Biomaterials 34 (2013) 9082–9088

Contents lists available at ScienceDirect

Biomaterials

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

Splitting culture medium by air-jet and rewetting for the assessment of the wettability of cultured epithelial cell surfaces

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ARTICLE INFO

Article history:
Received 6 June 2013
Accepted 12 August 2013
Available online 3 September 2013

Keywords:
Wettability
Epithelial cell
Cell culture
Mucosa
Surface analysis

ABSTRACT

This study found that the phenomenon of rewetting after squeezing culture medium varied in different culture conditions for rat oral mucosal epithelial cells. When culture medium covering over cultured cells was squeezed by an air-jet application, the motion of squeezed culture medium was able to be observed by using a commercially available movie camera. Squeezed width on cells cultured in keratinocyte culture medium (KCM), which contained with fetal bovine serum, was one-sixth of that in FBS-free KCM. This result corresponded to the mucous layer staining statuses of cultured cells in both cases; positive in KCM and negative in FBS-free medium. Furthermore, the gene expression of mucous glycoprotein MUC4 in KCM was 100 times higher than that in FBS-free medium, and the expression of MUC4 protein only showed on the apical surface of cells cultured in KCM. The relative gene expression levels of MUC1, 13, 15, and 16 in both the normal and FBS-free medium were found to be no more than one-thirtieth of that of MUC4 in KCM. The main factor of the wettability difference between KCM and FBS-free medium was speculated to be the difference of MUC4 expression between both media. This method can be a simple technique for testing not only the surface wettability but also the mucous formation of cultured cells.

1. Introduction

Generally, the surfaces of digestive tract [1], eye [2], etc. are covered with mucosal epithelium, which secretes and retains mucous layer. Mucous layer is a valuable structure for keeping an epithelial barrier function against pathogens and chemical irritants [3–5]. Mucous layer is composed of mainly mucin proteins, which is a glycoprotein with a structure where core protein is conjugated with hydrophilic glycans [6–8]. Therefore, the surface of mucous epithelium is a high wetting characteristic. The lost of the mucous layer on the surface of epithelia immediately gives infections, an ulcer, and the diseases of poor surface lubrication such as dry eye to the layer of mucous epithelium [9,10]. For dry eye syndrome, a noninvasive system for assessing the stability of tear on cornea has been investigated and applied to clinical use [11,12].

To date, regenerative medicine using cell sheets has been successfully established for replacing a dysfunction tissue such as the skin [13], cornea [14,15], esophagus [16], heart [17,18], and periodontal tissue [19]. Cell sheets are a thin membrane composed of cultured cells at 37 °C and harvested from a temperature-responsive cell-culture surface by reducing temperature to 20 °C. For preparing various types of cell-sheets, various types of cells are cultured at 37 °C on the surfaces [20]. Especially, oral mucosal epithelial cell sheets have been transplanted on the lost part of mucosal epithelia surface such as the damaged cornea epithelia and the esophageal ulcer after cancer dissection. In mucosal epithelial cell culture, the gene expression of some glycoprotein composing a mucous layer has changed between primary (uncultured) and cultured tissues [21,22]. Because the appearance of mucous protein affects the wettability of cell surface, wettability characterization by the measurement of contact angle on the surface of corneal epithelial explants has been reported [23,24]. However, it is difficult to measure the contact angle of cultured-cell surface in culture status. Therefore, a new method for assessing the wetting characteristic of cultured-cell surface has been strongly demanded.

