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A Microfluidic Chip Designed for Stimulating Drug Diffusion

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ABSTRACT

Two-dimensional, three-dimensional or microfluidic chip-based cell cultures have been extensively used to identify effective compounds for drug development in the past few decades. Nevertheless, these traditional strategies of cell cultures are unable to capture the *in vivo* process of drug administration and diffusion. Here, we exploited the techniques of microfluidic chips and developed a new platform to dynamically simulate drug delivery and diffusion. Using this platform, we designed two experiments to quantify the diffusivity from source channel to target channel: (1) the pH value in target channel regulated by the diffusion of input solution from source channel; (2) the oxygen concentration in target channel regulated by the diffusion of oxygen produced by oxygen-enriched water in source channel. The input channel and the target channel were separated by nanoporous membranes mimicking biological tissue walls. These two experiments as proof-of-concept demonstrated that our platform can simulate the in vivo process of drug diffusion and be applied to study drug diffusivity.

Introduction

Cell culture, a technique for *in vitro* maintenance of cells isolated from tissues, has been used in enormous areas, including assessing toxicity, nurturing viruses for vaccine manufacture, investigating the viral infection cycle, employing them for genetic engineering, and so on. Wilhelm Roux pioneered cell culture by preserving a portion of an embryonic chicken in a tepid saline solution for many days in 1885 (Zurlo, J., Rudacille, D., Goldberg, A., 2006). Since then, two major types of cell cultures have been developed, two-dimensional (2D) and three-dimensional (3D) cell culture. With respect to 2D cell cultures, cells grow on 2D surfaces and can extend only in 2D plane form, such as the cells cultured in flasks and petri-dishes. 2D platform is the easiest and most efficient way for culturing cells in term of the process of seeding, culturing, immunofluorescent staining, imaging and other phenotypic analyzing methods (Cassano, D., Santi, M., D'Autilia, F., Mapanao, A. K., Luin, S., & Voliani, V, 2019). Cell culture in three dimensions has been touted as "Biology's New Dimension". 3D cell cultures are though to better mimic the *in vivo* 3D living organs wherein cells reside (Barrila, J., Radtke, A. L., Crabbé, A., Sarker, S. F., Herbst-Kralovetz, M. M., Ott, C. M., & Nickerson, C. A., 2010). Therefore, there is an increase in use of 3D cell cultures in basic researches of life science , drug discovery, regenerative medicine etc. (Mapanao, A. K., & Voliani, V., 2020).

Based on these traditional 2D or 3D culture techniques, microfluidic chips emerged recently as a new novel tool with channels of micrometer size to mimic in vivo liquid flow during cell cultures. These chips achieve accurate regulation of fluid on a micro scale via precise control of pressure. Moreover, they provide a number of advantages, including replenishing medium continuously, applying hydrodynamic shear on cells, rapid analysis, reduced response times via short diffusion distances, low fluid consumption, large surface to volume ratios, lower fabrication costs, and instantaneous quality validation. In addition, cost-effective disposable chips could be fabricated in mass production. Therefore, microfluidic chips have become one of the most

popular devices in the fields of biology and medicine. However, all these conventional cell cultures, including 2D and 3D models, and the microfluidic chips miss the feature of mimicking *in vivo* drug diffusion (Guo, Q., Zhang, L., Liu, J., Li, Z., Li, J., Zhou, W., Wang, H., Li, J., Liu, D., Yu, X., Zhang, J., 2021).

