Journal of Bioscience and Bioengineering
VOL. 117 No. 1, 113–121, 2014

ELSEVIER

www.elsevier.com/locate/jbiosc

Noninvasively measuring respiratory activity of rat primary hepatocyte spheroids by scanning electrochemical microscopy

Ryosuke Takahashi,[1,*] Yuanshu Zhou,[2] Yoshiko Horiguchi,[2] Hitoshi Shiku,[2] Hiroshi Sonoda,[1,3] Naoshi Itabashi,[4] Jiro Yamamoto,[4] Taku Saito,[3] Tomokazu Matsue,[2,5] and Akiko Hisada[1]

Central Research Laboratory, Hitachi Ltd., Hatoyama, Saitama 350-0395, Japan,[1] Graduate School of Environmental Studies, Tohoku University, Aramaki-Aoba 6-6-11, Sendai 980-8579, Japan,[2] Social Innovation Business Project Div., Hitachi Ltd., Sotokanda, Chiyoda, Tokyo 101-8608, Japan,[3] Central Research Laboratory, Hitachi Ltd., Higashi-koigakubo, Kokubunji, Tokyo 185-8601, Japan,[4] and WPI Advanced Institute for Materials Research, Tohoku University, Kitahara 2-2-1, Sendai 980-8577, Japan[5]

Received 22 May 2013; accepted 20 June 2013
Available online 25 July 2013

Construction of an *in vitro* drug screening method for evaluating drug metabolism and toxicity by using cells is required instead of the conventional *in vivo* one that uses animals. In order to realize the *in vitro* study, analyzing the cellular activity or viability noninvasively in advance of the screening is essential. The aim of the current study is to establish a method that can evaluate the cellular activity depending on spheroid sizes by means of oxygen consumption and to determine the valid diameter of hepatocyte spheroids. To measure the respiratory activity of the spheroids, which were formed on a nanopillar sheet, we applied scanning electrochemical microscopy (SECM). From the viewpoint of high respiratory activity and its small variation, we determined that spheroids with 70 μm in diameter were adequate. We then performed a gene expression analysis by using a real-time PCR to evaluate the correlation with respiratory activity. As a result, a higher expression level of Hnf4α, which is essential for hepatocytes to fulfill many liver functions and is the indicator of well-differentiated hepatocytes, showed relatively higher respiratory activity. We concluded that the noninvasive SECM technique could evaluate the cellular activity of a single spheroid. Noninvasively measuring cellular activity by SECM makes it possible to evaluate the cellular activity prior to a nonclinical test and enables the continued monitoring of the drug response by using single spheroid. SECM becomes a powerful tool to satisfying the increasing demand for an *in vitro* system in the course of new drug development.

[**Key words:** Spheroid; Nanopillar sheet; Scanning electrochemical microscopy; Real-time PCR]

