

# 3D TUMOR SPHEROID CHIP USING BALANCED DROPLET DISPENSING FOR PHARMACOKINETIC DRUG ELIMINATION MODEL

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## ABSTRACT

This paper presents a three-dimensional (3D) tumor spheroid chip where the pharmacokinetic (PK) drug elimination model is developed by balanced droplet dispensing. The PK model should reflect the exponential decrease of drug concentration in a body to improve the reliability of cell-based screening assay. The previous cell chips, however, maintain the concentration of anti-cancer agents at constant or discretely decrease the concentration level by manual process. In the present chip where medium droplet dispensers are integrated on a well array, the small droplets of fresh medium and well waste are autonomously replaced each other, the exponential elimination of drug concentration in the well is provided. In the experimental study, we demonstrated that the present chip successfully develops the exponential elimination model using fluorescein isothiocyanate (FITC). The present chip has potential for analyzing the response of 3D tumor spheroids in the pharmacokinetically relevant drug elimination model for high-reliability spheroid-based drug screening chip.

## KEYWORD

Balanced Droplet Dispensing, Pharmacokinetic Drug Elimination Model

## INTRODUCTION

High-reliability anti-cancer drug screening is important to select optimized drug candidates among numerous agents for effective cancer chemotherapy. In screening process, the cellular response to various anti-cancer drugs and its combination is analyzed. In order to improve the reliability of the *in vitro* cell-based anti-cancer drug screening, the pharmacokinetic (PK) parameters of the drug should be considered so that the screening results can precisely reflect the time course effects of drugs to tumor [1]. In general PK models of drugs, body consists of several compartments [2] and the drug concentration in plasma or serum is generally regarded as the exponentially decreasing model due to the reaction of a drug within or between the compartments as shown in Fig. 1 (solid line). The previous cell-based drug screening chips, however, characterized the cellular response to anti-cancer agents without any chemical dilution of drug. The drug concentration in these chips shows the constant value throughout the drug screening process as the dashed line in Fig.1. In the some other cell-based screening methods [3,4], the drugs were manually diluted by the periodic medium renewal. However, the manual medium renewal process is time-consuming and results in the well contamination and large deviation of measured results caused by human factors. In addition, the medium renewal at the specific interval results in the discrete reduction of drug concentration (dotted line), different from the continuous exponential profile in the PK model (solid line). In this work, we present 3D spheroid chip capable to apply the exponential drug elimination model in Fig.1 (solid line) based on the balanced droplet dispensing. Since the small droplets of fresh medium and well waste are autonomously replaced each other, the exponential decrease of drug concentration in the well is obtained. This paper focuses on the design and experimental demonstration of the 3D tumor spheroid chip for analyzing the response of spheroids to the anti-cancer drug in the PK model of exponential drug elimination.

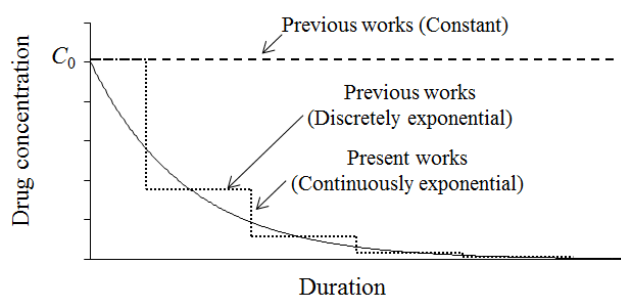


Figure 1: Comparison of the drug elimination models in the previous and present works.

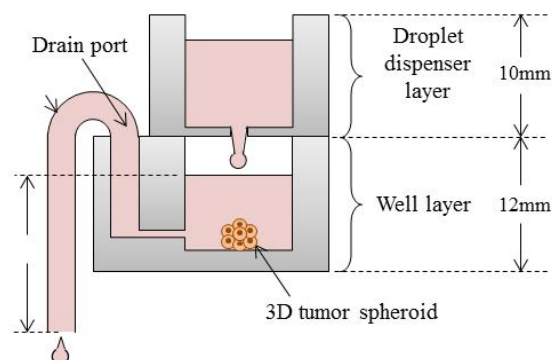


Figure 2: Cross-section view of 3D tumor spheroid chip using the balanced droplet dispensing.

