The Use of the Integrated Discrete Multiple Organ Co-culture (IdMOC®) System for the Evaluation of Multiple Organ Toxicity

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Summary — The application of the Integrated Discrete Multiple Organ Co-culture (IdMOC®) system in the evaluation of organ-specific toxicity is reviewed. *In vitro* approaches to predict *in vivo* toxicity have met with limited success, mainly because of the complexity of *in vivo* toxic responses. *In vivo* properties that are not well-represented *in vitro* include organ-specific responses, multiple organ metabolism, and multiple organ interactions. The IdMOC system has been developed to address these deficiencies. The system uses a 'wells-within-a-well' concept for the co-culturing of cells or tissue slices from different organs as physically separated (discrete) entities in the small inner wells. These inner wells are nevertheless interconnected (integrated) by overlying culture medium in the large outer containing well. The IdMOC system thereby models the *in vivo* situation, in which multiple organs are physically separated but interconnected by the systemic circulation, permitting multiple organ interactions. The IdMOC system, with either cells or tissue slices from multiple organs, can be used to evaluate cell type-specific or organ-specific toxicity.

Key words: *alternative methods, cytotoxicity screening, drug development, human drug toxicity, IdMOC,* in vitro *toxicity, precision-cut slices, primary cultures, tissue slices.*

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Introduction

Experimental approaches for the evaluation of *in vitro* toxicity include the use of tissue slices (1, 2), primary cells (3–9) and cell lines (10–12). They are generally viewed as useful for initial toxicity screening and for the mechanistic evaluation of toxic effects observed *in vivo*. For instance, in the pharmaceutical industry, *in vitro* toxicity screening assays are used routinely during the early phases of drug development, to help in the design of new chemical entities with an acceptable safety profile (13, 14), and in the later phases of drug development, to aid the definition of the human safety profiles of drug candidates with animal toxicity. It is also generally recognised that, due to the complexity of *in vivo* biology and physiology, *in vitro* approaches are inadequate as definitive tools for the assessment of *in vivo* toxicity.

For *in vitro* systems to be further developed as replacements for the use of animals in toxicity testing, it is necessary to understand the key *in vivo* parameters that are critical for the manifestation of toxicity, and to logically and systematically develop experimental systems to model such parameters. The development of the Integrated Discrete Multiple Organ Co-culture (IdMOC®) system is based on our previous success with the application of *in vitro* hepatic systems for the evaluation of drug metabolism and drug–drug interactions in drug development (15, 16). Our current experience with the IdMOC system (17–19) as an *in vitro* model of some of the key *in vivo* parameters for toxicity, is described. The potential of this system to refine, reduce and replace the use of animals in toxicity evaluation will also be discussed.

The Integrated Discrete Multiple Organ Co-culture (IdMOC) System

We consider the following properties to be important, if *in vitro* experimental systems are to successfully model *in vivo* xenobiotic toxicity (20–24):

— xenobiotic metabolism by key organs;
— target cells representative of key organs;
— multiple organ interactions; and
— predictive endpoints.

The IdMOC system (17–19) was developed as an *in vitro* experimental system to model these critical properties. This unique system is based on the concept that, in the animal or human body, there are multiple organs that are physically separated, but are interconnected by the systemic circulation. The systemic circulation permits multiple organ inter-

actions, which may be critical to the final manifestation of toxicity.

The IdMOC system is designed to evaluate organ-specific toxicity that cannot be readily examined by using current *in vitro* systems, due to the following phenomena:

