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Morphological and functional studies of rat hepatocytes on a hydrophobic or hydrophilic polydimethylsiloxane surface

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Abstract

This study describes the morphological and functional behavior of rat hepatocytes on a polydimethylsiloxane (PDMS)-coated surface. Hepatocytes were cultured on hydrophobic or hydrophilic PDMS-coated surfaces in serum-free and serum-containing media. In the serum-free medium, almost all hepatocytes adhered onto the surface irrespective of the wettability, with a cell adhesion ratio of >90% at 24 h. In the serum-containing medium, although they strongly adhered onto the hydrophilic surface (cell adhesion ratio >85%), the ratio on the hydrophobic surface was <15%. Furthermore, hepatocytes in the serum-free medium gradually formed spheroids irrespective of the surface characteristics; however, on the hydrophilic surface in the serum-containing medium, they maintained a monolayer configuration for up to 10 days, and their numbers gradually decreased over time. Expression levels of the functional activities (albumin secretion and ammonia removal) and the cell–cell adhesion molecules (cadherin and connexin-32) were higher in the hepatocytes that formed spheroids compared to those which assumed a monolayer configuration, and these levels were maintained for at least 10 days. These results suggest that the wettability of PDMS and the composition of the culture medium together control the cell adhesion, morphology and expression of functional genes in hepatocytes.

Keywords: Rat hepatocytes; PDMS; Hydrophobic; Hydrophilic; FBS

1. Introduction

Primary hepatocytes display different morphological and functional characteristics, depending on the surface properties of the biomaterials. They spread to assume a monolayer configuration when cultured on high-affinity materials such as collagen and fibronectin [1–3]. In contrast, in the case of materials on which cell–cell interaction is greater than cell–material interaction, hepatocytes form spheroids (spherical multicellular aggregates), derived from the rearrangement and compaction of a cell aggregate [2–7]. The expression of differentiated functions in hepatocytes is closely related to cell morphology, and it is known that cells can express highly differentiated functions in three-dimensional configurations, such as spheroids, rather than just in a monolayer configuration. Therefore, the design of biomaterials is important in applications for liver tissue engineering, bioartificial liver, hepatocyte-based chips and the study of cell biology.

Polydimethylsiloxane (PDMS) has several advantages over other biomaterials: it can be used to design various microstructures based on templates; it is biocompatible; it is elastic; it has high gas permeability; and it has properties such as optical clarity and lack of autofluorescence. Because of these advantages, PDMS has been used in the field of biotechnology for the following applications: (i) as a prominent component of microfluidic devices, including microchannels and microvalves [8,9]; (ii) as a tool for soft lithography, including microcontact printing, to form various cell patterns [10,11]; and (iii) as a promising material for cell culture. It has already been reported that various cell lines and primary cells can be cultured on PDMS, and the cells display good morphological and functional characteristics [12–15].

PDMS can architect and mass replicate complex two- or three-dimensional substrata because it can easily transfer

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the microstructures of templates. Therefore, if the properties of cell adhesion and cell morphology on the PDMS surface can be controlled, it could become a superior culture material compared to previously developed materials, and the fields of microchips and microfluidic culture devices may advance dramatically. The simplest method to control cell adhesion and cell morphology on a material is to vary the surface wettability of that material or the medium components. Normally, PDMS has a hydrophobic surface; so, for use in cell culture, its surface is made hydrophilic by treatment with air or oxygen plasma to facilitate cell adhesion.

It is known that cell adhesion and protein adsorption are affected by the surface wettability of the materials [16–20]. Furthermore, some researchers have reported that cell adhesion and metabolism in hepatocytes are enhanced on more wettable materials [21–24]. However, most reports have discussed the relationship between cell behavior and surface wettability of materials in serum-containing media. Although hepatocyte culture in a serum-free medium has been reported by some researchers [2–7], very little is known regarding the relationship between cell behavior and surface wettability of materials in such a medium.