## DESIGN AND FABRICATION

The present chip (Fig.2) consists of two Polydimethylsiloxane (PDMS) layers. In the balanced droplet dispensing (Fig.3), the hydraulic-head difference,  $\Delta h$ , between the well-inlet and drain-outlet, cause the removal of a waste droplet at the drain [5,6]. The decreased pressure in the well due to volume expansion provokes a fresh medium droplet supply from the dispensing layer. Therefore, the medium droplet is autonomously supplied to the well in the exact amount of the removed waste droplet without any pumps.

In Fig.3, the wells are initially filled with a drug at the specific concentration of  $C_0$ . Then, the drug solution in the well is gradually diluted based on the balanced medium droplet dispensing. Assuming that the medium droplet supplied from the droplet dispenser results in a uniform drug concentration,  $C$ , in the well, we derive the differential equation of  $\dot{C}(t) + QC(t)/V = 0$  where  $Q$  and  $V$  are the perfusion rate and well solution volume, respectively. By solving the equation, the drug concentration,  $C$ , is expressed as an exponential function of  $C(t) = C_0 \exp(-Qt/V)$ . As a result, the balanced droplet dispensing realized the pharmacokinetically relevant exponential drug elimination model [1].

Figure 4 shows the fabrication process for the prototype of Fig.5. Both droplet dispenser and well layer were fabricated from the molding and bonding processes of two PDMS plates. The fabricated droplet dispenser layer and well layer were sterilized by an autoclave and dried overnight. The bottom surfaces of the wells were treated with 2% (wt) bovine serum albumin (BSA) solution for 1hr at room temperature to prevent the non-specific binding. Finally, we stacked the PDMS layers and tightly sealed them using an acrylic jig with bolted joint.

## EXPERIMENTS

To demonstrate the capability for the exponential drug elimination, we measured the concentration profile of fluorescein isothiocyanate (FITC) gradually diluted from the balanced medium droplet dispensing. We supplied 140 $\mu$ l of FITC solution in the wells, and perfused the medium at the rate,  $Q$ , of 0.1 $\mu$ l/min using the balanced droplet dispensing. Then we collected the FITC solution from the drain outlet and measured the fluorescence intensity at the intervals of 1 day for the total duration of 5 days. Figure 6 shows the measured relative FITC concentration as a portion of the initial value during the medium perfusion for 5 days. The average and standard deviation of the FITC concentration were obtained from three measurements. Based on Eq.(3), the half-life,  $t_{1/2}$ , of the FITC is calculated as 16 hrs for the well solution volume,  $V$ , of 140 $\mu$ l and the perfusion rate,  $Q$ , of 0.1 $\mu$ l/min. In the previous pharmacologic study [7], the half-life of in-vivo cisplatin administration was measured as 21.7 $\pm$ 14.1 hrs. Therefore,

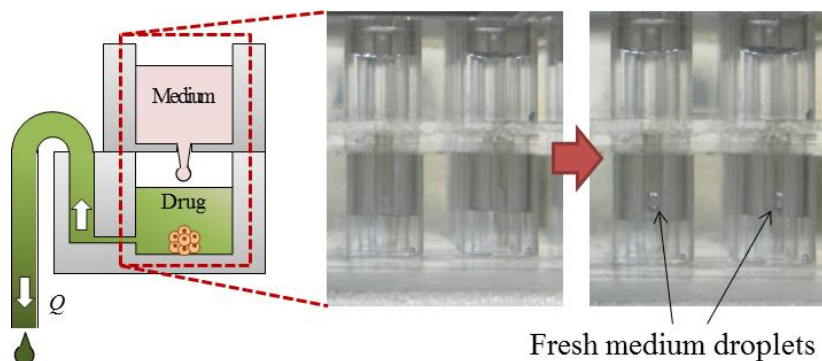


Figure 3. Balanced droplet dispensing for exponentially decreasing drug concentration.