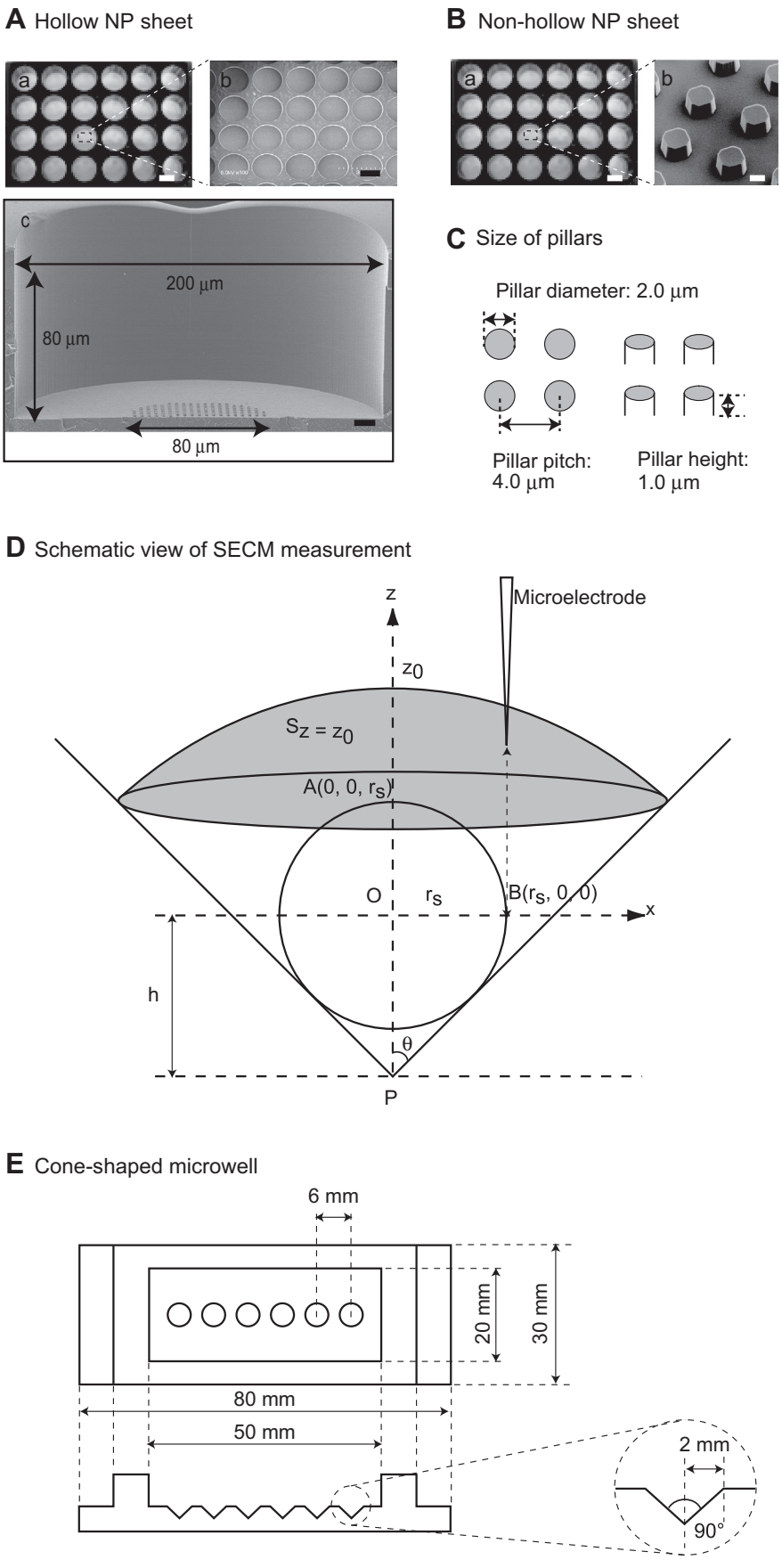
Existing methodologies for evaluating drug metabolism and toxicity in the development of new drugs are performed using animals. However, testing in animals does not always predict drug metabolism in humans because of species differences, which often results in unacceptable clinical efficacy and poor pharmacokinetics (1–3). An alternative *in vitro* screening system that uses human hepatocytes is required instead of the conventional *in vivo* study (4). In order to establish an *in vitro* screening system, we need hepatocytes that possess a polarized structure and differentiated functions close to those of the native liver. Moreover, when using the cells in a drug screening, we also need to develop a method that can measure the cellular activity and quality noninvasively, which provides a benefit that the viability of the spheroid can be evaluates prior to the nonclinical test and the drug response can be monitored continuously at single spheroid level. To resolve these two issues would surely become a great advantage for establishing an *in vitro* system.

In order to obtain the hepatocytes as above, we have developed a culture device for forming three-dimensional (3-D) spheroids, the nanopillar (NP) sheet. 3-D hepatocyte spheroids sustain viability for extended culture periods and enhance high levels of liver specific functions, such as albumin secretion, urea synthesis, or cytochrome P450 (P450) activity, better than does conventional two-dimensional (2-D) monolayer tissue (5–13). Accordingly, spheroid culture is an alternative culturing technique for predicting the metabolic process in all native environments (3,6). We previously reported that the spheroid formation of rat primary hepatocytes was able to be controlled by optimizing the pillar diameter and the pillar pitch (pillar center-to-center distance) of the NP sheet and that the spheroids possessed higher viability and polarity (9). The functions of hepatocytes were enhanced by combining spheroid formation and Matrigel (BD Bioscience, MA, USA) overlaying (9).

For measuring the cellular activity, we have been interested in scanning electrochemical microscopy (SECM). SECM is a technology for measuring the local distribution of electroactive species by scanning with the tip of a microelectrode used as a probe with higher spatial resolution (14–18). Application to a biological environment at the single-cell level is a promising field for SECM to quantitatively characterize various biological functions under physiological conditions (16,17,19–22). Oxygen consumption around the cells measured by SECM directly reflects the cellular respiratory activity. In the previous study, SECM was applied to

* Corresponding author: Tel.: +81 49 296 6111; fax: +81 49 296 5999.
E-mail address: ryosuke.takahashi.jr@hitachi.com (R. Takahashi).

1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2013.06.021


A Hollow NP sheet
a
b
c
200 μm
80 μm
80 μm
B Non-hollow NP sheet
a
b
C Size of pillars
Pillar diameter: 2.0 μm
Pillar pitch:
4.0 μm
Pillar height:
1.0 μm
D Schematic view of SECM measurement
z
Microelectrode
z0
Sz = z0
A(0, 0, rs)
O
rs
B(rs, 0, 0)
x
h
θ
P
E Cone-shaped microwell
6 mm
20 mm
30 mm
80 mm
50 mm
2 mm
90°