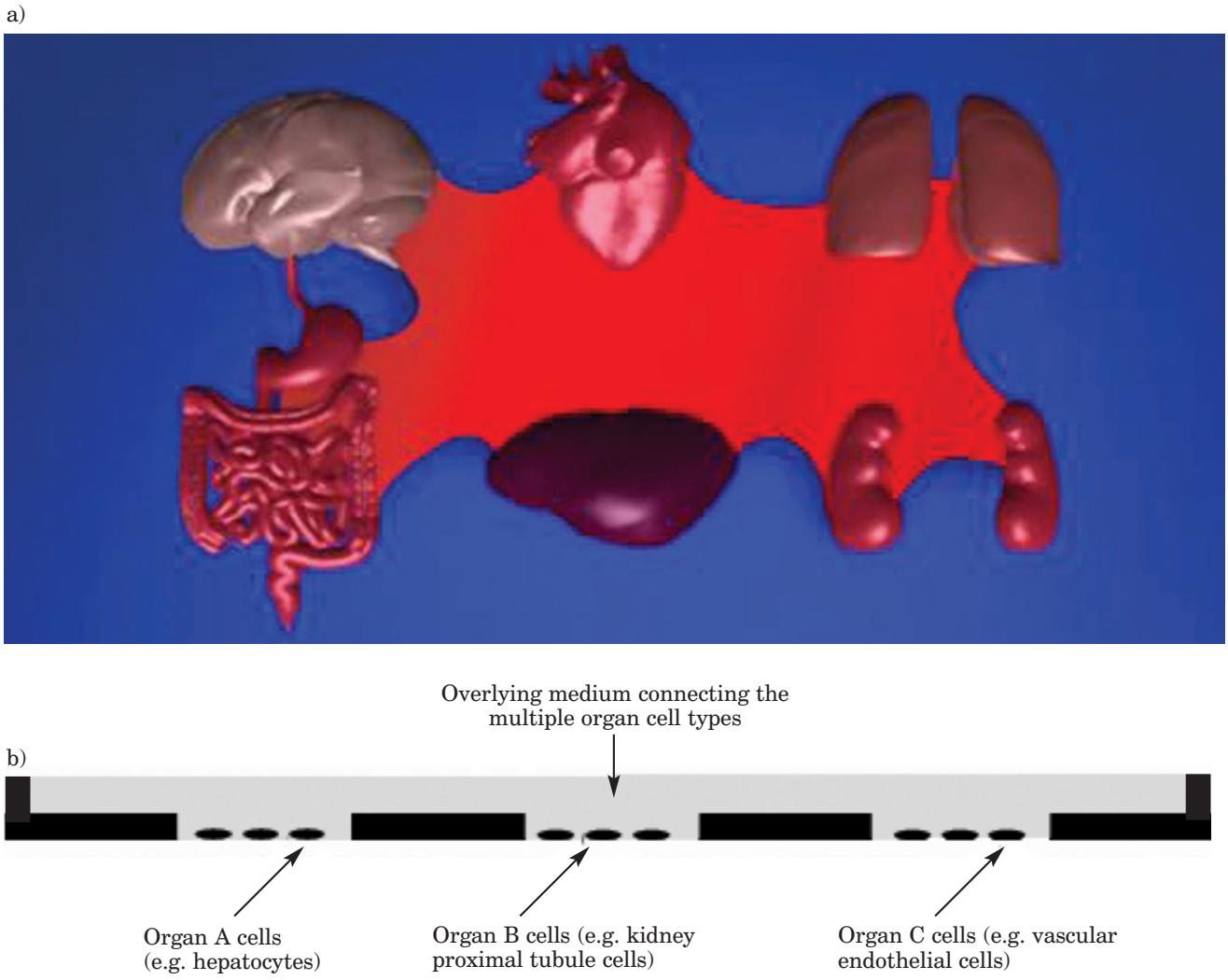
— A toxicant entering the systemic circulation may have differential distribution in various organs, with the organs in which the highest concentrations are sequestered being the most affected.

— A toxicant that has specific toxicity to a certain organ (e.g. the heart) may be less toxic, due to metabolic detoxification, to a different organ (e.g. the liver).

— A xenobiotic that is relatively non-toxic may be metabolised by one or more organs (e.g. liver, kidney, lung) into toxic metabolites (metabolic activation), which can travel to organs distal to the site of metabolism (e.g. peripheral neurons, brain), where they then cause toxic effects.

