Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Biomaterials 26 (2005) 5624–5631
Biomaterials
www.elsevier.com/locate/biomaterials

A synthetic nanofibrillar matrix promotes in vivo-like organization and morphogenesis for cells in culture

Melvin Schindler[a], Ijaz Ahmed[b], Jabeen Kamal[b], Alam Nur-E-Kamal[b], Timothy H. Grafe[c], H. Young Chung[c], Sally Meiners[b,*]

[a]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA
[b]Department of Pharmacology, Robert Wood Johnson Medical School-UMDNJ, 675 Hoes Lane, Piscataway, NJ 08854, USA
[c]Donaldson Company, Inc., P.O. Box 1299, Minneapolis, MN 55440, USA

Received 12 November 2004; accepted 8 February 2005
Available online 18 April 2005

Abstract

The purpose of this study was to design a synthetic nanofibrillar matrix that more accurately models the porosity and fibrillar geometry of cell attachment surfaces in tissues. The synthetic nanofibrillar matrices are composed of nanofibers prepared by electrospinning a polymer solution of polyamide onto glass coverslips. Scanning electron and atomic force microscopy showed that the nanofibers were organized into fibrillar networks reminiscent of the architecture of basement membrane, a structurally compact form of the extracellular matrix (ECM). NIH 3T3 fibroblasts and normal rat kidney (NRK) cells, when grown on nanofibers in the presence of serum, displayed the morphology and characteristics of their counterparts in vivo. Breast epithelial cells underwent morphogenesis to form multicellular spheroids containing lumens. Hence the synthetic nanofibrillar matrix described herein provides a physically and chemically stable three-dimensional surface for ex vivo growth of cells. Nanofiber-based synthetic matrices could have considerable value for applications in tissue engineering, cell-based therapies, and studies of cell/tissue function and pathology.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Biomimetic material; Nanotopography; ECM; Cell culture

1. Introduction

Cell development, organization, and function in tissues are regulated by interactions with a diverse group of macromolecules that comprise the extracellular matrix (ECM). For various mesenchymal and tumor cells [1,2], the ECM provides a surrounding coat of fibrils that is in contact with both apical and basal cell surfaces. The basement membrane, a structurally compact form of ECM, uniquely makes contact with the basal surfaces of cells that form tissues, e.g. epithelial and endothelial cells [1,2]. The three-dimensional structure of the basement membrane/ECM has been shown to be as important as the chemistry in its influence on cellular processes such as tumor development and drug sensitivity [3,4].

The vast majority of culture work has been performed using two-dimensional plastic and glass cell culture surfaces. However, such culture media do not reflect the three-dimensional geometry and porosity observed for the cell attachment sites and migration pathways within and between tissues. Nonetheless, two-dimensional culture surfaces have the advantage that they are uniform and can be reproducibly manufactured to precise tolerances. It is now generally acknowledged that the movement from cell to true tissue culture will require new three-dimensional environments, preferably synthetic, to faithfully recapitulate cell-basement membrane/ECM interactions within tissues [4–6].

*Corresponding author. Tel.: +1 732 235 2890.
E-mail address: meiners@umdnj.edu (S. Meiners).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.02.014

To pursue the goal of creating chemically and physically stable synthetic three-dimensional surfaces that mimic the structural geometry and porosity of basement membrane/ECM, we have utilized the technique of electrospinning to produce nanofibers that self-assemble into three-dimensional nanofibrillar networks [7–9]. The physical form of the nanofibrillar matrices provides unprecedented porosity ($>70\%$), high spatial interconnectivity, and a high surface to volume ratio for cell attachment. In the current study, we demonstrate that nanofibrillar matrices promote in-vivo-like cell morphology and organization of cytoskeletal components in NIH 3T3 fibroblasts and normal rat kidney (NRK) cells. In addition, these nanofibrillar surfaces are permissive for the morphogenesis of breast epithelial cells (T47D) into multicellular spheroids containing lumenal cavities.

Most significantly, nanofibers can be incorporated into: (a) large-scale cell culture protocols such as roller cultures and cell reactors/fermentors; (b) high throughput drug screening protocols; and (c) scaffolds for ex vivo culturing of cells for use in various applications of tissue engineering and cell-based therapies. In addition, the fibrillar structure of the nanofiber matrices structurally resembles the connective tissue and basement membrane through which tumor cells migrate in the processes of metastasis and intravasation [10,11]. This physical similarity suggests a potential role for nanofibrillar surfaces in studies of amoeboid tumor cell migration [10]. Thus polyamide nanofibers provide a new three-dimensional cell culture surface for more physiologically relevant cell growth that can be incorporated into a variety of applications.

