A Dynamic In Vivo Surrogate Assay Platform for Cell-Based Studies

by Gregory T. Baxter and Robert Freedman

Traditional methods of predicting human response to potential therapeutics utilize surrogates: either in vitro cell culture assays or in vivo animal models. In vitro cell-based assays are often of limited predictive relevancy because they do not mimic with sufficient realism the complex environment to which a drug candidate is subjected within a living organism. In vivo animal studies can account for complex intercellular and intertissue effects not observable from in vitro tests, but animal studies are expensive, labor intensive, time consuming, and unpopular. In addition, there is considerable concern whether animal studies can predict human risk precisely enough because, first, there is no known mechanistic basis for extrapolation to low doses, and second, cross-species extrapolation has been found to be frequently problematic with respect to toxicity and pharmacokinetic characteristics.

An in vitro alternative that more precisely simulates in vivo (especially human) response would be beneficial. Such an alternative assay platform would enable a greater proportion of drug candidates to "fail early" in vitro, and, by accurately selecting a smaller proportion of compounds for promotion to in vivo studies, would help debottleneck preclinical testing, accelerating time to market. A cell-based, microfluidic assay platform that addresses this need is discussed here.

Technology

A patented Hurel™ microfluidic circuit (**Hurel Corp.,** Beverly Hills, CA) comprises an arrangement of separate but fluidically interconnected "organ" or "tissue" compartments.* Each compartment contains a culture of living cells drawn from, or engineered to mimic (and thereby represent), the primary function(s) of the respective organ or tissue of a living animal. Microfluidic channels between the compartments permit a culture medium that serves as a blood surrogate to recirculate as in a living system. Drug candidates of interest are added to the culture medium and allowed to recirculate through the device; they then distribute to the cells in the organ compartments and interact with them much as they would in the human body. The effects of drug compounds and their metabolites on the cell type within each respective organ compartment are detected by measuring or monitoring key physiological events such as cell death, cell proliferation, differentiation, immune response, or perturbations in metabolism or signal transduction pathways. The cell types employed may be adherent or nonadherent, and derived from either standard cell culture lines or primary tissue.

*The company and its technology are both referred to as Hurel, and the company's individual devices are similarly known as Hurels.

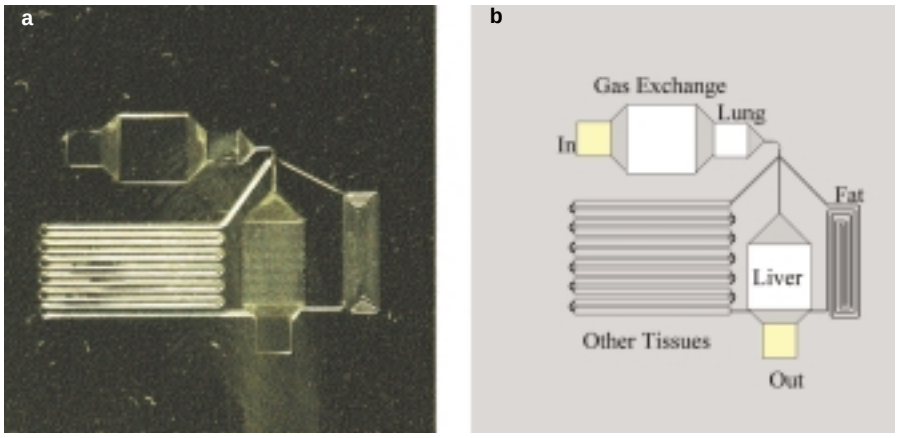

Figure 1 a) Photograph of a four-compartment Hurel, b) schematic diagram of this chip. This four-compartment Hurel was fabricated in silicon using standard microfabrication techniques. In this example, there is a liver compartment, a fat compartment, a lung compartment, and an other tissues compartment.

The physical features of a Hurel device (a Hurel) embody parametric values derived from a physiologically based pharmacokinetic (PBPK) model. The geometry and fluidics of the device are fashioned to replicate the values for drug residence time, circulatory transit time, organ cell density, tissue size, shear stress, and certain other physiological parameters found in the living animal, so as to mimic the fluid-mediated interactions of the organ systems represented in the microfluidic circuit.

