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Theoretical and experimental study of a membrane-based microfluidics for loading and unloading of cryoprotective agents

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ABSTRACT

Cells are routinely cryopreserved for various applications. Successful cryopreservation requires the loading of cryoprotective agents (CPAs) to prevent cells from cryoinjury during freezing and thawing, as well as the unloading of CPAs before use since CPAs are often toxic. Moreover, the CPA loading/unloading processes are usually effortful and error-prone. In this study, a membrane-based microfluidic device is introduced to aid the CPA loading and unloading processes. The device consists of two helical microfluidic channels with a sandwiched layer of microfiltration membrane. CPA exchange across the membrane via diffusion and filtration is realized when cell suspension and replacement fluid flow counter-currently in the channels. CPA concentration in the cell suspension can be regulated in a well-controlled, progressive manner. A theoretical model has been developed to better characterize the dynamic change and distribution of the CPA concentration, as well as the cell volume response, with respect to various mass transfer parameters within the device during CPA loading and unloading steps. Physical experiments have been conducted to validate the proposed model. Furthermore, operating conditions of the device have been optimized under the guidance of the established model and different devices with various sizes have been fabricated to evaluate their performance characteristics with respect to different levels of throughput. The optimized condition enables the loading of DMSO (from 0% to 10%, v/v) at a rate of 180 ml/h and unloading (from 10% to 1%, v/v) at 5 ml/h with a single pass through the initial prototype. A second enlarged device further improves the DMSO unloading rate to 180 ml/h with the washing solution consumption rate at 225 ml/h. The higher throughput and reduced washing solution requirement demonstrate the significant advancement in efficiency with the presented approach. Lastly, the system is easy to operate and the process is robust, thus provides a reliable solution for loading/unloading of CPAs for various sized cell suspensions.

1. Introduction

Cryopreservation is an enabling technology for a wide variety of fields including cellular therapy, regenerative medicine and biobanking [1–3]. In cryopreservation, cells or tissues are cooled down to subzero temperatures in an effort to discontinue biological activity. This is followed by the subsequent thawing procedure to bring the sample temperature back to physiological level. Successful cryopreservation requires the loading of cryoprotective agents (CPAs) to prevent cells from cryoinjury during freezing and the subsequent thawing process. Unfortunately, CPAs are toxic to cells or patients [3–6]. Therefore, a post-thaw washing step to remove the excess amount of CPAs from preserved cells is mandatory prior to any scientific or medical usages. During the loading and unloading steps, CPAs and water flow across the cell membrane and cause cell volume excursion [4,5]. In view of the limited ability of cells to withstand swelling and shrinkage, the osmotic disequilibria induced in the steps is a critical factor that often inadequately considered [2].

Routinely, CPAs are loaded and unloaded by centrifugal separation. Significant cell loss is often related to these steps due to centrifugation and osmotic forces. For instance, it was reported that

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cell loss of 27–30% is typical when removing dimethylsulfoxide (DMSO) from cell suspension by centrifugation [7]. Moreover, these steps are usually labor intensive and vulnerable to run-to-run variations in technical operation [8], which are major risk factors to the standardization and consistency requirements in modern biobanks. Aiming to reduce manual effort to improve process efficiency and controllability, several automated systems have been developed [9–11]. However, these systems are designed to only accommodate large quantity of cell suspensions (eg. usually a few hundred ml). With the current advancement in medicine and scientific research, precious biological samples are typically cryopreserved in small vials (less than 100 ml). T Therefore, an automated approach specifically optimized to safely and efficiently load and unload CPAs for handling small quantity of samples is in urgent demand.

Over the past decade, microfluidic systems have led to significant breakthroughs in a variety of biological applications [12–14]. Its ability to manipulate cells and the chemical environment surrounding them has made microfluidic devices very promising for cryopreservation applications. Studies on the CPA transport behavior across cell membrane using microfluidic channels have been extensively reported in the literature [14–17]. Recently, several diffusion-based microfluidics have been reported for effective loading or unloading of CPAs [1,12,18–21]. The developed systems involve the utilization of multiple parallel streams (including cell stream) that flow through a microfluidic channel, allowing CPAs to diffuse across the liquid interface due to concentration gradient. By assuming cells were moving along the channel and experiencing changes in CPA concentrations progressively, thereby osmotic shock is minimized. However, the process relies on direct contact of cell stream with solution containing different concentration of CPAs, thus is likely to induce osmotic shock for cells located near the interface between the streams. Acceptable cell recovery from the device is only achieved under a very narrow range of flow conditions, which has limiting the operating window for efficient CPA removal [1].