Efficient drug diffusion makes great contributions to clinics by simplifying the ways of drug delivery, increasing patient convenience, and, more importantly, improving therapeutic outcomes via reducing off-target accumulation and increasing drug concentration at target locations. Drug diffusion refers to the migration of effective drug components from a high-concentration zone to a low-concentration region (Gómez-Sjöberg, R., Leyrat, A. A., Pirone, D. M., Chen, C. S., & Quake, S. R., 2007). The rate of diffusion, also known as diffusivity, is a crucial metric of the drug delivery system. It is a proportionality constant between the molar flow, owing to molecular diffusion and the gradient in the concentration of species, The quicker they diffuse into distance, the greater the diffusivity. Numerous factors influence diffusivity, such as the condition of the substances, temperature, pressure, population dynamics, pore diameters, and so forth (Cussler, E. L., 1997; Grathwohl, P., 1998). To reach target site in body, the effective drug components are released after a delivery process, and then, migrate to pass through multiple barriers which are comprised of numerous semipermeable cell membranes (Maiti, S., Sen, K. K., 2017). Membranes are generally made up of a bi-molecular lipid matrix, determining the membrane permeability. As biological barriers, cell membranes potentially prevent exogenous foreign bodies from passing through. Thus, besides passive diffusion, other ways of drug diffusion are designed to travel across cell membranes, including enhanced passive diffusion, active transport, and pinocytosis (Le, J., 2020). Therefore, high efficiency of drug diffusion can promote medication molecules pass through the cell membranes to reach the systemic circulation and target locations in body (Nahler, G., 2017; He, H., Liang, Q., Shin, M. C., Lee, K., Gong, J., Ye, J., Liu, Q., Wang, J., Yang, V., 2013; Park, K., 2014). Yet, quantifying drug diffusivity is still challenging due to lack of proper methods. Here, based on the technique of microfluidic chips, we designed a platform to provide a strategy for the quantification of drug diffusivity.

Methods

Microfluidic Chip Design

The conventional two-dimensional or three-dimensional cell cultures, and microfluidic chip-based cell cultures are not capable of the quantification of drug diffusion efficiency. Also, traditional polymer microfluidics manage flows for low-cost cell culture and biological specimen analysis, yet they fail to effectively recreate the complicated 3D micro-structure of *in vivo* tissues wherein chemical drugs can transport. Therefore, we designed an origami-enabled microfluidic chip to mimic the process of drug diffusion in the complicated micro-structure of *in vivo* tissues. Moreover, we can quantify the drug diffusivity via this design. The origami-based technology is used in conjunction with folded tape. In this way, we can stimulate the process of drug diffusion by establishing pathways through nanoporous membranes (Lockwood, S. Y., Meisel, J. E., Monsma, F. J., & Spence, D. M., 2016).

The fundamental origami-folded construction is shown schematically in Figure 1. The 3D multi-material structure in the chips simulates the circulatory system of the drug from blood (red part in Fig 1A and 1B) to target location (blue part in Fig 1A and 1B) through biological cell membranes (yellow part in Fig. 1A and 1B). One pair of source micro-channels is served by one inlet and one outlet to carry one substance. Another pair of target micro-channels is served by the second inlet and the second outlet, interleaved with the source micro-channels to carry a different substance. Pressure is provided in the source inlet channel to create a continuous flowing channel mimicking the blood flow *in vivo*. The source channels and the target changes are separated via a folded nanoporous membrane mimicking biological tissue walls. The chemical components can diffuse through the nanoporous membranes from inlet channel to target channel, and vice versa (Xie, X., Kelly, C., Liu, T., Lang, R. J., Gandolfo, S., Boukataya, Y., & Livermore, C., 2018).