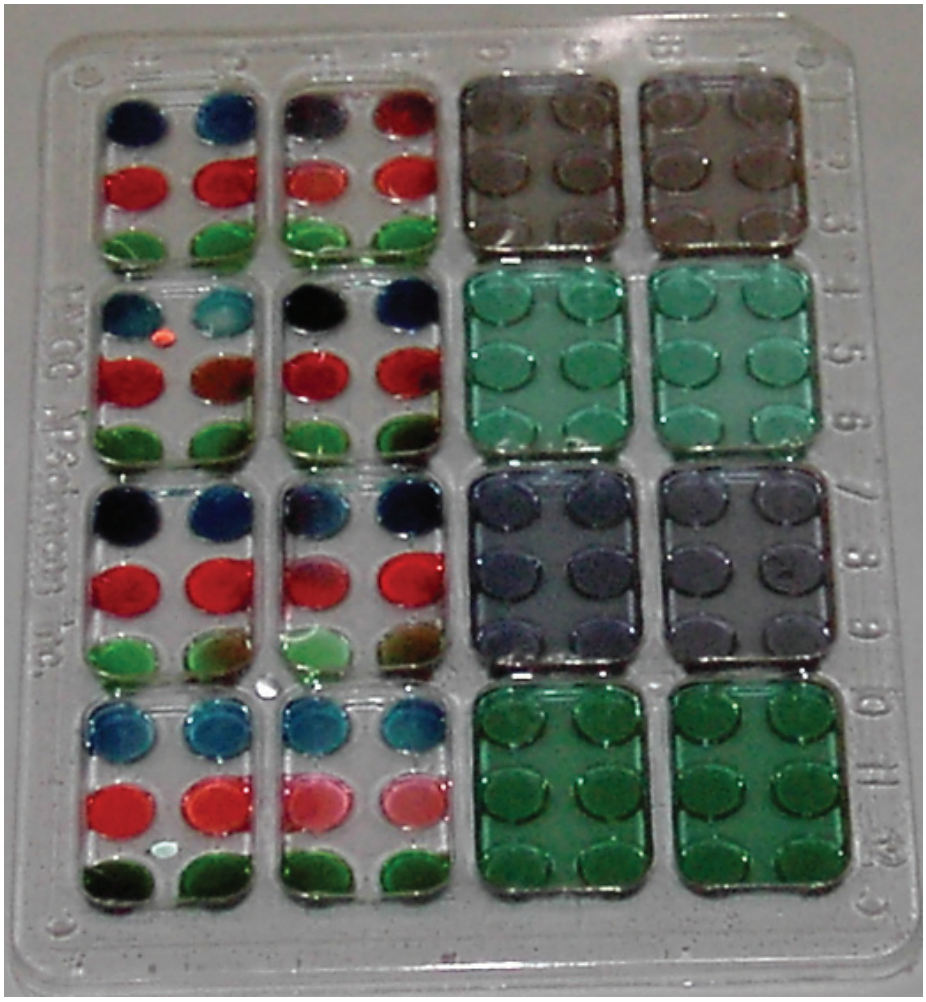
A schematic presentation of the scientific concept and configuration of the IdMOC system is presented in Figure 1, with a photograph of an IdMOC plate shown in Figure 2. The IdMOC system uses a wells-within-a-well concept. Cells from individ-

Figure 1: Schematic representation of the principles of the IdMOC experimental system



The human body is envisioned as consisting of multiple organs which are physically discrete, but are interconnected by the systemic circulation (a). This concept is applied in the development of the IdMOC system (b), which comprises multiple inner wells, with cells from a specific organ cultured in each of the wells. The overlying culture medium overflows from each of the inner wells. Therefore, the IdMOC system has multiple organs that are physically discrete, but are interconnected by an overlying culture medium. This characteristic of the system makes it more akin to the situation in vivo.

Figure 2: A photograph of an IdMOC plate with 6 small inner wells per large outer well, with 16 outer wells per plate



The separation of the inner wells from each other is illustrated by the coloured fluids on the left half of the IdMOC plate. The use of an overlying medium to integrate the inner wells in each outer well is illustrated on the right half of the plate. Therefore, this IdMOC plate has 16 independent units for experimentation.

ual organs are cultured separately in the inner wells. The inner wells are then interconnected by filling the outer well with an overlying medium to cover all of the inner wells. A xenobiotic introduced into the overlying medium will interact with the multiple cell types in each of the inner wells, and will be exposed to the metabolites collectively generated by the cells. The IdMOC system therefore satisfies the requirement for xenobiotic metabolism (via the use of hepatocytes) and non-hepatic target cells (via the use of cell types from non-hepatic organs). An advantage of the use of the IdMOC system over conventional mixed-cell type cultures is that, following treatment, the cells from each well can be evaluated for cytotoxic effects, thereby allowing the evaluation of cell type-specific effects after co-culturing, that is extremely difficult to perform with mixed-cell type co-cultures.