This study developed an assessing method for evaluating the wetting characteristic of culture-cell surface (Fig. 1). After an air-jet application, which is inspired by our previous study [25], to the

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http://dx.doi.org/10.1016/j.biomaterials.2013.08.029

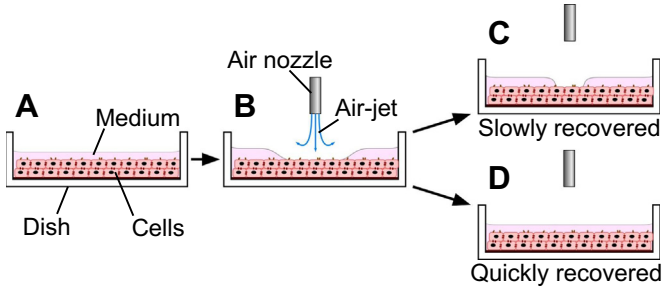

Fig. 1. Procedure of wettability assessment of cultured-cell surface. (A) Confluent cells on a cell culture dish were covered with a constant volume of culture medium. (B) Air-jet was given from an air-nozzle to the surface of culture medium, and the culture medium was squeezed by the air-jet. (C) In the case of hydrophobic surface, the squeezed area of culture medium was still remained. (D) On the other hand, in the case of hydrophilic surface, the culture medium recovers quickly.

surface of culture medium covering over cultured cells, the squeezed area of culture medium by air-jet was measured (Fig. 1B). After the cease of air-jet application within 1 s, medium on a low-wettability cell-surface was still squeezed or slowly recovered (Fig. 1C). On the other hand, culture medium recovered quickly over the cultured cells having a high wettability (Fig. 1D). Furthermore, the relationships between the expressions of mucous glycoprotein of cells, which were cultured in either fatal-bovine-serum-contained or eliminated cell-culture media, and the squeezed area were investigated.

2. Materials and methods

2.1. Development of measurement setup

The measurement setup was mainly composed of both an air-jet application unit (Fig. 2A) and observation unit (right side in Fig. 2B). In the observation unit, a handy digital movie camera (HDR-SR1) (SONY, Tokyo, Japan) (item No. 5 in Fig. 2B) was installed for observing and recording experiments, and an LED lamp (GH-LED08CLK) (GREEN HOUSE, Tokyo, Japan) (item No. 6 in Fig. 2B) illuminated the air-jet application unit area for obtaining clear images. The air-jet application-unit consisted of an air-nozzle (0.5 mm i.d., 20 mm long) (Air Blow Nozzle, ABNZNL5-1.0-20) (MISUMI, Tokyo, Japan) (item No. 2 in Fig. 2A) and a high speed solenoid valve (VA01PSP23-1P) (KURODA Pneumatics, Chiba, Japan) (item No. 3 in Fig. 2A) with a maximum switching frequency of 333 Hz. The air pressure of air source was controlled by an electro-pneumatic regulator (ITV2050-312CSQ) (SMC, Tokyo, Japan) before passing through the solenoid valve. Both solenoid valve and regulator were controlled by a laptop computer (ThinkPad X61) (Lenovo Japan, Tokyo, Japan) via an analog input/output interface module (CSI-360112) (Interface, Hiroshima, Japan). Compressed air as an air source was supplied from an oil-free type air-compressor (DPP-AYAD) (Koganei, Tokyo, Japan) through a membrane filter (Millex-GV, 0.22 μm, PVDF, 33 mm) (EMD Millipore, Billerica, MA). The air-jet application unit was attached on an *x*-*y*-*z*-axes linear slider device (custom-made item) (Sigma-koki, Tokyo, Japan) (item No. 4 in Fig. 2B) for obtaining fine alignment within a position accuracy of 0.1 mm. The movie camera and the linear slider with the air-jet application unit were rigidly fixed to a home-made base-plate made of aluminum frame (item No. 7 in Fig. 2B). The lamp was mounted on an appropriate position for observing around the air-jet application unit and a measurement object. To keeping temperature around the object, hot plate was installed on the base plate directly under the object.