2. Materials and methods

2.1. Materials

The reagents and dilutions employed in this study were as follows. Phalloidin-Alexa Fluor 488 (1:100), monoclonal cellular fibronectin antibody (1:500), monoclonal beta tubulin antibody (1:500), monoclonal actin antibody (1:500), and monoclonal vinculin antibody (1:400) were from Sigma Chemical Co. (St. Louis, MO). CY3-goat anti-mouse IgG (H + L) (1:500), CY3-donkey anti-rabbit IgG (H + L) (1:500), CY3-streptavidin (1:500), normal goat serum (1:10), and normal donkey serum (1:10) were from Jackson Labs (West Grove, PA). FAK (PY^{397}) rabbit polyclonal antibody (1:500) was obtained from Biosource (Camarillo, CA). Biotin-mouse anti-hamster IgM (monoclonal) (1:100) was obtained from Pharmingen (San Diego, CA). Hamster anti-rat/mouse CD29 (Integrin beta 1) IGM (1:100) was obtained from RDI (Flanders, NJ). Gel/Mount (aqueous mounting medium with antifading agents) was obtained from Biomeda (Foster City, CA). Dulbecco's Modified Eagle's Medium (DMEM) (high glucose), calf serum, and fetal calf serum were all obtained from Invitrogen (Carlsbad, CA). Coverslips (18 mm, No.1) were purchased from Fisher Scientific (New Brunswick, NJ). NRK cells were a gift from Dr. Sanford Simon (Rockefeller University, New York, NY), and T47D breast epithelial cells were a gift from Dr. Leroy Liu (Robert Wood Johnson Medical School-UMDNJ, Piscataway, NJ). Cell culture plates were purchased from Corning (Corning, NY). Nanofibrillar matrices are commercially available as Ultra-Web™ Synthetic ECM and were obtained from Donaldson Co. (Minneapolis, MN).

2.2. Nanofiber production by electrospinning

The nanofibers were electrospun using an adapted industrial electrospinning process with a field strength of 30 kV as described [12]. The nanofibers were electrospun onto glass coverslips (18 mm, No.1) in a controlled thickness and fiber density. The nanofibers are composed of two kinds of polyamide polymers, A $(C_{28}O_4N_4H_{47})_n$ and B $(C_{28}O_{4.4}N_4H_{47})_n$, that have been cross-linked in the presence of an acid catalyst.

2.3. Cell culture and fluorescence staining

NIH 3T3 and NRK cells were seeded at 5×10^4 cells/ml and cultured in DMEM (4.5 g/l glucose) in the presence of 10% calf serum at 5% CO_2 and 37 °C, while T47D breast epithelial cells and MCF-7 cells were seeded at 5×10^4 cells/ml and cultured in DMEM (4.5 g/l glucose) in the presence of 10% fetal calf serum at 5% CO_2 and 37 °C. All cell types were grown on either glass or nanofiber-coated glass coverslips in 12-well cell culture plates.

Staining for F-actin was performed in the following manner. Cells were rinsed once with phosphate-buffered saline (PBS), fixed with 4% paraformaldehye in PBS (15 min), washed with PBS, treated with 0.5% Triton X-100 (5 min), washed with PBS, incubated with phalloidin-Alexa Fluor 488(diluted with PBS containing 0.3% Tween-20) for 1 h, washed with PBS (3 ×, 5 min per wash), and then mounted on a slide with Gel/Mount. Imaging was performed with a Zeiss Axioplan Epi-Fluorescent Microscope.

Mouse monoclonal antibody staining was performed as described for phalloidin-Alexa Fluor 488 except after the treatment with Triton X-100, cells were washed with PBS, blocked with normal goat serum (diluted with PBS/0.3% Tween-20) for 30 min at room temperature, washed with PBS (3 ×, 5 min per wash), incubated overnight with primary antibody, washed with PBS (3 ×, 5 min per wash) followed by incubation for 1 h with the secondary antibody goat anti-mouse IGg*CY3

Download English Version:

https://daneshyari.com/en/article/11068

Download Persian Version:

https://daneshyari.com/article/11068

Daneshyari.com