One embodiment of the device, depicted in *Figure 1*, consists of four organ compartments: a "liver" compartment to represent the organ primarily responsible for xenobiotic metabolism, a "lung" compartment to represent a target tissue, a "fat" compartment to provide a site for bioaccumulation of hydrophobic compounds, and an "other tissues" compartment to assist in mimicking the circulatory pattern in nonmetabolizing, nonaccumulating tissues. Alternative device geometries can be configured to mimic various animal species, additional organs, or particular experimental applications such as drug absorption or bioavailability.

Experimental methods

For initial validation studies, three-compartment Hurels were fabricated on 22 × 22 mm coverslip-size biochips that were engineered to receive cultures of human cell types. These devices comprise a liver compartment, a target tissues compartment, and an other tissues compartment. The liver compartment was seeded with the human hepatocyte cell line HepG2-C3A, the target tissues compartment was seeded with the human colon cancer cell line HCT116, and the other tissues compartment contained no cells. Once the cells were seeded into their respective compartments, the chips were sealed inside acrylic chip housings. The top of the housings contain fluid interconnects to provide cell culture medium to the chip. Stainless steel tubes are connected to microbore peristaltic pump tubing and inserted into a small hole in the top of a microcentrifuge tube containing culture medium with or without test compound. The pump tubing is connected to the peristaltic pump, primed with the culture medium, and connected to the inlet and outlet ports of the chip housing, thus completing the fluidic circuit. The entire instrument is placed in a CO_2 incubator at 37 °C.

Validation studies: Tegafur

Among the validation studies of the Hurel technology that have been undertaken for the circuit was one using the cancer chemotherapeutic prodrug tegafur (5-fluoro-1-[2-tetrahydrofuryl]-2,4-pyrimidi-nedione). Tegafur is inactive and requires metabolic activation by cytochrome P450 enzymes present in the liver to generate the active metabolite 5′-fluorouracil (5-FU). Like most cancer chemotherapeutic agents, 5-FU induces marked apoptosis in sensitive cells through generation of reactive oxygen species.

Tegafur was added to the recirculating culture medium and pumped through Hurel devices at various concentrations for 24 hr. Following drug exposure, the Hurels were treated with the membrane-permeable nucleic acid dye Hoechst 33342 (**Molecular Probes, Inc.,** Eugene, OR) and the membrane-impermeable nucleic acid dye ethidium homodimer. Using these stains, all cells appear fluorescent blue, but dead cells are marked by the fluorescent red ethidium homodimer (*Figure 2*). In a comparative test, HCT116 cells were cultured on glass coverslips placed in 35-mm culture dishes, and various concentrations of tegafur were added to the culture medium.

With the Hurel system, tegafur was found to be cytotoxic to HCT116 cells in a dose-dependent fashion. However, tegafur was ineffective when tested using the traditional, static cell culture assay. In addition, although the active metabolite 5-FU triggered cell death in the traditional assay, cytotoxicity was not observed until after 48 hr of exposure. In comparison, using Hurel, cytotoxicity was observed after 24 hr of exposure to either 5-FU or tegafur.

To demonstrate that the Hurel liver compartment was responsible for the bioactivation of tegafur, in a separate study Hurels were seeded with HCT116 cells only (i.e., while cells were cultured in the target cell compartment as previously, no cells were cultured in the liver compartment). Either tegafur or 5-FU was added to the recirculating culture medium for 24 hr, and the Hurel biochip was then treated as described above. These experiments found that in the absence of a functional liver compartment, tegafur had no effect on the HCT116 cells, whereas the active metabolite 5-FU caused significant cell death. These results confirm that tegafur is metabolized to an active drug in the Hurel liver compartment, wherefrom it circulates to another organ compartment and is cytotoxic to the target cancer cell.