2.2. Characterization of air-jet application

The pressure distribution of air-jet application was measured by a pressure sensor (PA-400) (Nidec Copal Electronics, Tokyo, Japan). By using the position adjuster, the horizontal distance between the air-nozzle and the probe hole of pressure sensor was able to be changed from 0 to 3 mm with a step of 0.1 mm (the inset in Fig. 3A), and the vertical distance between the air-nozzle and the probe hole was able to be changed from 1 to 3 mm with a step of 1 mm (the inset in Fig. 3B). Then, one air-jet was given from the air-nozzle with a specific horizontal and vertical distance. The pressure of air source was set to be from 0 to 0.4 MPa with a pressure step of 0.05 MPa. After the measurement of pressure distribution, the pressure was converted into fluid force by calculating the following equation:

$$f = \sum_{i=1}^{N} \left\{ p_i (2i-1)\pi \left(\Delta r^2 \right) \right\} \quad (1)$$

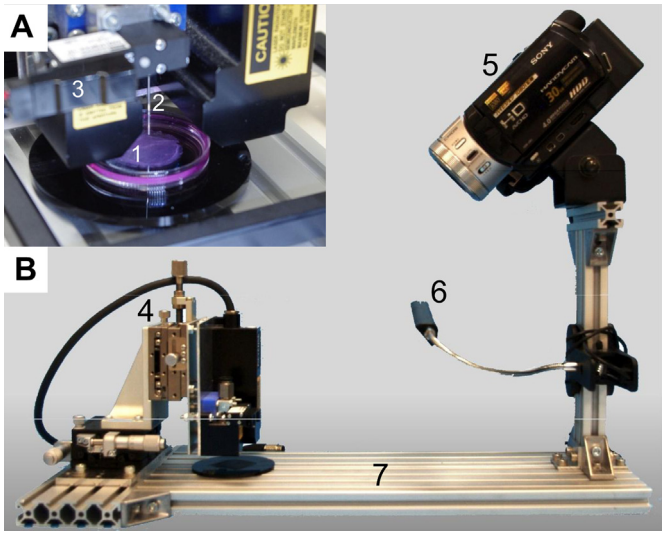

Fig. 2. Experimental setup for assessing the wettability of cultured cells. (A) Close-up view of air-jet application unit. (1) Cultured cells on a cell-culture substrate, (2) 0.5-mm-inner-diameter Air-nozzle, and (3) A electrical solenoid valve. (B) General view of experimental setup. (4) A position adjuster for *x*-*y*-*z*-axes directions, (5) A full-hivision movie camera, (6) An LED illumination, and (7) A home-made base-plate made of aluminum frames.

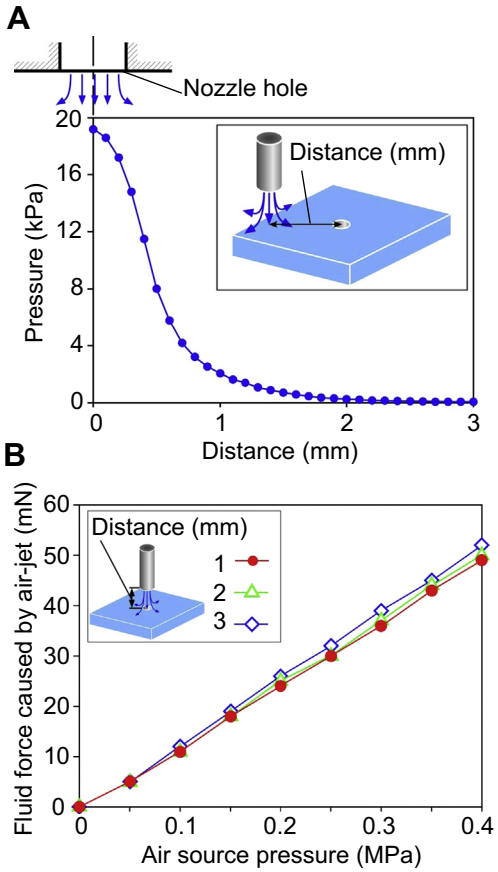

Fig. 3. Air-jet characterization of the wettability assessment device. Graph (A) The pressure distribution of air-jet was measured by a pressure sensor, which was able to move to the horizontal direction with an interval of 0.1 mm. The vertical axis is the pressure measured by the pressure sensor. The horizontal axis is the distance between a specific point under the air-nozzle and the probe hole of pressure sensor. Graph (B) shows the relationship between fluid force caused by air-jet and air source pressure at the inside of air-nozzle. Red, green, and blue lines show distances between air-nozzle and an object of 1, 2, and 3 mm, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)