micropatterned nanofiber enabled us to prepare uniform HepG2 spheroids because micropatterning of PEG hydrogels in the presence of PS nanofibers underscored the clear contrast between the adhesion-resistant, hydrophilic hydrogel region and the adhesion-promoting hydrophobic PS fiber region, which allowed us to confine cells only within the PS nanofiber region. Because cell spheroids smaller than 200 μm are desirable to prevent hypoxia or malnutrition, we used microwell-type hydrogel patterns with lateral dimension of 200μm × 200μm to create a microarray of HepG2 spheroids. Figure 2 shows that HepG2 cells formed spheroids with high circularity only within the nanofibrous microwell regions, and the hydrogel walls served as effective barriers to cell adhesion, proliferation, and crossover.

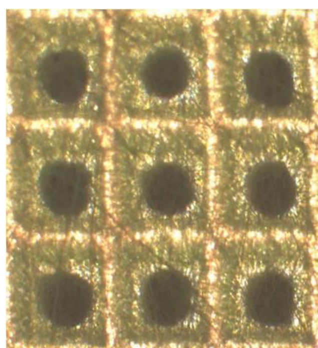

Fig. 2 Optical image of HepG2 spheroids formed within micropatterned nanofibers

To investigate the potential use of micropatterned fibers for immunoassay, IgG was immobilized onto PS/PSMA fibers. After the immobilization step, fibers containing both covalently attached and physically adsorbed IgG were thoroughly washed to remove weakly-bound IgG until no leaching of IgG was detected. Due to the exclusion effects of PEG hydrogel against proteins and other small molecules, probe proteins (IgG) would exist only in the fibrous region and subsequently target proteins (anti-IgG) would bind only in the same region with minimal adsorption in the PEG hydrogel region. Figure 3 shows the results of a representative experiment in which FITC-labeled anti-IgG was used to visualize the immunoassay. Fluorescent images demonstrate that anti-IgG bound specifically to the IgG-immobilized fibrous region (Figure 3a). Figure 3b demonstrated that more anti-IgG could bind to 3D nanofiber than 2D substrates

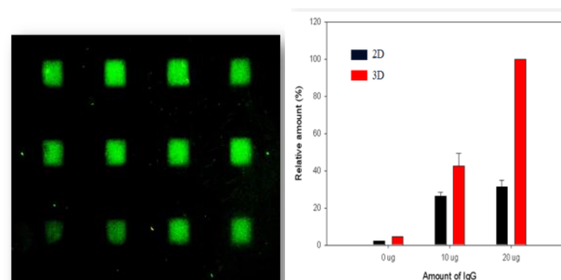

Figure 3. Immunoassay with micropatterned nanofiber. (a) Fluorescence image and (b) amount of bound FITC-labeled anti-IgG.

Finally, we fabricate three dimensional (3D) fibrous scaffolds for controlled release of two or more growth factors. Here we confirmed the differentiation of human mesenchymal stem cell (hMSC) to osteoblast efficiently using basic fibroblast growth factor (bFGF) and bone morphogenetic protein 2 (BMP-2). The electrospun fibers and hydrogels were used as reservoir of growth factors respectively. The crosslinked collagen has negative charge, so the bFGF could be attached on the nanofibers electrostatically. The BMP-2 were encapsulated in the PEG hydrogel, the release rate could be controlled via molecular weight of PEG. Because each has distinguished release profile, the amounts and periods of release were regulated by location of growth factors.

4 CONCLUSION

In this study, we successfully fabricated hydrogel micropatterned 3-D nanofiber scaffolds. Because of nonadhesive property of PEG hydrogel, we could control protein adsorption and subsequent cell adhesion. Immunoassays revealed that the nanofibrous substrates emitted higher fluorescence signals and were more sensitive than the corresponding planar substrates, most likely due to higher protein-loading capacity resulting from increased surface area. We are sure that this new system will provide new platform for cell-based biosensor, tissue engineering and other basic cell biology.

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