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A novel effect of parylene-based surface coating on HepG2 cell function

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ABSTRACT

Parylene-C (diX C) has been used as a surface coating material with many biological applications; diX AM, a member of the diX C parylene family, retains biocompatible features. Previously, it has been reported that diX AM shows high cell adhesiveness; however, the effect of diX AM on the function of cells remains unknown. In this study, we investigated cell morphology and gene expression in human hepatocellular carcinoma (HepG2) cells cultured on diX AM. Our results show that HepG2 cells adhered to the surface of diX AM, and retained morphology similar to that of the cells cultured on collagen-coated surfaces. Furthermore, microarray analysis has revealed that the expression of CYP1A1 and CYP1A2 was highly induced in HepG2 cells cultured on diX AM without any additional factors. Moreover, CYP1 enzymatic activity measured by ethoxyresorufin-*O*-dealkylase (EROD) assay corresponded with the induction of gene expression. These results indicate a novel effect of diX AM on HepG2 cell function for the first time and diX AM could be used as non-animal-derived material for cell culture.

1. Introduction

Function and morphology of the cells are highly dependent on the character of the surface to which the cells are attached. In cell-based assays, extracellular matrix (ECM) components, such as collagen, laminin, and fibronectin, are the compounds most widely used for attaining cellular adhesiveness and induction of cell function. However, the ECM is chemically and functionally unstable because it is biologically produced.

Parylene, commercially known as diX, has been widely used in various applications. In particular, poly(chloro-para-xylylene) (diX C) has been used as a coating material for insulating implantable biomedical devices, such as stents, defibrillators, pacemakers, and other devices permanently implanted in the body [1]. In cell biology, diX C has been used mainly as a coating to insulate neural electrodes [2–4]. Chemical vapor deposition of parylene provides highly conformal, transparent, and homogeneous coatings on virtually any substrate, which can protect substrates from electrical charge, moisture, and chemicals [1,4]. However, diX C cannot support and promote neuronal cell growth because of its hydrophobic characteristics, which results in extremely low adhesiveness of neuronal cells [5,6]. Thus, diX C is not suitable as a scaffold for cell culture.

Other functional materials in the diX family, such as diX N, diX SF, diX A, and diX AM, have become commercially available. These materials retain physical characteristics similar to those of diX C; in addition, they have other features that improve biocompatibility over that demonstrated by diX C. Recently, it has been reported that poly(monoaldehyde-para-xylylene) (diX H) and poly(monoaminomethyl-para-xylylene) (diX AM) (see Fig. 1 for chemical structure) achieved high cellular adhesiveness in neuronal cell cultivation and were capable of supporting rat adrenal pheochromocytoma (PC12) cells [5,6]. However, the effect of these materials on cellular functions remains unknown.

In this study, we investigated the effect of diX AM on the cellular functions of human hepatocellular carcinoma (HepG2) cells. Adhesiveness and morphology of the cells cultured on diX AM were examined and compared with the cells cultured on tissue culture-treated plates and those on collagen-coated plates. Furthermore, we performed global gene expression analysis using microarray to determine the differences in gene expression between the HepG2 cells cultured on collagen and those on diX AM. In addition, the effect of diX AM on liver-specific cell functions, such as activity of cytochrome P450 (CYP) and albumin secretion was investigated.

2. Materials and methods

2.1. Preparation of parylene-coated cell culture plates

A single layer of diX AM coating on the surface of 24-well PS culture plates (Asahi Glass, Tokyo, Japan) was obtained by chemical vapor deposition using a parylene deposition system (KISCO, Tokyo, Japan). The diX AM dimer was vaporized at 110 °C and the temperature increased gradually to 180 °C, and pyrolyzed into its monomer form at 550 °C, then the monomer molecules in vapor phase were deposited and polymerized at room temperature on the substrate surface to

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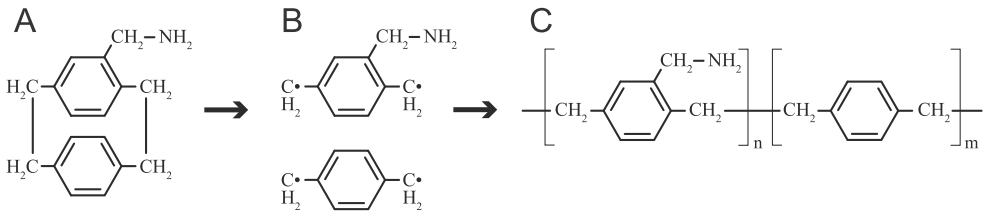

Fig. 1. Chemical structure of poly(monoaminomethyl-para-xylylene) (diX AM) and schematic diagram of chemical vapor deposition method. (A) Dimer structure of diX AM. (B) The dimer is vaporized and pyrolyzed into monomer gas. (C) The monomer molecules polymerize on the entire surface of substrate to form a conformal coating.

form a conformal coating. The details of the coating method have been described in [1]. The thickness of the diX AM coating on the culture plate surface was controlled by the deposition time. The optimal thickness was determined to be 1 μm, which is sufficient for cell adhesion. The diX AM-coated cell culture plates were provided by Daisan Kasei (Tokyo, Japan).