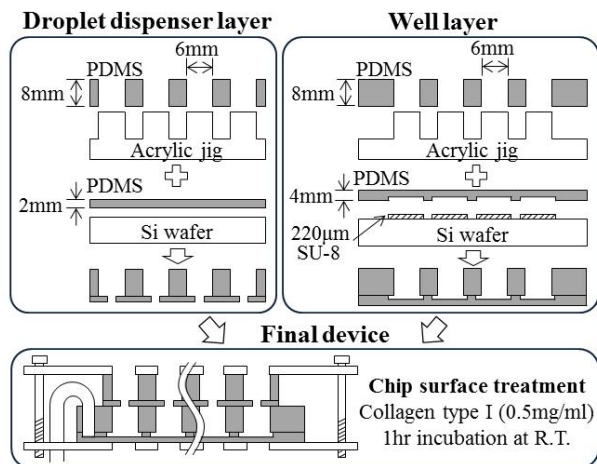


Figure 4: Fabrication process.

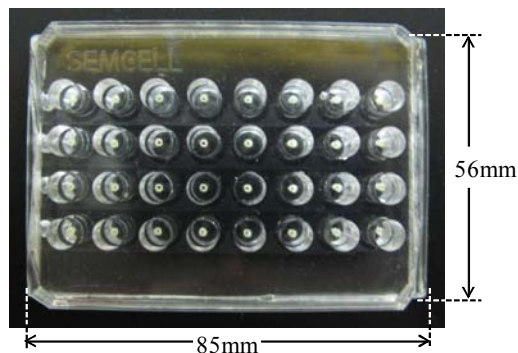


Figure 5. Fabricated 3D tumor spheroid chip.

further modification of the well solution volume,  $V$ , and the perfusion rate,  $Q$  is required to precisely adjust the present half-life to that of in-vivo profile. As a result, we observed that the measured FITC concentration profile agrees well with the exponential function. Therefore, we experimentally verified that the present chip implements the exponential drug elimination in the one-compartmental PK model using the balanced droplet dispensing.

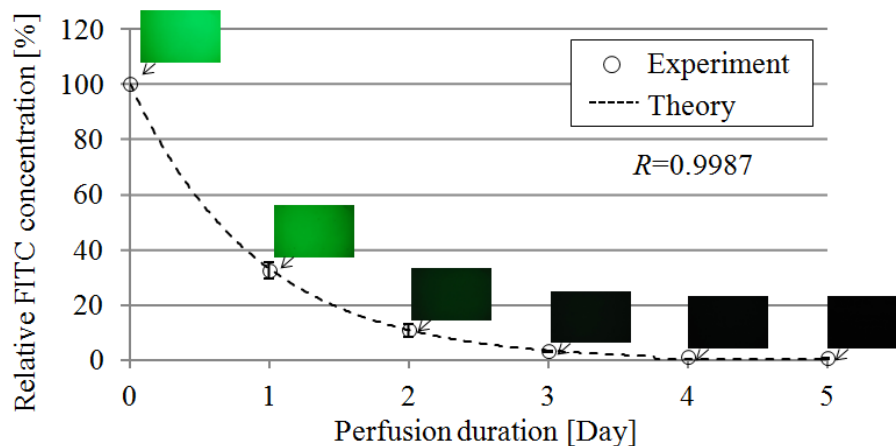


Figure 6. The exponentially decaying relative FITC concentration depending on the perfusion duration, illustrated by the inserted fluorescence images.

## CONCLUSION

We proposed and demonstrated the 3D tumor spheroid chip for the drug-response analysis of spheroids in the PK drug elimination model developed by balanced droplet dispensing. The present chip achieves the drug-response analysis of 3D tumor spheroids in the pharmacokinetically relevant drug elimination model for highly reliable spheroid-based drug screening chip.

## ACKNOWLEDGEMENT

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