Evaluation of organ-specific cytotoxicity with the IdMOC system

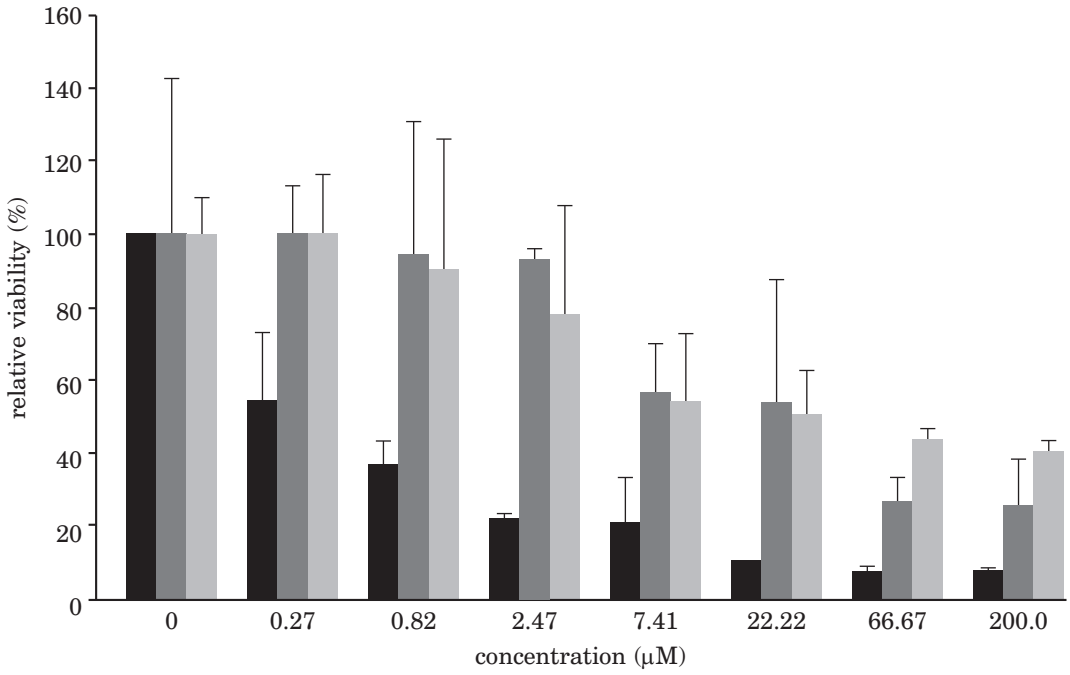
Since cells co-cultured within a chamber in the IdMOC system are exposed to the same overlying medium, the system is ideal for the evaluation of differential toxicity, largely because well-to-well or plate-to-plate differences are eliminated. To illustrate that the IdMOC system can be used to evaluate organ-specific toxicity, co-cultures of hepatocytes, renal proximal tubule cells and pulmonary epithelial cells were used to evaluate the toxicity of a known

liver-specific toxicant, aflatoxin B1 (AFB1). We have previously shown that AFB1 toxicity can be evaluated in primary human hepatocyte cultures, and that its cytotoxicity is mediated by cytochrome-P450 (CYP) metabolism (24). In the IdMOC system, AFB1 demonstrated differential cytotoxicity toward the three co-cultured cell types, with significantly higher dose-dependent cytotoxicity toward hepatocytes than to the renal proximal tubule cells and the airway epithelial cells (Figure 3). The selective cytotoxicity of AFB1 toward hepatocytes is consistent with the hepatotoxicity of AFB1 observed *in vivo*. The results thereby support the application of the IdMOC system in the evaluation of differential effects of a toxicant toward multiple organs.