2.2. Cell culture

HepG2 cells were obtained from the American Type Culture Collection (ATCC) and were used in all experiments. HepG2 cells were maintained on 100-mm tissue culture-treated plates in Dulbecco's modified Eagle medium (DMEM, Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Sigma–Aldrich, St. Louis, MO), 1% penicillin–streptomycin–amphotericin B suspension (Wako Pure Chemical, Osaka, Japan), and 1% MEM Non-Essential Amino Acids Solution (Life Technologies) at 37 °C in a humidified atmosphere of 5% CO_2. For experiments, cells were placed in 24-well tissue culture-treated plates (Becton, Dickinson and Company, New Jersey, USA), collagen-coated plates (Sumitomo Bakelite, Tokyo, Japan) or diX AM-coated plates (Daisan Kasei) at a density of 8.0×10^4 cells. The number of cells was determined using a Countess Automated Cell Counter (Life Technologies) with the dye exclusion using 0.2% trypan blue dye.

2.3. Microarray analysis

Total RNA from cells cultured on tissue culture-treated, collagen-, or diX AM-coated plates for 3 days were prepared using a RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Synthesis of first-strand cDNA from 250 ng of total RNA and in vitro transcription (IVT) reaction to generate biotinylated cRNA were carried out using the 3′ IVT Express kit (Affymetrix, Santa Clara, CA). For hybridization, 10 μg of fragmented cRNAs were incubated in hybridization solution at 45 °C for 16 h. cDNA synthesis, labeling, and hybridization were carried out according to the manufacturer's protocol. All experiments were performed using a Human Genome U133 Plus 2.0 array chip (Affymetrix). The expression data obtained were normalized to the median, and raw expression data were cut off at 100 to eliminate nonspecific background. The expression data of genes flagged as present or marginal and at least 2-fold changes in expression levels compared to that in cells cultured on tissue culture-treated plate were extracted.

2.4. Quantitative RT-PCR

Total RNA was prepared from cells cultured on tissue culture-treated, collagen-, or diX AM-coated plates using a RNeasy kit (Qiagen) according to the manufacturer's instruction. Reverse transcription of the total RNAs was performed using an oligo-dT primer and PrimeScriptII RTase (Takara Bio, Shiga, Japan) according to the manufacturer's instruction. Quantitative RT-PCR was performed using Power SYBR Green PCR Master Mix (Life Technologies) on the Applied Biosystems 7500 Real-Time PCR System (Life Technologies) under the following conditions: 2 min at 50 °C; 10 min at 95 °C; 40 cycles each consisting of 15 s at 95 °C and 1 min at 60 °C. To calculate the relative expression levels of target genes, glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) transcripts were used as an endogenous reference. Primer sequences used in this study are listed in Table 1.

2.5. Ethoxyresorufin-O-deethylase (EROD) assay for CYP1 activity

Culture media from HepG2 cells cultured on 24-well tissue culture-treated, collagen-, or diX AM-coated plates for 3 days were changed to assay media containing 10 μM ethoxyresorufin. After incubation for 1 h in a CO_2 atmosphere, the assay media were collected. Fluorescence produced as a result of the conversion of ethoxyresorufin to resorufin by CYP1 family enzymes was measured using a fluorescence spectrophotometer with an excitation wavelength of 530 nm and an emission wavelength of 610 nm. The number of cells was determined using a Countess Automated Cell Counter, and the data of EROD activity obtained were normalized to the number of cells.

2.6. Enzyme-linked immunosorbent assay (ELISA) of human albumin

Culture media from HepG2 cells cultured on 24-well tissue culture-treated, collagen-, or diX AM-coated plates for 3 days were collected. The concentration of human albumin was determined using a human enzyme-linked immunosorbent assay kit according to the manufacturer's instruction (Bethyl Laboratories, Montgomery, TX). The obtained data of ELISA measurements are normalized to the number of cells.

2.7. Statistical analysis

All the data in this study are reported as mean with standard deviation. Statistical analyses were performed with KaleidaGraph 4.1 software using ANOVA. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Cell growth and morphological characterization

HepG2 cells were cultured on tissue culture-treated, collagen-, or diX AM-coated plates and the morphology of the cells examined. HepG2 cells cultured on tissue culture-treated plates proliferated and formed cell clusters (Fig. 2). In contrast, cells cultured on collagen- or diX AM-coated plates proliferated without forming cell clusters. The

Table 1
Primer sets used for quantitative RT-PCR analysis.

Gene	Forward primer	Reverse primer
CYP1A1	ggagctagacacagtgattggc	ggtgaaggggacgaagga
CYP1A2	gggcacttcgacccttacaa	gcacatggcaccaatgacg
AKR1C1	agacattgttctggttgcctat	aagggtcaaatatcgcacat
HMGCR	taccatgtcaggggtacgt	caagcctagagacataatcat
GSTA1	agagccctgattgatatgta	gttgccaacaaggtagtctt
GCLM	atcagtgggcacaggtaaaa	tgaccgaataccgcagtag
ALB	cgttcccaaagagtttaatgc	aagctgcgaaatcatccataac
GAPDH	gcggggctccagaacatcat	ccagccccagcgtcaaggtg