Metabolic Characterization of Primary Rat Hepatocytes Cultivated in Parallel Microfluidic Biochips

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ABSTRACT: The functionality of primary rat hepatocytes was assessed in an Integrated Dynamic Cell Cultures in Microsystem (IDCCM) device. We characterized the hepatocytes over 96 h of culture and evaluated the impact of dynamic cell culture on their viability, inducibility, and metabolic activity. Reverse Transcription quantitative Polymerase Chain Reaction (RTqPCR) was performed on selected genes: liver transcription factors (*HNF4α* and *CEBP*), nuclear receptors sensitive to xenobiotics (*AhR*, *PXR*, *CAR*, and *FXR*), cytochromes P450 (CYPs) (*1A2*, *3A2*, *3A23/3A1*, *7A1*, *2B1*, *2C6*, *2C*, *2D1*, *2D2*, and *2E1*), phase II metabolism enzymes (*GSTA2*, *SULT1A1*, and *UGT1A6*), ABC transporters (*ABCB1b* and *ABCC2*), and oxidative stress related enzymes (*HMOX1* and *NQO1*). Microperfused-cultured hepatocytes remained viable and differentiated with *in vivo*-like phenotype and genotype. In contrast with postadhesion gene levels, the first 48 h of perfusion enhanced the expression of xenosensors and their target CYPs. Furthermore, *CYP3A1*, *CYP2B1*, *GSTA2*, *SULT1A1*, *UGT1A1*, *ABCB1b*, and *ABCC2* were upregulated in IDCCM and reached above postextraction levels all along the duration of culture. Metabolic activities were also confirmed with the detection of metabolism rate and induced mRNAs after exposure to several inducers: 3-methylcholanthrene, caffeine, phenacetin, paracetamol,, and midazolam. Finally, this metabolic characterization confirms that IDCCM is able to maintain rat hepatocytes functions to investigate drug metabolism. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3264–3276, 2013

Keywords: hepatocytes; hepatic metabolism; drug delivery system; cytochrome P450; phase II metabolism; efflux pumps; *in vitro* models

INTRODUCTION

Hepatic metabolism is a major pathway to improve or inhibit toxic effect of some chemical compounds. Several researches aim at developing new *in vitro* powerful methods to predict the liver xenobiotic metabolism and toxicity. New systems based on microscale bioartificial organs have been proposed using recent developments in the field of microtechnology and allow designing *in vitro* systems on a very microenvironment.[1–6] Previous works in our laboratory developed microfluidic-biochip-based approach that create a specific microenvironment where the surface to volume ratio and the microstructuration of cell-culture chamber contribute to enhance specific tissue properties.[7] Thus, when hepatocytes (human primary cells or HepG2/C3A cell lines) are cultivated in microenvironment, activities and mRNA levels of cytochromes P450 (CYPs) and drug transporters, related to the drug metabolism and multidrug resistance phenotype, respectively, were upregulated compared with the static cultures.[4,8–10]

Moreover, dynamic perfusion circuit provides a continuous cell supplying and/or xenobiotic exposure in biochip.[1,11,12] Indeed, perfusion can reproduce some liver zonation aspects via nutrient gradients along the tissue and via specific liver differentiation according to the cell position in the biochip.[13–15] Furthermore, *in vitro* data issued from microfluidic systems can be used for *in silico* pharmacokinetic model to

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extrapolate *in vivo* exposure.[5,16] Finally, a first attempt to use the liver microfluidic biochip for biomarker researches and predictive toxicology applications has been proposed.[17,17] To use these microfluidic biochips for high-throughput screening, parallelized systems were created by integrating conventional multiwell plates formats, which are compatible with others emerging or conventional technologies of bioanalyses (such as microscopes, robotic handlers,[6] integrated peristaltic actuators,[19] or external fluid pumping system[20]). In addition, when compared with our first generation of biochips,[10,21] the extension of the number of cell-culture conditions allowed testing simultaneously different concentrations of chemical compounds.

