LIVER-KIDNEY MICROFLUIDIC BIOREACTOR FOR CELL CO-CULTURE IN DRUG STUDIES

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ABSTRACT

A microfluidic bioreactor for cell co-culture has been developed to study the cytotoxicity of xenobiotics in multi-organ interaction models. In this paper, we investigate the drug metabolism of ifosfamide, an anticancer drug, in liver and its impact on kidney. HepG2/C3A (liver) and MDCK (kidney) cells are cultured in two separate PDMS chambers connected by a perfusion loop mimicking blood flow. We show that the metabolism of Ifosfamide in liver induces a significant effect on the MDCK cell proliferation in the bioreactor on contrary to monoculture controls.

KEYWORDS: Microfluidic, Bioreactor, Co-culture, Metabolism, *In vitro* Model, Ifosfamide

INTRODUCTION

In order to minimize animal testing, we investigate an alternative method to screen the effect of xenobiotics. The method is based on the conception of a microfluidic bioreactor integrating the progresses in tissue engineering and micro technology [1, 2]. To highlight functionality of the bioreactor in drug research, we chose a liver kidney co culture model (liver being the major site of xenobiotics biotransformation and the kidney being the major site of their elimination). In this study, we worked with the ifosfamide, an antineoplasic alkylant drug, used to treat a wide range of tumors in both adult and children. By itself, ifosfamide is not nephrotoxic however its liver and tubular metabolisms lead to the formation of the chloroacetaldehyde and acrolein which are active and nephrotoxic metabolites.

EXPERIMENTAL

Microfabrication

To fabricate the bioreactor, we use the PDMS, a widely used polymer for microfabrication and construction of microstructures. This biocompatible and gas permeable material allows the cell oxygenation in culture. As transparent material, it permits morphological observations of cells. The microfabrication details based on replica molding have been reported previously in [3]. The bioreactor is composed of two cell cultures chambers in which liver and kidney cells can grow separately (Fig 1).

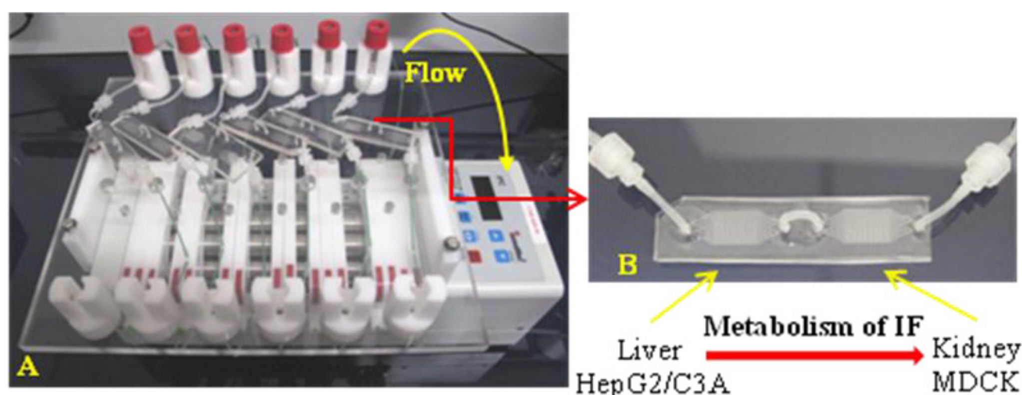

Figure 1: Photo of the perfusion system (A) and the details of the liver-Kidney microfluidic bioreactor (B).

Cell culture

Human hepatocarcinoma cells (HepG2/C3A) and kidney epithelial cells MDCK (Madine Darby canine kidney) were provided by the American Type Culture Collection (ATCC, USA). The cells were grown in plastic flasks at 37°C in a humidified atmosphere of 5% CO_2-95% air, using Minimum Essential Medium (MEM Eagle) supplemented with 10% fetal bovine serum, 2 mM L-Glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 10 mM of HEPES buffer and 100 IU of penicillin/ml and 100 µg of streptomycin /ml. The cell culture medium was changed every 48h, and the cells were passaged weekly at confluency with 2.5 mg of trypsin/ml and 100 µg of EDTA/ml. The HepG2/C3A and MDCK cells were seeded between $3\pm1 \times 10^5$ and $3\pm1 \times 10^5$ cells/cm² in the bioreactor or Petri dishes (use as conventional flat control) after 1 h of coating with fibronectin solution (10 µg/ml).

Experiments

Following 24 h of adhesion, the cell culture inside the bioreactor were perfused at a rate of 10 µl/min using a sterile loop, containing a culture medium tank and a peristaltic pump (Fig 1). The culture is performed in a humidified CO_2 incubator at 37°C. After diluting ifosfamide (IF) in sterile culture media (MEM Eagle) at a final concentration of 50 µM the cells were exposed (treated) or not (control) for 72h. Mono cultures of MDCK and HepG2/C3A with and without treatment were performed in parallel to identify the effect of the co-cultures. Petri flat cultures were also performed for comparative purposes. Characterization of each culture was performed by monitoring the cell activity in terms of cell proliferation and basal metabolism (glucose, albumin, ammonia). The set of experiments was repeated 3 times in triplicate (n=3x3). We use the Mann-Whitney test as statistical method.

RESULTS AND DISCUSSION

After 24h of adhesion, the cells colonized the entire surface of each chamber of the bioreactor. They grew up over the microstructures creating a 3D tissue during 96h of cultures (including 72h in perfusion). The cells morphology is shown in Fig 2.