The IdMOC system as an *in vitro* model of a tumour-bearing man

By co-culturing normal cells of the major organs and tumour cells, the IdMOC system can be used as an *in vitro* model of a tumour-bearing man. This model can assist with the discovery of compounds that are toxic to cancer cells, but non-toxic or less toxic to normal cells. Such compounds would have the potential for further development into anticancer drugs with lower toxicity toward normal tissues than many existing anti-cancer therapeutics. This application is illustrated by our study with the anti-mammary cancer drug, tamoxifen. We evaluated the toxicity of tamoxifen toward the MCF-7 mammary cancer cell line and cells from five major organs — hepatocytes (liver), kidney (proximal tubule epithelial cells), nervous system (astrocytes), vascular endothelium (human aortic endothelial cells), and lung (airway epithelial cells). The results (18) showed that tamoxifen, while being cytotoxic to all six cell types, had the highest cytotoxicity toward MCF-7 cells. Of the multiple cell types from the various organs, the hepatocytes were the most resistant to tamoxifen toxicity, which probably is a result of the high detoxifying metabolic capacity of these cells. The astro-

Figure 3: Organ-specific cytotoxicity determination in the IdMOC system, illustrated with the known hepatotoxic agent, aflatoxin B1



The co-cultured cells (and their respective organs) were: human hepatocytes (liver); human renal proximal tubule epithelial cells (kidney); and human small airway epithelial cells (SAEC; lung). The various cell types were cultured in the IdMOC system (6 wells per chamber; 2 wells per cell type) and treated with a range of three-fold increasing concentrations of aflatoxin B1 (AFB1) for 48 hours. The results are shown as relative viability (mean + SD, n = 3), based on cellular ATP content (calculated as the viability of treated cells divided by that of the solvent-treated control cells). The results show that AFB1 was selectively cytotoxic to hepatocytes, thereby demonstrating the potential of the IdMOC system to be used for the evaluation of organ-specific toxicity.

= Human hepatocytes; = human renal proximal tubule cells; = human small airway epithelial cells.

cytes were the most sensitive (after the MCF-7 cells). It should be noted that tamoxifen is used at hormonal levels as an anti-oestrogen for the treatment of oestrogen-dependent breast cancer, so clinical toxicity is not a complicating factor. Our results on the cytotoxicity of tamoxifen are consistent with its known *in vivo* toxicity when used at relatively high levels, which include hepatotoxicity (25) and renal toxicity (26).

The IdMOC system with tissue slices from multiple organs

Tissue slices have been used extensively for toxicology studies (1, 2). A major advantage of tissue slices is that multiple cell types are present in relatively uninterrupted substructures (except for the cut surface, where the cells are damaged), thereby allowing the evaluation of toxicity toward one of more cell types within the organ studied.

We have recently started to use organ slices in the IdMOC system, to achieve the intended "multiple organ co-culture". Precision-cut slices of heart, liver and kidney from male Sprague-Dawley rats were prepared and co-cultured in the IdMOC system (2 slices per chamber). The co-cultures and control mono-organ cultures were evaluated for xenobiotic metabolism, via the quantification of luciferin-isopropyl acetal (LIPA) metabolism. We have recently shown that LIPA is readily metabolised by the CYP3A4 expressed by intact human hepatocytes, to luciferin, which can be quantified, based on luminescence via luciferase activity in the presence of ATP, since luciferin is the enzymatic substrate (27). In this study in the IdMOC system, LIPA metabolism was evaluated as a function of time, with co-cultured tissue slices from three organs: liver, heart and kidney (two slices [one slice per well] of each organ per chamber). The IdMOC system containing single organs: liver only, heart only, and kidney only, were used as controls. As shown in Figure 4, time-dependent LIPA metabolism was observed in the IdMOC system co-culture of liver, heart and kidney slices, and in the single organ culture of liver slices. Single organ cultures of heart and kidney had no measurable LIPA metabolism. The results demonstrate the provision of hepatic metabolism in the IdMOC system with multiple organs via the use of liver slices. Studies are now being conducted in our laboratory with co-cultures of tissues slices from multiple organs, as an experimental system for the evaluation of non-hepatic organ toxicity in the presence of hepatic metabolism.