In this paper, we have evaluated the performance of such parallelized biochips with the culture of primary rat hepatocytes. Indeed, primary human hepatocytes are preferentially used to predict *in vivo* toxicity but are limited by availability of donor material and the large variability between the donors. Therefore, primary hepatocytes isolated from other mammals (mouse, rats) are used as an alternative to human tissue.[22] Nevertheless, the maintenance of differentiation of primary hepatocytes is a major requirement to achieve physiological metabolism and toxicity.[23,24] We characterized phenotype and genotype of hepatocytes cultivated in an "Integrated Dynamic Cell Cultures in Microsystems" (IDCCM) to demonstrate the advantages of this new parallelized system applied to toxicology assessment.

MATERIAL AND METHODS

Microfluidic Biochips and IDCCM Box

The entire setup was called the IDCCM. The biochips design and microfabrication were described in Baudoin et al. (2011).[25] The biochips are made in polydimethylsiloxane (PDMS) conventional moulding (Fig. 1a). The concept and detail of the IDCCM box are presented in detail in our previous work.[20,26] Briefly, the PDMS biochips were connected at the bottom of the IDCCM box by a simple series of "plugging" ports. This format allowed an "easy plug and display" of the biochips to external setup such as microscopes. IDCCM is a manufactured polycarbonate box using the conventional format of 24-well plate. Each microfluidic biochip was connected between two wells. The 24 wells were used as entrance and outlet reservoir leading to the parallelized culture of 12 biochips (Figs. 1b and 1c). A specific cover was designed to allow the hermetic closure of the polycarbonate box for continuous flow perfusion. The cover includes ports for fluid perfusion and sampling. The hermetic closure of the IDCCM box and the pressure in the IDCCM box avoid any leakage or reservoir drain (Fig. 1c).

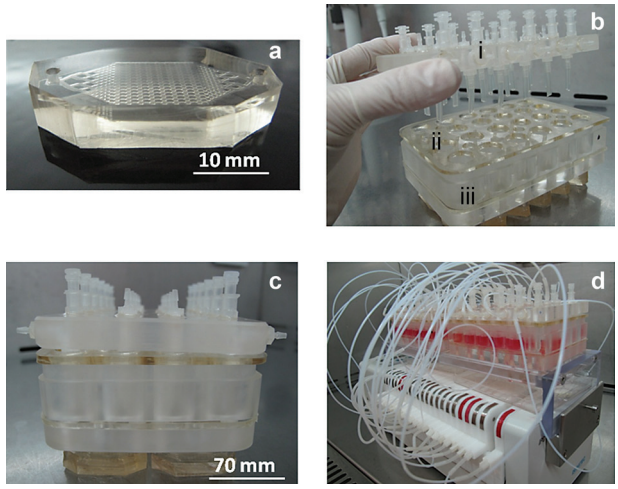

Figure 1. The IDCCM box with (a) PDMS biochip and (b) three compartments of IDCCM, from top to bottom: (a) the fluidic cover, (b) the silicon joints, and (c) the 12 biochips connected under the 24-well polycarbonate plate. (c) The side view of IDCCM and (d) two IDCCMs connected to the peristaltic pump with 24 PTFE tubes. Corresponding bars were indicated in mm in views a and c.

Primary Rat Hepatocytes Culture in IDCCM

The experiments were performed for 5 days. It included three different phases: the day of hepatocyte extraction (day 0), the adhesion phase (day 1), and 72 h of perfusion phase (from day 2 to day 4). The sterilization of the biochips of the IDCCM box and the perfusion circuit was performed by autoclaving all the setup. The biochips were then connected to the box under sterile condition.

Primary Rat Hepatocytes Isolation

Primary hepatocytes from 5-week-old male Sprague–Dawley rats weighing about 250 g were isolated by a modification of the two-step *in situ* collagenase perfusion according to the method of Seglen.[27] Animals were provided by the Janvier Elevage Animal Center (Le Genest Saint Isle, France). They were housed at the University of Compiegne with a 12 h light/dark cycle at 22°C with food and water *ad libitum*. All experiments were approved by the Animal Experimental Committee of the University of Compiegne. The animal was anesthetized by intraperitoneal injection of sodium pentobarbital (Centravet, Gondreville, France). Briefly, after *in situ* washing and perfusion with collagenase solution at 30 mL/min, the liver was extracted, and the digested liver tissues were filtered through cotton gauze, then 400 and 100 µm filters. The cell suspension was centrifuged and washed three times. Percoll isogradient centrifugation was performed to isolated dead cells and a significant portion of the nonparenchymal cells in a floating top