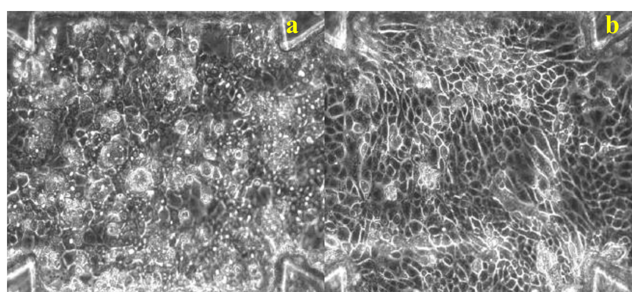

Figure 2: Morphological observation of the cells in the microfluidic biochip HepG2/C3A cells (a), MDCK (b) after 96h of culture (72h in perfusion).

The cell number in co-culture was multiplied by 2,1±0,8 for HepG2/C3A and 2,3±1,5 for MDCK in co-culture or mono cultures in the bioreactors without treatment (Figs 3 and 4). These results are consistent with previous works of mono cultures in a bioreactor [4]. After 72h treatment in perfusion, the HepG2/C3A or MDCK monocultures treated by IF did not show a significant effect of the molecules when compared to the untreated case. This result was similar in Petri flat cultures in which IF has no effect on the cell proliferation (Fig 3). On the other side, in co-culture, a significant reduction of the proliferation is observed in the renal cells compartment. This can be attributed to the IF that has been metabolized by the liver cells resulting to the production of toxic metabolites on kidney cells (Fig 4). Our results are consistent with several studies where it has been shown that only metabolites of IF are toxic to the kidney, whereas the prodrug is not. The IF might be oxidized by cytochrome P450 3A5 and 2B6 which are present in liver cells. Future investigations will be performed to characterize the metabolite production and CYP expression in our model. Finally, the ifosfamide loading leads to a higher ammonia production and a higher albumin synthesis. These productions might be explained by a higher metabolism induced in the cell via the cellular detoxification processes (Table 1).

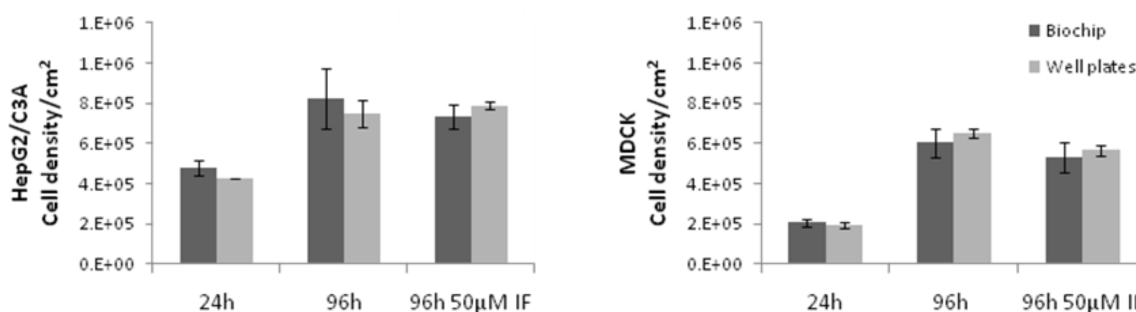

Figure 3: Comparison of the effect of ifosfamide on HEPG2/C3A and MDCK cells in monoculture biochips.

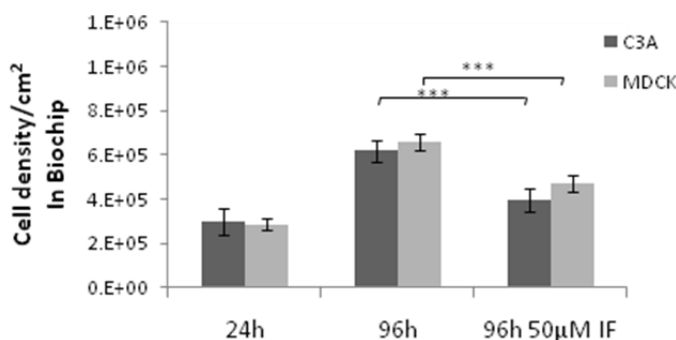

Figure 4: Comparison of the effect of ifosfamide on co-culture HepG2/C3A-MDCK cells in co-culture biochips.

Table 1: Metabolism activity: Glucose consumption, ammonia production by MDCK and HepG2/C3A, and synthesis of albumin by HepG2/C3A (n=3x3).

	Control		IF 50µM	
	24h	**96h**	**24h**	**96h**
Glucose (µg/h/10^6 cells)	34±8	33±8	20±8	26±8
Ammonia (nmol/h/10^6 cells)	31±2	27±4	56±10	72±2
Albumin HepG2/C3A (ng/h/10^6 cells)	21±4	21±2	55±13	85±7

CONCLUSION

The HepG2/C3A /MDCK co-culture model creates an anti-proliferative effect of Ifosfamide onto kidney cells. The ifosfamide effect was not detected in MDCK monoculture illustrating the potential of the co-culture. This can be probably attributed to the liver metabolism and will request confirmation. This first study demonstrate the potential of our co-culture liver-kidney model to investigate drugs with a novel alternative method. The model provides a larger physiological and realistic environment to mimic the tissue when compared to conventional flat Petri culture.

ACKNOWLEDGEMENTS

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