

“Cell Cross-talk” analysis in static and dynamic Multi-Compartmental Bioreactor

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Abstract— Our research is based on use of Multi-Compartmental Bioreactor (MCB) designed on the basis of allometric scaling laws in order to recreate physiological life conditions of four different human cell types (pancreatic, adipocyte, endothelial and hepatic cells), interconnected each other through media flow. In this work we have analysed the cross-talk of two different cell types: endothelial and hepatic cells, cultured in static and dynamic conditions without and with connection between them. Endothelial cells are derived by umbilical vein of human cord; while, hepatic cells are a purchased cell line called HepG2. We have set up optimal work conditions of both cell types before in static conditions and then in MCB system. The determination of cell behaviour alone and of their cross-talk has been determined by the analysis of urea, glucose, albumin and nitric oxide in collected samples of media culture. The results of this experiments have been showed that there is an increase in the release of urea and albumin and in the consumption of glucose, while there were not significant changes in nitric oxide levels, in the cells cultured in MCB system respect them cultured alone.

I. INTRODUCTION

In the last ten years bioengineering has supported biology in order to produce new research tools to improve knowledge in cell function and analyze the effects of different stimuli (chemical, physical, etc) on it. These new system are looking for to recreate more physiological conditions respect to those normally present during the classic cell culture experiment.

The main goal of Multi-Compartment Bioreactor (MCB) system is to reproduce an in vitro “human system” in order to understand the interactions between different cell types that compose it and the influence on them of physical and biochemical stimuli.

This bioreactor, moreover, represents a new biomedical device to replace or reduce the use of animals for drug testing and allows to study at cellular level the mechanism of physiological and pathological behaviour of human metabolism.

Ghanem and Shuler have developed PBPK-based systems to analyze products of metabolism of xenobiotics, as naphthalene, by three different cell types (hepatocytes, pulmonary cells and adipocytes) [1]. In this system, the cells were housed in fixed areas and through analytical instrumentation products of biotransformation were detected. Respect to this bioreactor,

MCB system has been developed joining biological knowledge, bioengineering concepts and allometric laws [ref], in order to correlate biological parameters (organs volume, blood flow, blood retention time and metabolic function) to mimic human metabolism. This approach allows to study not only kinetic but also metabolic relationships between cells. This study, in particular, have involved human primary (human umbilical vein endothelial cells, HUVEC) and immortalised cells (an hepatic cell line HepG2). Our purpose is to study if there is a cross-talk between these different human cell types without a their physical contact, as also shown in an our previous paper[2]. In this study some fundamental metabolites for cell life, as albumin, nitric oxide (NO) and glucose, and metabolic activity, with MTT, have been quantized, in static and dynamic conditions, and alone or correlated each other.

II. MATERIALS AND METHODS

CELL AND MEDIA CULTURE

Human Umbilical Vein Endothelial Cells (HUVECs) and Hepatic cell line HepG2 have been used.

HUVECs have been cultured in M199 media culture added with 1% Bovine Brain Extract (BBE), 2% of streptomycin/penicillin mixture, 2% of L-glutamine and 10% ml Fetal Bovine Serum (FBS). These have been extracted by human cord with enzymatic treatment.

HepG2 media culture was MEM added with 2% streptomycin/penicillin mixture, 2% of L-glutamine, 10% FBS, 2% MEM non-essential aminoacids and 2% MEM vitamins. This cell line is obtained by an human hepatoma and was purchased by Sigma.

ALGINATE COATING OF HEPATOCYTES

Hepatic cells are normally not subjected to a direct flow, but through capillary network absorb the metabolites necessary to their life. In MCB system, the endothelial barrier is not present, so, in order to supply to its lack and do not modify their function, the hepatic cells have been covered with an alginate coating.

A 4% alginate solution in Phosphate Buffer Solution (PBS) was diluted to 2% in MEM media, and then cross-linked with a solution 0.1 M Calcium Chloride (CaCl_2) in PBS. This modified protocol is commonly derived by alginate encapsulation of hepatic cells [3-6].