The IdMOC system with multiple cell types from a single organ

While the initial purpose of the IdMOC system is to evaluate multiple organ toxicity by co-culturing the representative cell types of the major organs, it can also be used to model a single organ by using the multiple cell types of the organ. We have evaluated the cytotoxicity of a key cigarette component, nicotine, in the IdMOC system, with co-cultures of three pulmonary cell types: small airway epithelial cells; bronchial epithelial cells; and pulmonary microvascular endothelial cells. Nicotine was found to cause a dose-dependent decrease in viability in all three pulmonary cell types, with the bronchial epithelial cells being slightly more sensitive to the cytotoxic effects of nicotine (Figure 5). The results show that nicotine, in additional to being an addictive agent (28), has intrinsic cytotoxicity toward multiple pulmonary cell types. This study illustrates the application of the IdMOC system for the co-culturing of multiple cell types to define single organ toxicity.

Discussion

While it is well-recognised that, because of species differences, results with animals do not always predict human effects (21–23), *in vivo* toxicity evaluation with whole animals continues to be the "gold standard" of toxicity testing. *In vitro* experimental systems, while making tremendous strides toward the replacement of animals in the evaluation of well-characterised events, such as drug metabolism and drug–drug interactions, are still considered to be inadequate for the accurate definition of xenobiotic toxicity.

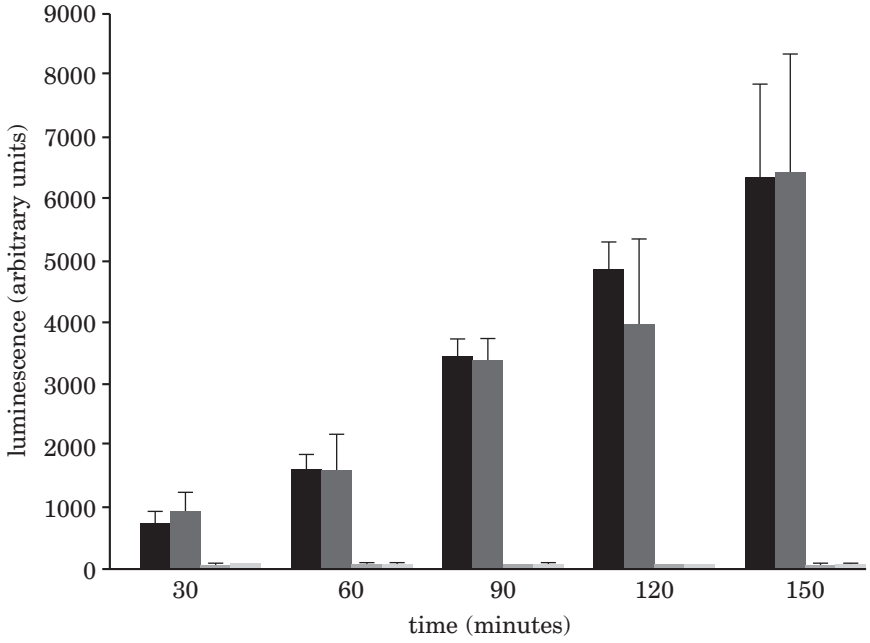
Issues with *in vitro* testing include the continual reliance on continuous, transformed cell lines, such as 3T3 cells and HepG2 cells. These cells can be used to detect general toxic agents (e.g. cyanide, strong acids and bases), but would be of little use for toxicity that, due to xenobiotic-metabolising enzyme activities or organ-specific biochemical pathways, would lead to organ-specific toxicity. Furthermore, toxicity to an organ can be caused by metabolites produced by a different organ. Such organ–organ interactions occur *in vivo*, but are not modelled in routine *in vitro* experimental systems. The IdMOC system was developed to overcome these deficiencies. It incorporates:

— hepatic metabolism via the use of hepatocytes or liver slices;

— hepatic and non-hepatic target cells via the co-culturing of hepatocytes or liver slices with key cell types or tissue slices from major organs; and

— multiple organ interactions via an overlying medium.

