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Characterization of rat or human hepatocytes cultured in microphysiological systems (MPS) to identify hepatotoxicity

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ABSTRACT

The liver is the main site for drug and xenobiotics metabolism, including inactivation or bioactivation. In order to improve the predictability of drug safety and efficacy in clinical development, and to facilitate the evaluation of the potential human health effects from exposure to environmental contaminants, there is a critical need to accurately model human organ systems such as the liver *in vitro*. We are developing a microphysiological system (MPS) based on a new commercial microfluidic platform (Nortis, Inc.) that can utilize primary liver cells from multiple species (*e.g.*, rat and human). Compared to conventional monolayer cell culture, which typically survives for 5–7 days or less, primary rat or human hepatocytes in an MPS exhibited higher viability and improved hepatic functions, such as albumin production, expression of hepatocyte marker HNF4α and canaliculi structure, for up to 14 days. Additionally, induction of Cytochrome P450 (CYP) 1A and 3A4 in cryopreserved human hepatocytes was observed in the MPS. The acute cytotoxicity of the potent hepatotoxic and hepatocarcinogen, aflatoxin B_1, was evaluated in human hepatocytes cultured in an MPS, demonstrating the utility of this model for acute hepatotoxicity assessment. These results indicate that MPS-cultured hepatocytes provide a promising approach for evaluating chemical toxicity *in vitro*.

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1. Introduction

There has been growing interest over the past decade in developing advanced *in vitro* and/or animal-free models such as *in silico* PK/PD modeling, pathway-based toxicity modeling, and microphysiological systems (MPS) (Esch et al., 2015; Huh et al., 2011) to improve the predictability of pharmacological and toxicological outcomes, and to reduce the use of experimental animals for systems-level analyses. MPS represents an interconnected set of cellular constructs designed to recapitulate the structure and function of human organs, frequently referred to as "organs-on-chips", "*in-vitro* organ constructs", or "organoids" (Wikswo, 2014). MPS platforms utilize a microfluidic device with flow driven by either a perfusion or pneumatic pump through 3D cell constructs imbedded in an extracellular matrix (ECM) gel. Microfluidics that have small volumes (microliters to attoliters) of fluids are integrated in these systems and are used to create microenvironments with dynamic fluid flow and gradients recapitulating human organs (Esch et al., 2015).

Currently, the most commonly used *in vitro* model for *in vitro* preclinical pharmacology and predictive toxicology is monolayer cell culture in plastic flasks or plates using mammalian cells, also known as conventional two-dimensional (2D) culture. Although conventional 2D culture has provided significant contributions to biological research, these basic approaches have numerous limitations, including limited nutrient and metabolite transportation by diffusion, poor mimicry of extracellular concentrations *in vivo*, and difficulties recapitulating heterogeneous tissue microenvironments (Wikswo, 2014). Unlike conventional 2D culture, MPS cultures have the potential to provide an optimal microenvironment for heterogeneous cell growth and differentiation that more closely mimics the physiological responses of tissues *in vivo* (Esch et al., 2015; Huh et al., 2011; Griffith et al., 2014; Huh et al., 2010). Furthermore, in contrast to standard 2D cell culture models, MPS are capable of modeling cell-cell, drug-cell, drug-drug, and organ-drug interactions *in vitro*. By potentially recapitulating *in vivo* physiology and

Abbreviations: MPS, microphysiological system; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; BNF, beta-naphthoflavone; Rif, rifampin; EROD, ethoxyresorufin-O-deethylase; CYPs, cytochrome P450s; HNF4α, hepatocyte nuclear factor 4 alpha; MDZ, midazolam; AFB, aflatoxin B_1; BSEP, bile salt export pump; MRP2, multidrug resistance-associated protein 2.

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biochemistry, MPS may be able to more accurately predict chemical toxicity than traditional 2D cultures (Knudsen et al., 2015).

The liver is the main organ that metabolizes drugs and environmental chemicals and the most important organ when it comes to predictive evaluation of compounds for efficacy and toxicity, including both detoxification and bioactivation of drugs and other xenobiotics. In order to improve the predictability of drug safety and efficacy in clinical development, and to have a clear perspective of the potential human health effects from exposure to environmental chemicals, there is a critical need to accurately model liver function using the MPS approach. Here we describe the development and basic validation of rat and human 'liver-on-chip' *in vitro* models using a commercial MPS (Nortis, Inc.). The major goals of this study were to characterize how primary hepatocytes function in the MPS culture system compared to conventional 2D culture, and to validate this liver MPS as a potentially new *in vitro* approach for assessing chemical toxicity and metabolism that allows direct comparison of human and experimental animal responses. As a proof of concept experiment to demonstrate liver MPS bioactivation of a hepatic toxin, we exposed the liver MPS to AFB and evaluated cell viability and toxicity.

2. Materials and methods

2.1. Sources of rat and human primary hepatocytes

Freshly-isolated human hepatocytes or cryopreserved human hepatocytes were purchased from Triangle Research Labs (TRL; donors HUM4037, HUM4038, HUM 4055A, HUM4080 and HUM4096A), or received from The Liver Tissue Cell Distribution System (LTCDS; NIH service; donors 14-018, 15-001,15-002,15-004). All demographic information on hepatocyte donors is listed in Supplementary Table S1. Primary rat hepatocytes were isolated by two-step collagenase digestion following isolation of the liver from male Sprague Dawley® rats (Charles River Laboratories, Seattle, WA) (Shulman and Nahmias, 2013).