MCB CELL CULTURE CHAMBER

Cell chamber (Fig. 1) have been designed on the basis of allometric scaling laws [7-9]. These rules allow to correlate mathematically different physiological parameters in a non linear way. The dimensions of cell chamber system were 15.4 cm length, 12.3 cm width and 1.8 cm height, and it was realized by casting in a mold Polydimethylsiloxane solution (PDMS, Dowcorning®, USA), and then placed in an oven in order to favour its polymerization. This polymer is biocompatible, sterilisable and commonly used to realize microfluidic components. The system have four different cell chambers inter-connected each other just by one channel in order to allow the flow of media culture in a closed loop. The distances between chambers and flow, and the cell number (HepG2 and HUVECs ratio is 3:1) are related through allometric principles, in order to replicate the same conditions in human body.

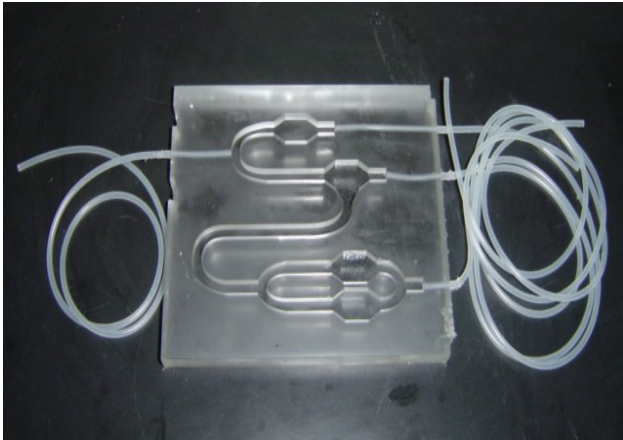


Fig. 1. MCB cell culture chamber

METABOLIC TESTS

Albumin has been measured with an ELISA kit (Bethyl Laboratories, USA). Urea has been determined with an enzymatic assay kit (Sigma, Italy). Glucose has been measured with a colorimetric assay kit (Megazyme, USA).

In order to quantify metabolic activity MTT test has been used.

III. RESULTS

CELL LIFE BASELINE

Initially, cell life baseline are evaluated in static environment. HepG2 and HUVECs have been tested isolated and in co-culture in ratio 10:1 respectively. The ratio, used in static conditions, was different respect to that used in MCB system, where this value was 1:3 (liver vs. vascular system), as normally present in human body. The difference was led that in static conditions just liver tissue has been replicated, while in MCB system the number of all endothelial cell present in human body was correlated to hepatocytes cells. In static conditions cell density was 8000 cells/cm² and 80000 cells/cm² for HUVEC and HepG2 respectively, while in MCB cell density was 240.000 cells/cm² and 80.000 cells/cm² or HUVEC and HepG2 respectively.

Because the HepG2 cells present different cell behaviour using different media culture, it was decided do not change their media culture and test if the cell proliferation of HUVEC was altered by change the media culture. The cell proliferation of HepG2 increases as increasing time, as predictable for an immortalized cell line (Fig.2). HUVECs proliferation is not modified by change of media culture. Finally, co-culture proliferation is higher than the single cell type and also of their mathematical addition. This results have been suggested the presence of a synergic effect between these two cell types on cell behaviour.

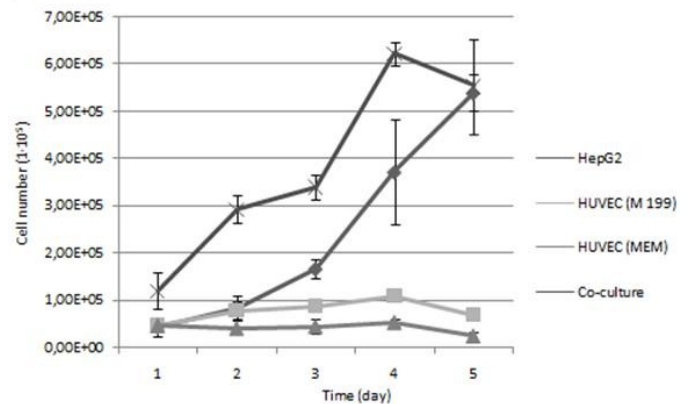


Fig. 2. Cell growth number in static conditions..

The metabolic analysis have showed that:

- 1) glucose consumption was higher in co-culture than in single cell types (Fig.3); this results is also confirmed by MTT test (Fig. 4).
- 2) albumin release (Fig. 5) has showed that in the first days of experiment the co-culture presented a more constant release than single culture of HepG2 probably due to development of a metabolic equilibrium between the two cell types. This state was altered in the other days by the different cell growth between the two different cell types.

- 3) The same trend of albumin was observed by urea release (Fig. 6) , confirming the theory that there is a cross-talk between different cell type cultured together, as normally happens in human body.

