Comparison of Cell Culture in Renal Microfluidic Bioreactor and Petri Dishes
L. Choucha-Snouber¹, JM. Prot¹, R. Baudoin¹, L. Griscom², C. Legallais¹, E. Leclerc¹
Corresponding author: eric.leclerc@utc.fr
1 CNRS UMR UMR 6600 Laboratoire de Biomécanique et BioIngénierie, Université de Technologie de Compiègne, France 2 CNRS UMR 8029, BIOMIS, ENS Cachan, France

Introduction
The directive REACH aims at reducing the risks to human health by improving the identification of the toxicity of the chemical substances. In this frame, we develop an alternative method of screening xenobiotics in order to minimize toxicological animal testing. The method is based on tissue engineering and microtechnology. A biochip that mimics a kidney organ and its metabolism was developed (Baudoin et al., 2007). This tool is an original cell growth model in a miniaturized system with dynamic culture conditions allowing continuous cell feeding and waste removal.

Materials and Methods
A PMDS biochip was fabricated by bonding two microstructured polymer layers. After a fibronectin surface treatment, MDCK (Madine Darby canine kidney) kidney epithelial cells were cultivated in the biochip. The biochip was included in a perfusion loop containing a media culture tank and a peristaltic pump. Characterization of the culture was performed by monitoring the cell activity in terms of cell proliferation and basal metabolism. The cell viability was monitored by a live dead assay. Cellular cycle repartition was quantified by flow cytometer. All assays were compared to Petri dishes. The set of experiments was repeated 3 times in triplicate.

Results
After 24h of adhesion, the renal cells colonized the entire surface of the biochip. They grew up over the microstructures creating a 3D tissue during 96h of cultures (including 72h in perfusion). The live dead assay demonstrated that the cells were alive. The cells number was multiplied by 3,1±1,2 in the biochip and by 2,8±0,7 in Petri dishes. The cell cycle analysis shows a delay in the proliferation during the first 24h of culture in the biochip. This was attributed to a time required by the cells to adapt to the biochip environment. After 96h of culture the cell cycle was similar in Petri and in the biochip. The absence of abnormality in the DNA in the biochip culture allowed the cells proliferation (Figure 1). A higher glucose and glutamine consumption, and a higher ammonia production were found in the biochip when compared to the Petri dishes after 96h of culture (Table 1).

Fig.1. Comparison of distribution of cells cycle phases between biochip and Petri dishes

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<th>Dishes</th>
<th>Biochip</th>
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<tr>
<td>Glucose (µg/h/10⁶ cells)</td>
<td>57±5</td>
<td>11±0,5</td>
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<tr>
<td>Ammonia (nmol/h/10⁶ cells)</td>
<td>34±6</td>
<td>16±1</td>
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<tr>
<td>Glutamine (nmol/h/10⁶ cells)</td>
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<td>36±13</td>
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Table 1. Metabolism activity: Glucose and glutamine consumption, ammonia production.

Discussion and Conclusions
According to the data of viability, proliferation and the distribution of cells in the cell cycle, the biochip appeared suitable for renal cell culture. Maintaining and even improving MDCK cell functions under the dynamic conditions, the biochip provide an adequate environment for renal cell cultures. Those results will be used as the reference cell status in the biochip for future studies of the effects of various nephrotoxic drugs.

References

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