Membrane-based systems provide new solutions for CPA processing. By introducing a semi-permeable membrane, direct contact of cell suspension with replacement fluid is avoided, and flexible mass transfer is achieved by controlled dialysis or filtration. Several systems have been investigated for unloading CPAs based on commercial hemodialyzers or hemofilters, and the benefits of improved efficiency and cell safety have been demonstrated [10,22,23]. Most recently, membrane-based microfluidics have also been attempted [8,24,25]. The studies opened a new path for easy and safe CPA processing. However, the current designs are far from ideal and system efficiency needs significant improvement to fulfill the practical requirement. For instance, the devices are difficult to provide sufficient CPA removal through a single-pass process [8,24,25].

Herein, we introduce a novel membrane-based microfluidic system to aid the processes of loading and unloading of CPAs. With the new device design, an improved system efficiency was achieved. Theoretical and experimental studies have been conducted with the presented membrane-based microfluidic device to evaluate its mass transfer characteristics, and then obtain optimized operation condition and system design. The obtained results will pave the way for development of a clinical protocol for safe and efficient CPA processing for samples with a wide range of sizes.

2. Material and methods

2.1. Design and fabrication

As shown in Fig. 1, a microfluidic device consists of two PMMA sheets with two laser engraved helical microfluidic channels.

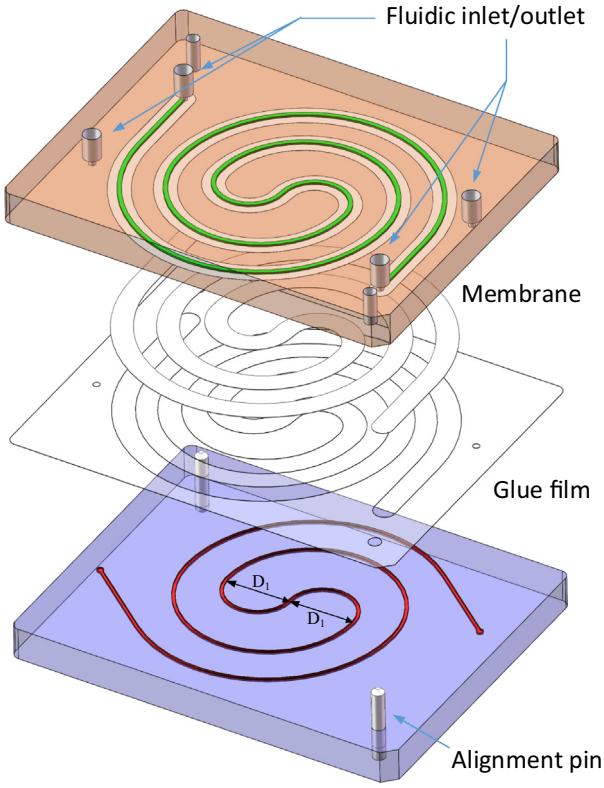

Fig. 1. The membrane-based microfluidic device for CPA loading/unloading.

Accordingly, a membrane slice used were also cut into a similar but wider helical geometry (with laser cutting) to cover the channels. Then all the components are sealed together by an epoxy glue thin film. When cell suspension and replacement fluid are introduced and flow counter-currently in the channels, CPAs exchanges between them through diffusion and microfiltration, and CPA concentration in the cell suspension can be increased or decreased in a controlled, progressive manner.

The helical geometry consists of a series of tangent semicircles, which are originated from the inner turns and extended to the outer turns. The diameter of the inner turns (D_1) of each channel was selected according to the width of channel and the required space to attach the membrane and glue film on the shoulder (typically $D_1 = W + 10$ mm). Then the diameter of each outer turn can be determined as $D_i = D_{i-1} + 2D_1$. Such topology of the channels was designed to obtain as long channel as possible while maintaining it pressure loss characteristics similar as a straight channel.

2.2. Modeling and simulation

To assist in selecting the appropriate geometry parameters and operating conditions for the microfluidic device, a theoretical model was proposed in both device-level and cell-level (as shown in Fig. 2) to predict the transport of water and CPAs as well as the resulting cell volume excursion, respectively. The following assumptions were applied for the ease of calculation: (1) flows in the microfluidic channels are one dimensional although the channel is non straight, because the impact of channel geometry on the inside flow should be negligible considering the characteristic size of the channel is very small and the flow rates are relatively low; (2) cell and the extracellular solution flow as a combined stream, neglecting the effects of cells; (3) both extracellular and intracellular solutions consist of water, a permeable CPA (e.g. glycerol) and