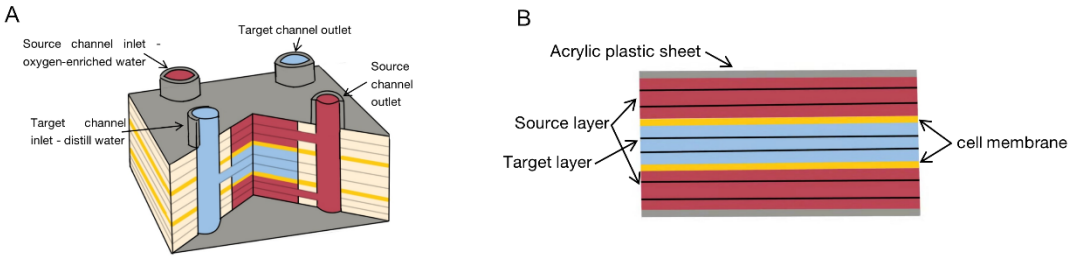

Figure 1. Schematic of the microfluidic device designed for quantification of drug diffusion. (A) illustrates the perpendicular interlocking of tape channels and nanoporous membranes. The functional chambers, and the inlet and outlet ports are shown by pale yellow tape; the functional chambers are linked to the inlet and outlet ports by red and blue tape; the nanoporous membrane is defined by yellow tape; and the acrylic plastic sheet at the top and bottom of the model is represented by gray tape in the diagram. The source channel that mimics blood vessels is depicted in red; the target channel that replicates the surrounding tissues is represented in blue. The inlet and outlet for source channels are shared, but the inlet and outlet for target channels are separated. The source and target channels are linked by diffusion across the nanoporous membrane. (B) shows the cross-section of the microfluidic device. The whole model is divided into three portions, each of which contains three layers. The first and third levels (in red) are connected to the inlet and outlet of the source channel. The target layer, i.e., the second layer, is connected to the entry and exit of the target channel. A nanoporous membrane in the center of each layer is connected to the entire model via diffusion.

The process of drug diffusion through living cell membranes, living organ walls, or vascular walls is critical for quantifying the drug diffusivity. Ideally, the nanoporous membrane could be replaced by culturing these biological tissues in the chips. For the sake of proof-of-concept, we used special nanoporous membranes to mimic the biological tissues. Two experiments were designed to quantify the diffusivity from source channel to target channel, (1) the pH value in target channel regulated by the diffusion of input solution from source channel, (2) the oxygen concentration in target channel regulated by the diffusion of oxygen produced by oxygen-enriched water in source channel.

By adjusting the pH value of the input in the inlet of the source channel, the first experiment assessed pH variations in the target channel. In the tests, we altered the pH input values by adding different quantities of citric acid (from 0.1 grams to 0.7 grams) in 50 milliliters of distilled water, providing citric acid solutions with varying concentrations. Then, the citric acid solutions can pass through the source channel to the target channel via nanoporous membrane. At the same time, we pumped 50 milliliters of distilled water into another layer through the target channel inlet, and measured pH values of all intake and outflow solutions with a pH meter. With these data, we can determine whether the model has diffusion capacity based on the pH values of outlet solution according to the diffusion principle.

We conducted another experiment to investigate oxygen diffusion in liquid. Oxygen was created by burning potassium permanganate, and immediately injected into 20 milliliters of distilled water, resulting in oxygen-enriched water. The source channel attained 20 milliliters of oxygen-rich water, meanwhile the target channel obtained 20 milliliters of distilled water. To make the experimental data more accurate, the control variable approach was utilized, including pumping both liquids into the model with the speed of 300 milliliters per hour, doing the experiment under room temperature (around 20 degree centigrade) and constant pressure. An oxygen meter was used to measure the oxygen level of these two liquids before pumping them into the chip, serving as control groups for the following experimental results. According to the diffusion principle, oxygen in oxygen-rich water would diffuse from the high concentration side to the low concentration side through the

nanoporous membrane. Hence, we were able to monitor the variation of oxygen concentration of the liquid flowing out of both outputs. The membrane in the chip was crucial to the entire experiment procedure. It linked all of the layers in the chip and accurately replicates the structure and mechanism of a more realistic process of drug diffusion in vivo.

Results

Using our chips, we performed experiments to quantify the diffusivity of citric acid solution by measuring the variation of pH. By pumping citric acid solutions of different concentrations into the source channel inlet and distill water into the target channel inlet, the citric acid solutions diffused through nanoporous membranes in the model, and then we measured the change in pH values in both source channel and target channel, as shown in Table 1. The pH of the solution in the source channel inlet dropped from 2.16 to 1.82 when more citric acid was added to the same volume of distilled water, indicating that the liquid was becoming more acidic. The measured pH of distilled water was 7.34, a relatively neutral value. Furthermore, the lower the pH of the solution injected into the source channel, the lower the pH value from both channels' output tubes. The pH values from these 7 experiments ranged from 3.14 to 2.05 in the source channel outlet, and 3.81 to 2.54 in the target channel outlet. Of note, no source channel has a greater output value than that of the target channel.

