

LIVER-TUMOR MODEL BASED ON SPHEROIDS IN MICROFLUIDIC NETWORKS FOR PHARMACOKINETIC STUDIES

J.Y. Kim^{1*}, D. Fluri², J. Kelm², A. Hierlemann¹ and O. Frey¹

¹ETH Zurich, Department of Biosystems Science and Engineering, Bio Engineering Laboratory, Basel, SWITZERLAND and

²InSphero AG, Schlieren, SWITZERLAND

ABSTRACT

A simple in vitro model for physiological organ-to-organ interaction studies was realized by using spherical microtissues (MTs) cultured in a microfluidic network. We used primary rat liver (rLi) MTs as liver model and human colon carcinoma (HCT116) MTs as tumor model that were cultured in dedicated microchambers and that were fluidically connected with a microchannel. Medium was internally circulated by using gravity-driven flow. The in vitro model was validated by comparing the bioactivation of an anticancer pro-drug, cyclophosphamide (CPA), under perfusion conditions and under conventional static conditions.

KEYWORDS: 3D cell culture, microtissues, spheroids, microfluidics, gravity-driven flow

INTRODUCTION

Most of the currently used cell-based assays in drug development and toxicity testing are based on two-dimensional cell cultures, which are limited in mimicking *in vivo*-like environments and, therefore, in their relevance [1]. Recently, 3D cell cultures in microfluidic devices have been used to overcome some of these limitations [2]. However, simple experimental setups for tissue-like cell culturing are still needed. This holds particularly true for culturing, in the same setup, different organ models, which then will be interconnected through fluidics to establish multi-organ networks for pharmacokinetic studies. Here, a simple and robust microfluidic device is presented that can host various organotypic MTs and that enabled to investigate CPA pro-drug bio-activation processes: CPA has to be activated by liver tissue to have effects on tumor tissue growth.

EXPERIMENTAL

As illustrated in Figure 1a, the device consists of 4 MT compartments, 4 MT loading channels and a medium perfusion channel. Both channels are interconnected at the MT compartment (Figure 1b). 3 rLiMTs and 1 HCT116 MT were externally formed by using the hanging-drop method and then loaded into the compartments (Figure 1c). The device was operated in an incubator on an automated tilting platform enabling medium perfusion by gravity-driven flow (Figure 1d). This simple setup allows for loading multiple devices on the platform and for conducting multiple experiments in parallel (currently up to 48) with various drug concentrations, replicates, and controls. In this experiment, HCT116 MTs were cultured under static and perfusion conditions over 8 days with 4 different bio-activation conditions (Table 1).

Table 1. MT culturing conditions for CPA bio-activation experiment

Aim	CPA conc. (mM)	No. of rLiMT	No. of HCT116 MT	Note
Toxicity of CPA alone	0	0	1	control
	1	0	1	
Bio-activation of CPA by liver	0	3	1	control
	1	3	1	

For applying static conditions, the MTs were cultured in v-bottom well plates (GravityTRAP™, InSphero AG, Switzerland), and medium from wells containing rLiMT was manually transferred into

wells containing HCT116 MT every 2 days. For MTs cultured in the microfluidic device, the medium was inter-changed continuously between the MTs through the microfluidic perfusion channel by periodic tilting of the platform (every 2 minutes). Media were also exchanged every 2 days via the open reservoirs by simple pipetting. MT morphologies and diameters were monitored by an inverted microscope (Axiovert 25, Carl Zeiss AG, Japan) in bright-field mode over a period of 8 days. ATP for tissue viability was measured at the end of the experiment (day 8).

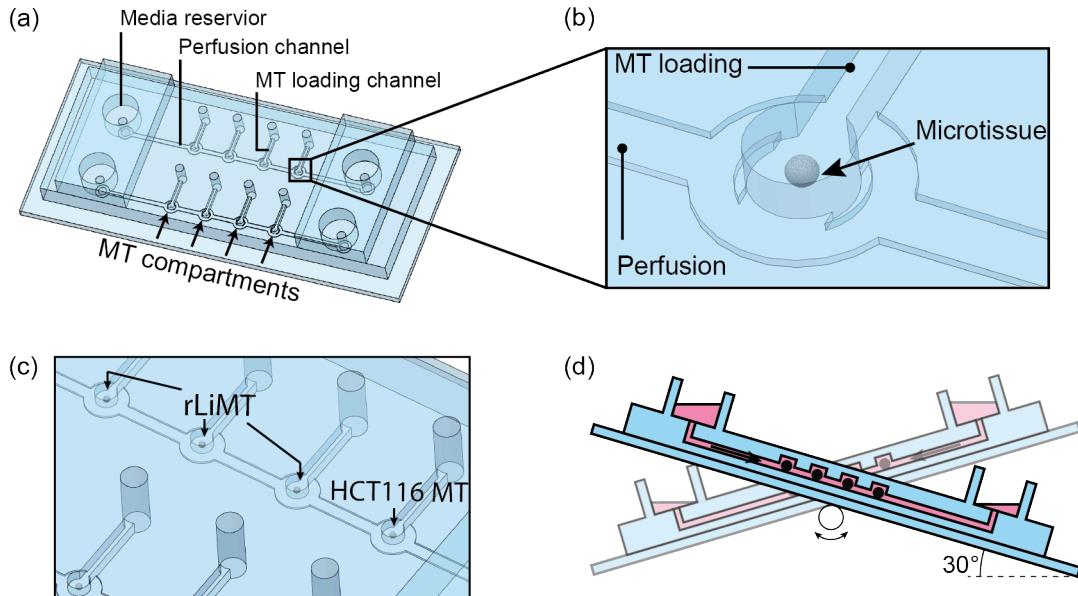


Figure 1. (a) Schematic of the microfluidic device (b) MT compartment at the junction of MT loading and medium perfusion channels, (c) MT configuration in the microfluidic channel for CPA bio-activation experiments. (d) Gravity-driven flow using a automated tilting platform. Tilting back and forth was executed every 2 minutes with an inclination angle of 30 degrees.