This results have been suggested us that it was possible to use only MEM media for experiments in MCB system.

MCB TEST

The differences between the cell experiment in static conditions and in MCB system are principally two. One is the ratio of cell density between HUVEC and hepatocytes: in fact, in MCB system, this ratio taking in account the number of endothelial cells present in all human vascular tree respect the hepatocytes that compose the liver of a standard man. This value is equal to 3:1 (3 endothelial cells vs. 1 liver cell). Instead, in normal co-culture experiment this ratio is inverted, because it is considered the number of endothelial cell present in the liver respect the number of hepatocytes. In this case the ratio is 1:10 (1 endothelial cells vs. 10 liver cell). The second difference is the cell-culture modality. In static culture the cells are in physical contact each other, while in MCB system the flow of media culture determines the interactions between the two different cell types. In this way the cross-talk is possible just through cell-specific mediators released by each cell type. In the experiments performed in MCB system, the cells are before seeded and cultured on glass slides in a normal incubator. After 24 hours MCB is assembled, inserting one glass slide of respective cell types in a fixed location, and then the cell culture chamber is connected to peristaltic pump (set up of 175 μ l/min) and media reservoir (total media volume 30 ml). When the hepatocytes are placed in their area inside the MCB chamber, in order to avoid the direct contact between the flow of media culture that can modify their cell function, they are coated by an alginate film.

Samples of media culture are collected every 2h 40', 4h 20', 6h and 24h and consumption of glucose, and release of albumin, urea and nitric oxide is analysed trough different commercial kit. Results of these analysis show important trends in time behaviour of these important metabolites. Glucose consumption (Fig. 7) has an increase of 5-6 times in MCB system respect to that obtained in static culture. Moreover, in dynamic and in static conditions connected culture and co-culture, respectively, present a decrease of consumption of glucose respect to that of monoculture.

The release of albumin (Fig. 8) has an increase in its production in dynamic conditions respect to static culture and, the results of experiments in MCB culture show that the presence of HUVECs not in direct contact with hepatocytes furnish an increase in its release. The urea secretion (Fig. 9) presents in MCB system respect to static culture an increase confirming an high anabolic and catabolic activity.

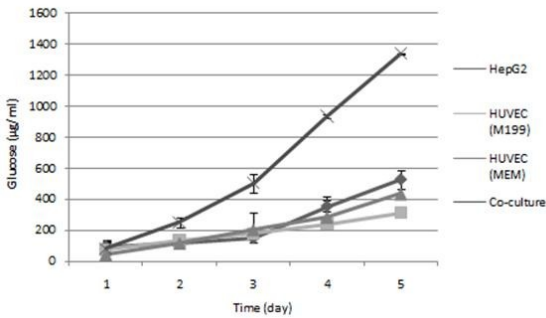


Fig. 3. Glucose consumption in static conditions.

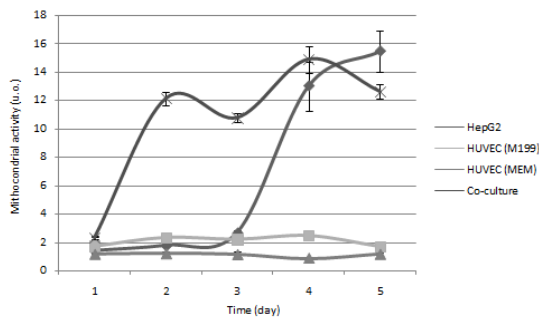


Fig. 4. MTT test for mitochondrial activity in static conditions.

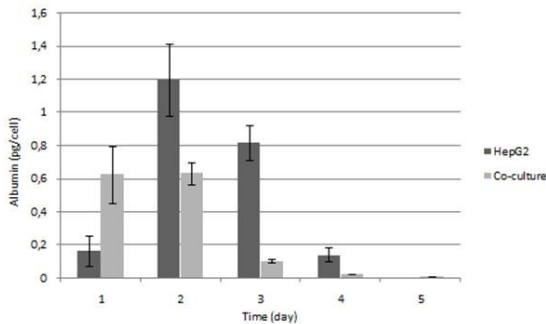


Fig. 5. Albumin release in static conditions.