Table 1. pH variation due to the diffusion of citric acid solution.

Distilled water (for both Inlet Channel)	Citric acid - Solid Citric Acid + 50ml Distilled Water (Source Channel)	Source channel		Target channel	
50ml		Inlet's pH	Outlet's pH	Inlet' pH	Outlet's pH
	0.1g	2.16	3.14	7.34	3.81
	0.2g	2.11	2.90	7.34	3.64
	0.3g	2.04	2.70	7.34	3.46
	0.4g	1.99	2.50	7.34	3.26
	0.5g	1.93	2.34	7.34	3.04
	0.6g	1.88	2.20	7.34	2.78
	0.7g	1.82	2.05	7.34	2.54

We performed another experiment to quantify the diffusivity of oxygen-enriched solution by measuring the variation of oxygen concentration. The oxygen levels of the liquid were recorded at both channel outputs by injecting oxygen-rich water and distilled water via source and target channel inlets, respectively. The oxygen-rich water traveled through the source channel, and the distilled water traveled through the target channel. Table 2 shows that at the beginning of this experiment, 20 milliliters of oxygen-enriched water with a 10 milligrams per liter oxygen content was supplied for the source channel inlet, and 20 milliliters of distilled water with a 1 milligram per liter oxygen content for the target channel inlet. The oxygen level was measured every minute for a total of five minutes. Over time, the oxygen content of the source channel fell from 9.35 to 2.98

milligrams per liter, whereas the oxygen content of the target channel raised from 1.18 to 2.74 milligrams per liter (Table 2). The variation of oxygen is plotted as the function of time, as shown in Figure 2. The overall absolute gradient of the oxygen concentration of the source channel outlet (red line) was substantially greater than that of the target channel outlet (blue line), indicating that the oxygen concentration in oxygen-enriched water fluctuated more than that of distilled water. Moreover, the oxygen concentration became similar between the source channel outlet and the target channel outlet, because of diffusion of the oxygen-enriched solution.

Table 2. Variation of oxygen concentration due to the diffusion of oxygen-enriched solution.

Time/minute	Source Channel - Oxygen-enriched Water		Target Channel - Distilled water	
	Inlet mg/L (20mL)	Outlet mg/L	Inlet mg/L (20mL)	Outlet mg/L
1	10	9.35	1	1.18
2		5.26		1.89
3		3.42		1.92
4		3.12		2.04
5		2.98		2.74

