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Predictive toxicology using systemic biology and liver microfluidic "on chip" approaches: Application to acetaminophen injury

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ABSTRACT

We have analyzed transcriptomic, proteomic and metabolomic profiles of hepatoma cells cultivated inside a microfluidic biochip with or without acetaminophen (APAP). Without APAP, the results show an adaptive cellular response to the microfluidic environment, leading to the induction of anti-oxidative stress and cyto-protective pathways. In presence of APAP, calcium homeostasis perturbation, lipid peroxidation and cell death are observed. These effects can be attributed to APAP metabolism into its highly reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI). That toxicity pathway was confirmed by the detection of GSH-APAP, the large production of 2-hydroxybutyrate and 3-hydroxybutyrate, and methionine, cystine, and histidine consumption in the treated biochips. Those metabolites have been reported as specific biomarkers of hepatotoxicity and glutathione depletion in the literature. In addition, the integration of the metabolomic, transcriptomic and proteomic collected profiles allowed a more complete reconstruction of the APAP injury pathways. To our knowledge, this work is the first example of a global integration of microfluidic biochip data in toxicity assessment. Our results demonstrate the potential of that new approach to predictive toxicology.

Nomenclature

Superscripts in figures denote information confirmed at
(1) the gene, protein and metabolite levels,
(2) at the gene and protein levels,
(3) at the gene and metabolite levels,
(4) at the gene level,
(5) at the protein level,
(6) at the metabolite level.

Capital letters (G6PD …) refer to gene symbols,
Chemical species in italics (as in *g6pd* or *glucose-6-phosphate dehydrogenase*…) refer to proteins or gene products,
Species in regular typeface (glucose…) refer to metabolites

Introduction

Currently, *in vitro* cell culture methods for screening molecules mainly use plates (Petri dishes). Hepatocytes are considered to be among the most difficult type of cells to maintain in vitro in such systems. However it is essential to use hepatocytes to understand and model metabolic phenomena (Guillouzo, 2008). That is why many tissue engineering processes have been developed to provide better environments for hepatocyte maintenance and development (Gebhardt et al., 2003; De Bartolo and Bader, 2001; Franklin and Yost, 2000; Guillouzo, 1998; De Kanter et al., 2002). Such environments must reproduce, as closely as possible, the in vivo conditions. Each one of the many in vitro hepatic culture systems, currently available or in development, can be used to answer toxicology or pharmacology questions, but they should be carefully selected to be able to meet the pursued objectives.

One such *in vitro* system, bioartificial organs, seems to be a suitable method for reproducing the behavior of an organ or group of organs as well as the conditions of in vivo exposure. Bioartificial organs can now take advantage of recent developments in microtechnology

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to produce systems on a very small scale (Griffith and Naughton 2002; Powers et al., 2002; Sivaraman et al., 2005; Chao et al., 2009; Prot et al., 2011a; Baudoin et al., 2007; Novik et al., 2010; Baudoin et al., 2011). The cellular organization brought about by the microtopography of these systems and their dynamic microfluidic culture conditions appear to be key features for reproducing *in vivo* environments. These systems can function equally well in closed or open circuit modes, and thus simulate either chronic or acute tissue exposures.

A variety of approaches are available for describing the behavior and activity of cells as they react to stress, such as during exposure to a drug). Transcriptomic, proteomic and metabolomic techniques are part of those (Boverhof et al., 2006). Genomic and transcriptomic methods can provide a near-complete analysis of the hereditary material of living organisms. Proteomics assay all the proteins contributing to the structure and function of a cellular compartment, a cell, a tissue or a whole living organism (Figeys, 2004). Lastly, metabolomics, have also been proposed (Nicholson et al., 1999) to analyze concurrently all the small intermediate or final metabolites produced by chemical reactions taking place in cells or whole organisms. Metabolomics can potentially identify all the changes in biochemical composition and metabolism occurring after exposure to a given substance (Nicholson et al., 1999; Bugrim et al., 2004; Madalinski et al., 2008). All these "omic" approaches can therefore help understanding how a substance acts, at various levels, on an organism.

In our previous work, we showed that hepatocytes cultivated in microfluidic biochip maintained the activity of their main enzymes for xenobiotic metabolism (various CYP, several SULT and UGT subfamilies and some phase 3 transporters such as MDR1 and MRP2) probably due to a stress response enhanced in the biochips (Prot et al., 2011a, 2011b). In a study of the well-known hepatotoxic drug acetaminophen (APAP) in HepG2/C3a cells, we demonstrated that the use of biochips helps reproduce some of its *in vivo* reported mechanism of toxicity, such as GSH depletion and mitochondrial damage (Prot et al., 2011c). To investigate the potential of integrating systems biology and microfluidic biochip technology, we present here the interaction between the transcriptomic, proteomic and metabolomic profiles of HepG2/C3a cells cultivated in a microfluidic PDMS biochip and exposed to APAP. From the integration of those profiles we identified the activation of some liver specific pathways related to drug metabolism. On the basis of our previous work we chose to work at 1 mM APAP, a concentration at which perturbations of cell proliferation and hepatic metabolism are detectable (Prot et al., 2011c). APAP is metabolized by the cytochromes CYP2E1, CYP1A2 and CYP3A4. Secondary metabolism is mediated by glutathione (GSH), sulfo and glucurono conjugations. Thanks to the microfluidic culture conditions, we were able to identify the major biological pathways involved in APAP toxicity to hepatocytes. Finally, a comparison with published *in vivo* studies finally lead to a similar interpretation of