2.2. Conventional 2D and MPS cell cultures

Cell culture supplies and materials were from Thermo Fisher Scientific, except for the aflatoxin toxicology experiments, where tissue culture medium from Triangle Research Labs (TRL)/Lonza was used. Plating medium composed of William's E medium supplemented with 5% fetal bovine serum (FBS), 100 IU/ml penicillin, 100 mg/ml streptomycin, 100 nM dexamethasone, 1 μg/mL of Gibco® Fungizone®, 100× diluted ITS + (final concentration of insulin/transferrin/selenium was 6.25 μg/mL), and 0.2 mM glutaMAX supplement (Thermo Fisher) was used. The maintenance media were the same formula as plating medium but without FBS.

Primary rat or human hepatocytes were cultured in 2D configuration on 24-well plates coated with type I collagen, or were seeded into Nortis ™ devices for MPS culture (Fig. 1). For the 2D culture, primary rat or human hepatocytes were seeded at a density of 2.1×10^5 cells/cm^2 into type I collagen coated plates. After 4 h of plating cell, the hepatocytes in 2D plates were switched to ice- cold Matrigel® containing (Corning®, Oneonta, NY, Cat#356234, lot#5131014, final conc. 0.23 mg/mL) maintenance medium. For MPS culture *via* chamber seeding (Fig. 1A), 0.2 to 0.3 mL of a cell suspension at a density of 1×10^6 hepatocytes/mL mixed with type I collagen (final conc. is 2–3 mg/mL, Ibidi USA, Inc. Madison, Wisconsin) was injected into each device *via* ablumenal ports. All devices were pre-coated with 0.1 mg/mL of collagen type I in PBS with 0.1% acetic acid at 37 °C for 1 h. For MPS culture *via* channel seeding (Fig. 1B), 2 to 5 μL of a cell suspension at a density of 1×10^7 hepatocytes/mL was injected into each chip with pre-gelled collagen type I (6 mg/mL) *via* the injection port using a sterile 5 μL Hamilton #65 syringe. After 4 h of seeding cells, the hepatocytes were switched to maintenance medium by initiating the lumenal or ablumenal flow at flow rates between 5 and 30 μL/h using an infusion syringe pump (KD Scientific Inc. model# KDS220). Following maintenance of cells at 37 °C in a 5% CO_2 incubator overnight, cells were subsequently maintained in an incubator in a humidified atmosphere at 37°C in 5% CO_2/95% air. The effluent media from the MPS cultures and conditioned media from 2D cultures were collected at various time points and stored at −80 °C for later measures of LDH or ALT, and albumin.

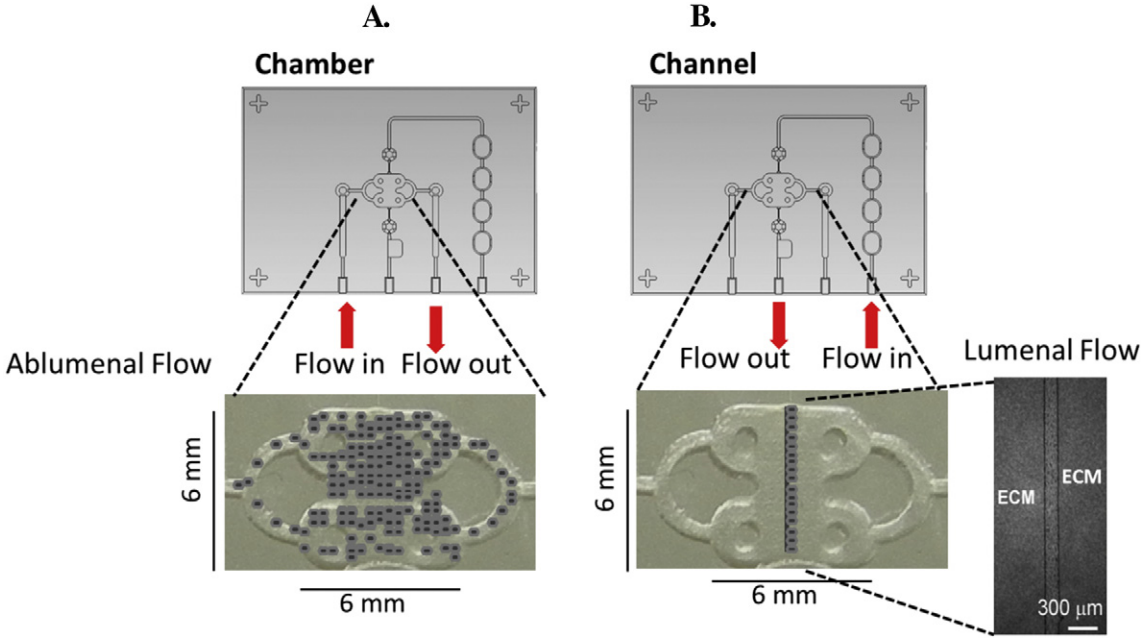

Fig. 1. The methods used in populating hepatocytes inside the MPS. Methods of injecting cells included (A) chamber seeding (ablumenal flow) and (B) channel seeding (lumenal flow) with high concentration (3 mg/mL), or low concentration (1.3 mg/mL) of type I collagen, or Matrigel™ (0.2 mg/ml). Extracellular matrix (ECM) such as type I collagen (6 mg/mL) were used for filling the MPS chips prior to channel seeding. Grey dots represent seeded hepatocytes.

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