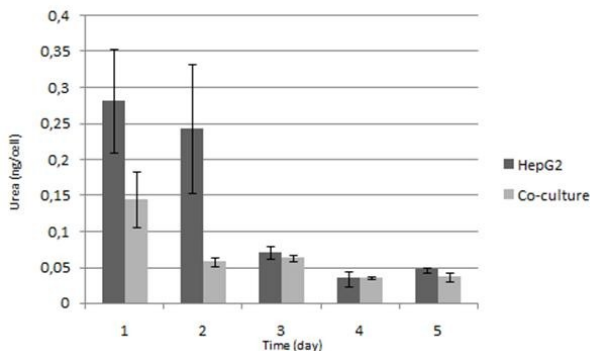


Fig. 6. Urea release in static conditions.

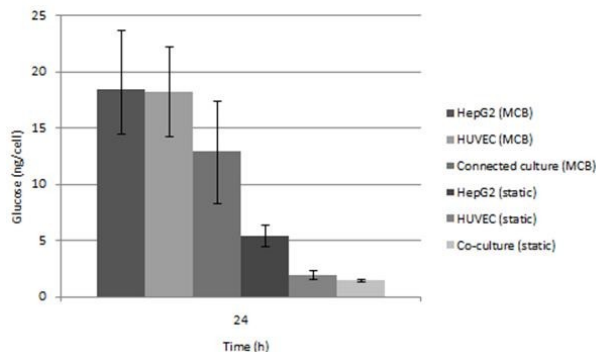


Fig. 7. Comparison of glucose consumption between static and dynamic culture.

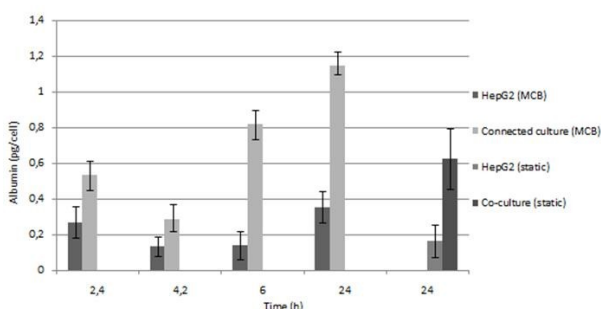


Fig. 8. Albumin production in MCB during 24 hours and comparison with values in static conditions.

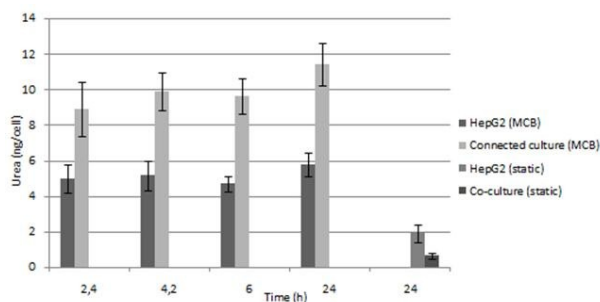


Fig. 9. Urea secretion production in MCB during 24 hours and comparison with values in static conditions.

IV. CONCLUSIONS

Cell culture techniques have improved knowledge on their biological function and response to extracellular stimuli, but static and mono cultures methods present limits due to the absence of a series of biological interactions present in each normal human tissue or system. This necessity has urged the development of new biotechnological tools able to study human biological environment in a more physiological way and to understand the relationship between different organs and

cell types. The Multi-Compartmental Bioreactor, showed in this work, is thought to furnish a device useful for this aim and for this reason was designed on the basis of allometric parameters in order to respect biological proportions. To validate our system we performed initially classic static culture experiments. During this first series of experiment the basal release of metabolites by monoculture and co-culture conditions was analysed. This experiment showed an increase of cell density and in the metabolic activity in co-culture conditions, showing a synergic effects between them. The albumin production and urea secretion, moreover, showed an increase in co-culture conditions, constant values in the first days of experiments. These results suggest that the direct contact between different cell types improve cell function of whole system as it was predictable. When these experiments were performed in MCB system the experimental results change. In fact, glucose consumption became generally higher than that in static culture but with a similar behavior for each cell culture condition. It was observed a decrease of consumption in connected cell culture and co-culture. The release of albumin and urea, in connected culture, increases from 2 to 6 times their concentrations respect both mono culture in MCB and static culture [ref]. These results suggest that interactions, mediated by metabolites present in media flow, have a remarkable effect more than the physical interaction and can lead to a restoration of physiological cell life conditions. This study opens new and interesting fields of research of the cell interaction between different cell types, and, furnishes an innovative tool for future study aimed to comprehension of patho-physiology of different metabolic diseases and their possible treatments with specific drugs.

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