Figure 4: The cytochrome P450 metabolic capacity of an IdMOC system comprising individual cultures or co-cultures of Sprague-Dawley rat liver, kidney and heart slices



The various tissue slices (two slices per chamber) were placed in the IdMOC system as single-organ cultures (slices from one of the three organs) or co-cultures (slices of each of the three organs). A P450 CYP3A substrate, luciferin-IPA (LIPA), was added to the IdMOC. LIPA is metabolised by CYP3A4 to luciferin, which can be quantified by luminescence upon incubation with a luciferase-ATP based detection reagent (Promega Inc., Madison, WI). Following an incubation period of 30, 60, 90 and 120 minutes, aliquots of media were removed for the quantification of luciferin formation. The results shown are expressed as arbitrary luminescence units (mean + SD, n = 3). Time-dependent metabolism of LIPA, as evidenced by time-dependent increases in luminescence, was observed for IdMOC co-cultures of three organs, as well as for the single-organ culture of liver slices. In contrast, single-organ cultures of heart and kidney slices had virtually no detectable LIPA metabolism. The results clearly demonstrate the main advantage of the IdMOC system, namely, the provision of hepatic metabolism to allow the evaluation of the effects of hepatic metabolites toward non-hepatic organs.

= co-culture of liver, kidney and heart slices; = single-organ liver slices; = single-organ kidney slices; = single-organ heart slices.

There are a number of challenges associated with the use of the IdMOC system in the evaluation of organ-specific toxicity:

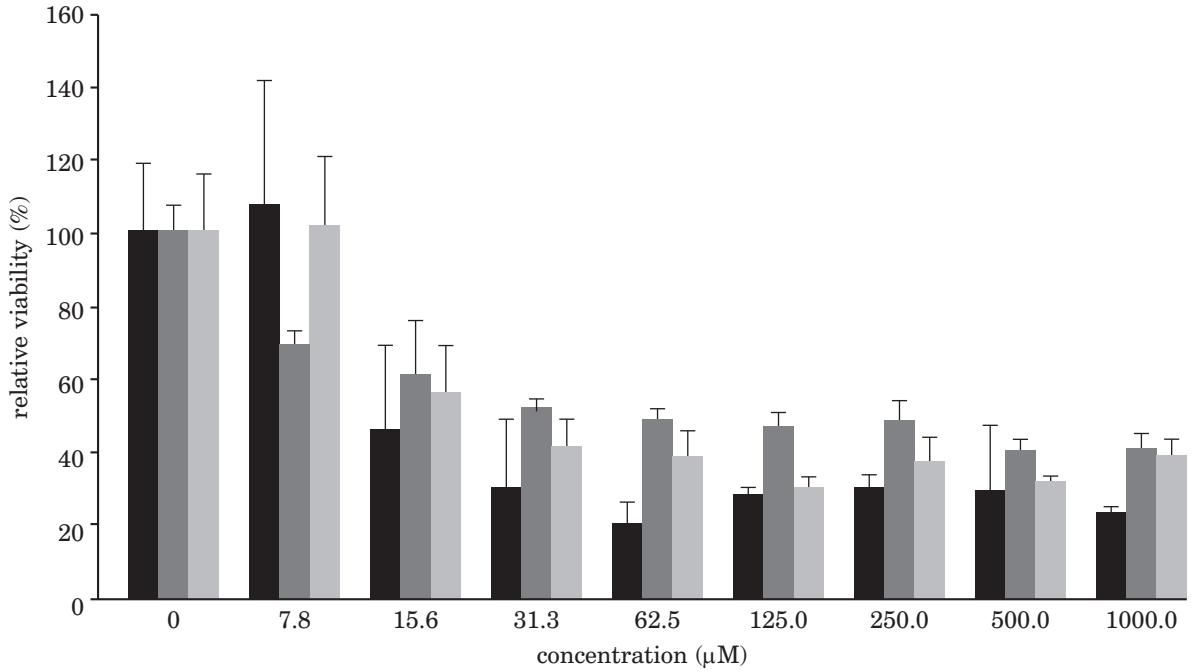
— *Primary cells:* The success of the IdMOC system relies on the physiological relevance of the cells being used to represent the different organs. At the time of writing, hepatocytes represented the best-characterised primary cell system for liver-specific metabolism. Hepatocytes, or liver parenchymal cells, are the major cell type responsible for xenobiotic metabolism, and are often the target cells for toxicants. This key liver cell type has been used successfully for hepatic metabolism and hepatotoxicity studies. The other cell types commonly used to model a single organ are renal proximal tubule cells (kidney), neurons (brain, central and peripheral nervous systems), cardiomyocytes (heart), airway epithelial cells (lung), and vascular endothelial cells (vascular endothelium). The usefulness of the information obtained with these primary cells will be dependent on the quality of the primary cells, particularly with regard the degree of retention of organ-specific properties. Non-hepatic primary cells are generally less well-defined, so one must choose the source of the cells with care. Furthermore, one single cell type may not adequately model the response of an organ to toxic substances. One may need to further define organ-specific effects by using co-cultures of multiple cell types for a single organ, as illustrated in our study of the IdMOC model of the lung (see the section entitled *The IdMOC system with multiple cell types from a single organ*).