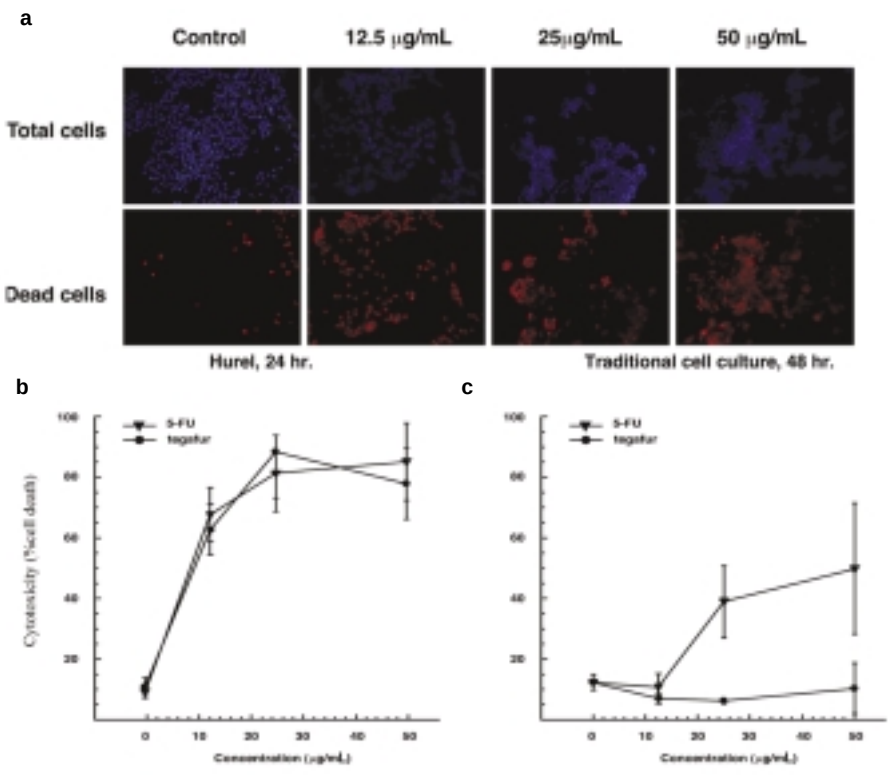

Figure 2 Effect of tegafur on HCT116 cells. a) Fluorescence image of HCT116 colon cancer cells in the target tissues compartment of the Hurel chip. Blue fluorescence (top row) indicates total cells; red fluorescence (bottom row) indicates dead cells. The chips were treated with indicated concentrations of tegafur for 24 hr. b) Graph is a plot of percentage dead cells vs tegafur or 5-FU concentration after 24 hr of recirculation on Hurel. c) Graph is a similar concentration response using a traditional in vitro cell culture assay with HCT116 cells seeded on glass coverslips. After a 48-hr incubation, coverslips were treated as described above and the percentage of cell death was determined.

Conclusion

Embodied on a microfluidic biochip, Hurel's multicompartmental and fluid-circulatory attributes enable it to simulate interorgan or intertissue interactions as found in a living animal. Traditional static cell-based assays do not possess this capability. For this reason, assays run on the Hurel platform may provide higher and more accurate informational content across a wider range of applications (e.g., information on drug absorption, distribution, bioaccumulation, metabolism, efficacy, and toxicity) than is obtainable from static in vitro assays. Hurel should therefore be seen not simply as a technological substitute for the static cell-based assay, but as a more broadly applicable in vivo surrogate assay platform that in various circumstances may precede, supplant, or complement in vivo tests.

The Hurel technology has been successfully tested using a variety of assays (immunohistochemical, immunofluorescent, and others); it is anticipated that it will be adaptable to many different experimental applications and compatible with virtually any type of assay used in traditional, static in vitro formats. Moreover, Hurels can be designed for plug-and-play compatibility with plate readers and other standard laboratory instruments, they can be manufactured and operated at 96-well scale (and smaller), and their use can be automated for increased throughput and reproducibility.

By affording dynamic assessment of potential toxicity, metabolism, and bioavailability, Hurels' capabilities hold the potential to markedly improve the prioritization of drug leads prior to the preclinical (animal) testing phase. Earlier prioritization will reduce the number of animals needed for toxicological testing, decrease the time and cost of preclinical studies, and increase the efficiency of clinical trials.

Dr. Baxter and Mr. Freedman are founders of ***Hurel™ Corp.,*** *8840 Wilshire Blvd., 2nd Fl., Beverly Hills, CA 90211, U.S.A.; tel.: 310-652-5900; fax: 310-358-3285; e-mail: info@hurelcorp.com.*