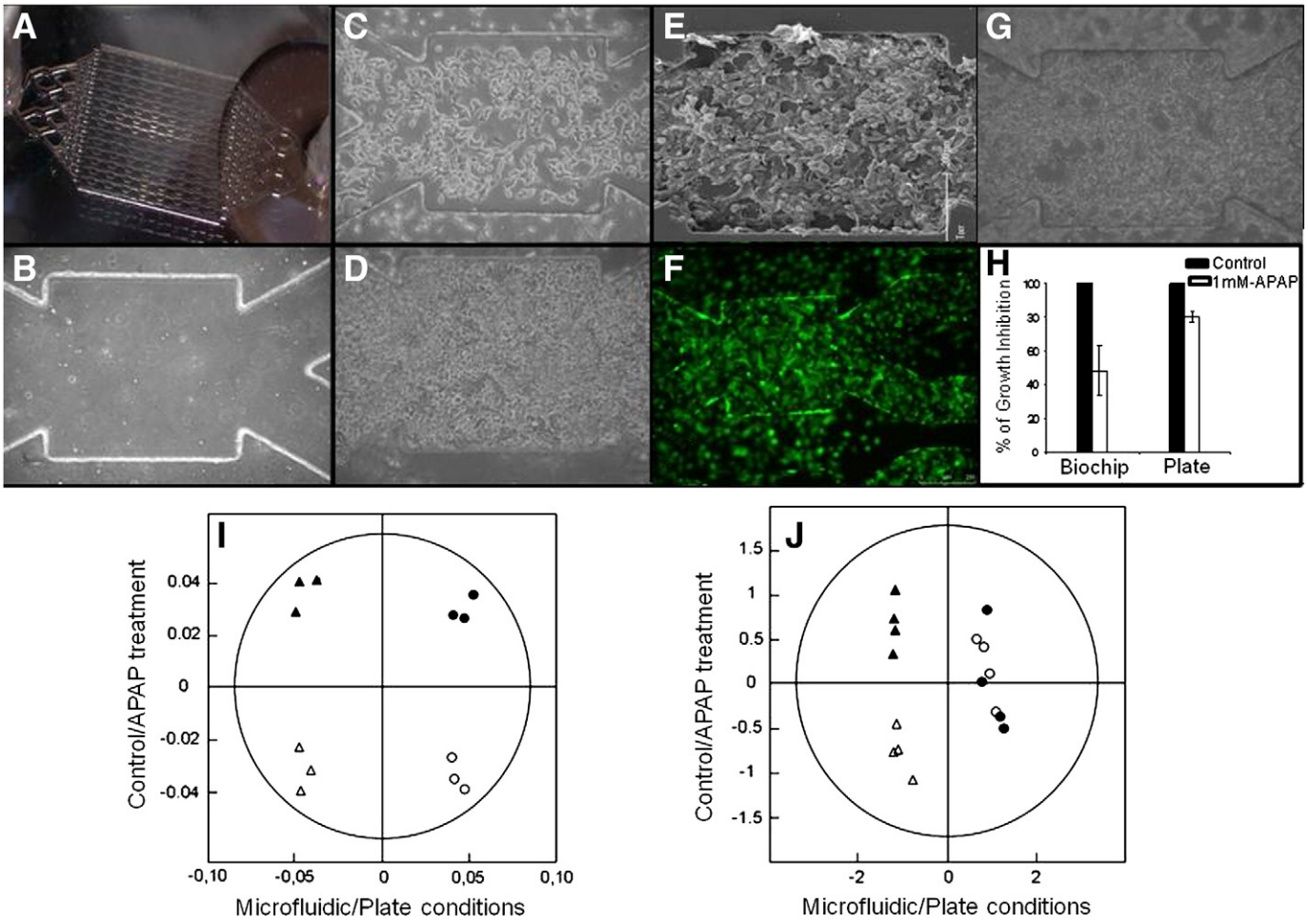

Fig. 1. (A) Microfluidic PDMS network; (B) Cell chamber before cell inoculation; (C) Cell after adhesion; (D) Cell after 96 h of cultures without APAP; (E) SEM view of the cell multilayers in the biochip after 96 h of culture without APAP; (F) viability of the cells after 96 h of culture without APAP; (G) Cells after 96 h of culture including 72 h of APAP treatment; (H) Cell number decreases in biochip and plate with 1 mM of APAP after 96 h of cultures including 72 h of treatment; (I) Results of the PCA of the transcriptomic analysis; (J) Results of the PCA of the proteomic analysis; circles denote plate data, triangles denote biochip data, black symbols are control data and white symbols are APAP data. (Fig. 1 is rebuild using several images coming from our previous works: Prot et al., 2011a, 2011c).