— *Organ slices:* The use of tissue slices offers advantages over the use of primary cells, as the

multiple cell types of the organ are fully represented. The limitations of tissue slices are that, while the cells on the outside regions of the slices are in direct contact with the medium, the cells inside the slices will be exposed to the toxicant in the medium via diffusion, which may be limiting. This need for diffusion is an *in vitro* artefact, due to the lack of blood circulation as it occurs *in vivo*. Furthermore, tissue slices in the IdMOC system are, as yet, only viable for approximately 24 hours, and as such do not permit longer-term studies to be performed. Another challenge in the use of tissue slices in the IdMOC system is that, while tissue slices from multiple organs can be routinely prepared from laboratory animals, it is highly improbable that multiple human organs can be available for experimentation on the same day, unless they are from posthumous research donation, when they are subject to the priority acquisition of organs for transplantation purposes. The cryopreservation of human tissue slices, if successful, would greatly enhance the use of human organ slices in the IdMOC system.

— *Pharmacokinetics:* Although in IdMOC models, multiple "organs" are connected by the systemic circulation, there is no actual directed flow of the overlying medium from one organ to another. Therefore, the system cannot model the sequential events of drug absorption (e.g. bolus dose to the liver, followed by systemic dis-

Figure 5: Evaluation of nicotine cytotoxicity toward multiple pulmonary cell types co-cultured in the IdMOC system: a lung model



One application of the IdMOC system is to model a single organ via the co-culturing of the multiple cell types of that organ. To model the lung, the major cell types of the lung: bronchial epithelial cells; small airway epithelial cells; and microvascular endothelial cells, representing the bronchus, alveolus, and capillary of the lung, respectively, were co-cultured in the IdMOC system. Nicotine, a pulmonary toxicant and key ingredient of cigarette smoke, was added at the various concentrations shown. After a treatment period of 24 hours, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was added directly to the IdMOC plate wells. MTT was metabolised by viable cells to insoluble blue formazan crystals, which, because of their insolubility, would localise within each of the inner-wells containing the individual cell types. After a 4-hour incubation, the medium in the IdMOC plate wells was completely removed. DMSO was added to each of the inner-wells, in order to dissolve the blue formazan crystals for absorbance determination by using a plate reader at 570nm. The results are shown as relative viability (mean + SD, n = 3), calculated as the viability of treated cells divided by that of the solvent-treated control cells. Nicotine was found to be cytotoxic to all three cell types, with apparently higher cytotoxicity toward the bronchial epithelial cells.

= human bronchial epithelial cells; = human small airway epithelial cells; = human microvascular endothelial cells.

tribution), or differential exposure due to organ-specific blood flow. Hence, it is important that results obtained with the IdMOC system are qualified with known pharmacokinetics parameters, in order to model *in vivo* effects.

Conclusions

The IdMOC system is a simple experimental system, which can be adopted in most laboratories without the need for specialised equipment. The physically-discrete cultures permit the evaluation of the effects of a toxicant on a specific type of cell or tissue slice, after co-culturing. The alternative co-culture approach, namely, the mixing of multiple cell types, renders the evaluation of cell type-specific effects extremely difficult. The IdMOC system has the potential to be a valuable, universally applicable *in vitro* system for the evaluation of xenobiotic properties, including metabolism, distribution, and toxicity. Valuable results can be obtained with the IdMOC system, via the careful selection of cell types or organ slices, choice of mechanistically-relevant endpoints, application of the system within its limitations, and evaluation of the data to include *in vivo* parameters.

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