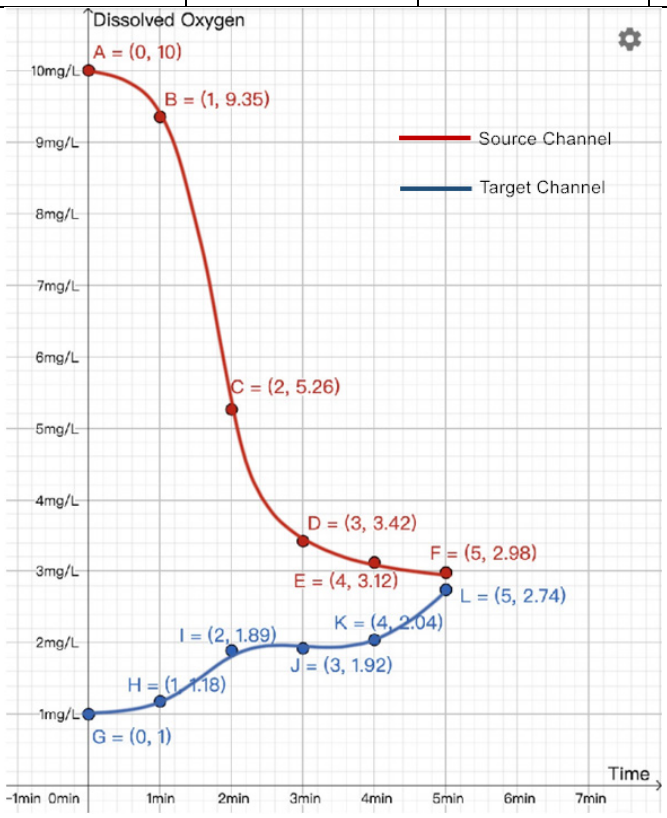

Figure2. Variation of oxygen concentration as a function of time. By pumping 20 milliliters of oxygen-rich water into the source channel inlet and 20 milliliters of distilled water into the target channel inlet, the solutions would enter the model and diffuse via nanoporous membranes, and then we will obtain the change in oxygen level per minute from the two outlets as shown in Table 2 and Figure 2. The pattern of oxygen content changes between the two channel outlets. The oxygen concentration of the source channel outlet is shown by the red

line, while the oxygen concentration of the target channel outlet is represented by the blue line. The red line on the graph represents a decreasing tendency, whereas the blue line represents an increasing trend.

Discussion

As a crucial technique, *in vitro* cell culture has been extensively exploited in basic researches and clinic studies. Using conventional 2D or 3D cell cultures, or microfluidic chip-based cell cultures, scientists have discovered enormous mechanisms by which living organisms maintain the homeostatic state or develop pathological state, and then, developed therapeutics to treat patients. Nevertheless, these conventional platforms for cell cultures lack the feature of mimicking *in vivo* process of drug diffusion. Therefore, we developed a new microfluidic chip-based design for cell cultures. The nanoporous membrane in the chip plays a critical role, which mimics the biological tissue walls or cell membranes. Following the principle of diffusion, the input solution diffuses in a trend from high concentration to low concentration through the nanoporous membrane.

Our two experiments demonstrated that the microfluidic chips we designed here can be used to simulate the process of drug diffusion and quantify the drug diffusivity. The first experiment showed that the pumped citric acid solution and distilled water have significant pH variations after the diffusion process. We would not be able to see any change in value at either outlet channel if this model doesn't allow the solution to diffuse. The significant pH variations at both outlets demonstrated the chips can mimic and quantify the process of drug diffusion. During the second experiment, the significant increase of oxygen concentration in the target channel outlets confirmed again the capacity of mimicking and quantifying the diffusion process using our chip. In addition, oxygen concentration in the source channel of oxygen-enriched water droped precipitously from the first minute to the second minute. This phenomenon was caused by oxygen in escapes from the distilled water when it passed through the channel, as the channel was not an enclosed space. A significant quantity of oxygen-enriched water went through the nanoporous membrane, inducing the sudden increase in oxygen concentration of distilled water in target channels from the first minute to the second minute. After 5 minutes, the oxygen concentration of either side of the nanoporous membrane eventually became equal due to the equilibration of diffusion between the source channel and the target channel.

Our chip design can better represent the process of drug diffusion inside the living body with some feasible modifications. For example, by filling the source channel inlet with the drug solution of the concentration used in clinics, we can quantify the drug diffusivity and the time required for the drug delivery. The nonporous membrane can be replaced by living cells or tissue walls, mimicking the real *in vivo* barrier through which drugs diffuse. We could also culture another type of cells in the target channels to quantify interaction between the two different types of cells with the drug diffusion from source channel to target channel. This chip design provides advantages of mimicking drug diffusion which are lacking in previous cell culture platforms and paves a promising avenue for the study of drug diffusion and potential application.

Conclusion

Here, we designed an origami-enabled microfluidic chip containing a nanoporous membrane through which drugs can diffuse from source channel to target channel. Our design can mimic the *in vivo* process of drug diffusion and quantify the drug diffusivity. Using this design, we performed two experiments: (1) the pH value in target channel regulated by the diffusion of citric acid solution; (2) the oxygen concentration regulated by the diffusion of oxygen-enriched water. The first experiment demonstrated that the diffusion between the source channel and the target channel led to the PH increasing the source channel outlet and decreasing in the target channel outlet, respectively, compared with their own corresponding inlet values. In the second experiment, the diffusion of oxygen-enriched water induced a continuous decreasing of oxygen concentration in the source

channel and an increasing in the target channel. Therefore, our design could simulate the *in vivo* process of drug diffusion, which can be used to quantify the drug diffusivity.

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