RESULTS AND DISCUSSION

No significant effect of bio-activated CPA on HCT116 MT was observed under static conditions, as depicted in Figure 2. Under all 4 conditions, we observed comparable growth rates for the HCT116 MTs (Figure 2a). The viability of the HCT116 MTs decreased to about 80% with respect to their initial value (Figure 2b). This decrease was observed regardless of using media (containing CPA) that had been incubated with rLiMT or media (containing CPA) that had not been incubated with rLiMT.

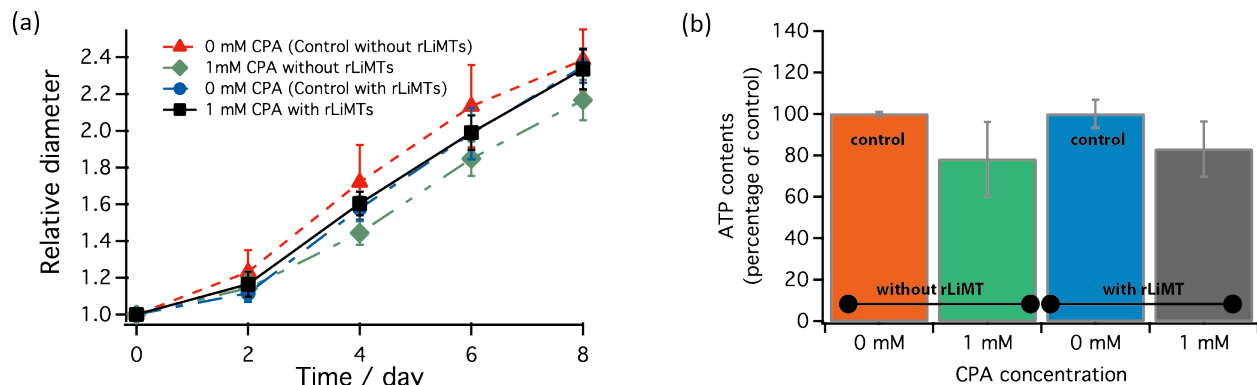


Figure 2. (a) Growth ($n \geq 3$) and (b) viability ($n = 4$) of HCT116 MTs cultured under static conditions in well plates.

In contrast, the bio-activation through rLiMTs is evident upon culturing rLiMTs and HCT116 MTs together in the microfluidic chip under continuous perfusion. We can observe a growth stagnation and even size reduction of HCT116 MTs in medium containing 1 mM CPA, whereas MTs under all other conditions behave similarly as in the static experiment (Figure 3ab). The tumor tissue viability decreased to about 50% over a period of 8 days with respect to the control group, in which rLiMTs were not present.

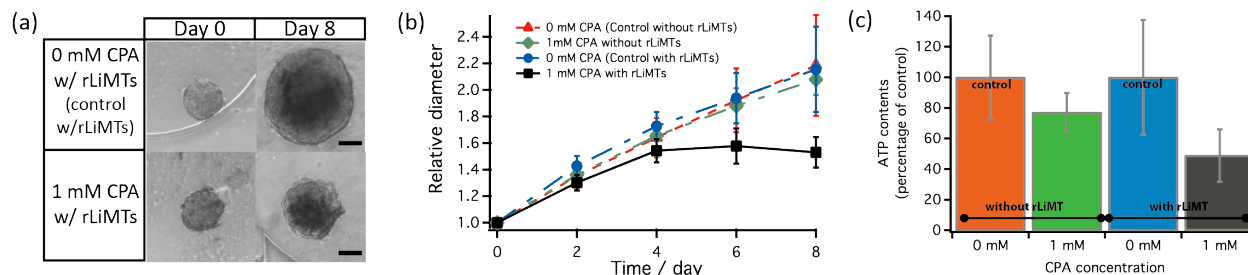


Figure 3. Growth and viability of HCT116 MTs cultured under perfusion conditions. (a) Bright-field micrographs (scale bar is 100 μ m) and (b) relative diameter over 8 days ($n=8$) and (c) viability at day 8 ($n=5$).

CONCLUSION

An on-chip *in vitro* 3D liver-tumor microfluidics-based model was successfully established by arranging spherical microtissues in microfluidic channels. We were able to reproduce the bio-activation of a cancer pro-drug in the microfluidic device, which we could not observe under static conditions. The results provide first evidence that continuous perfusion and rapid exchange of metabolites between different tissue types may be critical in multi-tissue setups and that systems based on the presented approach may be suitable “body-on a chip” models for compound testing.

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CONTACT

* J.Y. Kim; phone: +41 61 387 3314; jinyoung.kim@bsse.ethz.ch