In this study, we focused on the differences in cell behavior by using a combination of surface wettability of the materials and composition of the medium. Furthermore, we investigated the differences in the expression of cell adhesion molecules (integrin, cadherin and connexin-32) for cell–matrix or cell–cell interaction in hepatocytes in both serum-containing and serum-free culture media. To determine the application of PDMS in cell culture, the goal of this study was to understand the combined effect of surface wettability of PDMS and the composition of the medium on hepatocyte behavior.

2. Materials and methods

2.1. Preparation of hydrophilic and hydrophobic PDMS-coated dishes

The hydrophilic and hydrophobic PDMS-coated dishes were prepared as follows: the PDMS prepolymer (Sylgard™ 184 silicone elastomer; Dow Corning Corp., Midland, MI) was mixed with the curing agent at a ratio of 10:1 (v/v), and 0.5 ml of this mixture was poured in a 35 mm polystyrene dish (Falcon™, nontreatment type 1008; Becton Dickinson Labware, Franklin Lakes, NJ), which was then cured at room temperature for 48 h. This PDMS-coated dish had hydrophobic characteristics (contact angle, 120±3°). To prepare a hydrophilic PDMS-coated dish, the hydrophobic dish was placed in a plasma system (Plasma Cleaner; Harrick Scientific Co., Ossining, NY) and treated for 10 s at a high-power setting. By this procedure, the contact angle of the PDMS surface was changed to 28±3°. These hydrophilic characteristics were maintained for at least 48 h under dry conditions, and the hydrophilic PDMS-coated dishes were used within 24 h after preparation. Both types of PDMS-coated dishes were sterilized in 70% ethanol solution and then precultured in sterilized water prior to the cell culture.

2.2. Culture medium

Three kinds of culture media were prepared for this study: (i) a serum-free medium comprising Dulbecco's modified Eagle's medium (Invitrogen Corp., Carlsbad, CA) supplemented with 10 μg ml^{-1} insulin (Sigma, St. Louis, MO), 7.5 μg ml^{-1} hydrocortisone (Wako Pure Chemical Industries, Osaka, Japan), 50 ng ml^{-1} epidermal growth factor (Biomedical Technologies Inc., Stoughton, MA), 60 mg l^{-1} proline (Wako), 50 ng ml^{-1} linoleic acid (Sigma), 0.1 μM $CuSO_4 \cdot 5H_2O$, 3 ng ml^{-1} H_2SeO_3, 50 pM $ZnSO_4 \cdot 7H_2O$, 58.8 μg ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin; (ii) a serum-containing medium consisting of the serum-free medium supplemented with 10% fetal bovine serum (FBS); and (iii) an albumin-containing medium consisting of the serum-free medium supplemented with 0.4% bovine serum albumin (BSA medium).

2.3. Rat hepatocyte culture

This experiment was reviewed by the Committee of Ethics on Animal Experiments in our institute and was carried out under the Guidelines for Animal Experiments at our institute.

Hepatocytes were isolated from the whole liver of an adult Wistar rat (male, 7–8 weeks old, weighing approximately 200 g) by a two-step perfusing method [25]. Briefly, the portal vein of the liver was cannulated in situ, and then a washing solution of ethylene diamine tetraacetic acid (EDTA; Wako) at 37 °C was preperfused into the liver. Then, the liver was perfused with 0.05% collagenase solution (Wako) at 37 °C for approximately 10 min. The liver was minced, and large tissue fragments were removed by passing through a gauze mesh. The dense cell suspension was also passed through a stainless mesh with a 45 μm pore size. The cell suspension obtained was washed thrice with a culture medium using centrifugation at 50*g* for 90 s. Purity of the parenchymal hepatocytes, which was determined by cell size, was more than 97%. Cell viability was determined by the Tryptan blue dye exclusion method, and cells with >85% viability were used.

The hepatocyte suspension (2.5×10^5 cells ml^{-1} cell density) was inoculated onto each PDMS-coated dish (35 mm diameter) containing 2 ml of the culture medium. The media were changed at 24 h after inoculation and, subsequently, at 1 day intervals. All cells were cultured under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

2.4. Experimental designs

In this study, two types of experiments were designed. In the first experiment, to investigate the interaction between the hepatocytes and the PDMS surface, the cell adhesion