DESIGN AND DEVELOPMENT OF 3D ROTATIONAL BIOREACTOR FOR 3D CELL CULTURE

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Received: December 20, 2021; Revised: May 04, 2022; Accepted: May 05, 2022

Abstract

Currently, cell and tissue culture are a key to success for regenerative medicine and the pharmaceutical industry for implementing new therapeutic concepts. Cell culture is a complex process in which cells are grown under controlled conditions. Three-dimensional (3D) scaffolds provide space for cell adhesion and growth in 3D structures. However, in 3D cell culture, the ability to supply nutrients and oxygen, and the simultaneous removal of waste products and metabolites are the limiting factors for cells survival, due to nutrient starvation and metabolite toxicity. Therefore, an in-house 3D rotational bioreactor, for 3D cell culture, was designed, fabricated, and developed based on a chitosan porous scaffold. The 3D rotational bioreactor provides a continuously moving culture medium led to high rates of mass transfer throughout the scaffolds. The rotation speed and duration are programable and provide a continuous free flow during the rotating period. The results demonstrated the potential of an in-house 3D rotational bioreactor for better nutrient supply and waste transfer systems outweighs the conventional cell culture technique.

Keywords: 3D cell culture, 3D rotation, Bioreactor, pharmaceutical industry, programable

Introduction

The pharmaceutical industry discovers, develops, produces, and markets drugs for medications to save lives and enhance the quality of life (Scherer, 2000; Li and Li, 2021). Cell culture is one of most experimental pre-clinical testing in the pharmaceutical industry, particularly the utilization of animals or human cells for drug validation on the efficiency, toxicity, and intracellular targeting of the developed pharmaceutical compounds (Ozturk and Hu, 2005; Bang and Kim, 2021; Dasgupta *et al.*, 2021). A conventional cell culture approach has been performed in a 2D model (monolayer). This is less likely similar to the real human physiological condition, in which cells are lying in a three-

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dimensional (3D) structure. Therefore, a 3D cell culture has been developed to generate a testing condition that most fits human tissue (Jensen and Teng, 2020). To improve 3D cell cultivation conditions, biocompatible materials have been used as a 3D structural scaffold for cell adhesion and growth such as ceramics, 3D printed polymers, and lyophilized polymers (Nikolova and Chavali, 2019; Da Silva *et al.*, 2021; Liu *et al.*, 2021). Cells attach to the biocompatible fabricated structure and proliferate freely within 3D space (Agueda *et al.*, 2021). Resolution and improvement of an insufficient nutritional supply in the 3D cell culture model are challenging (Sun *et al.*, 2021). Thus, the engineered devices "bioreactors" have been developing (Lv *et al.*, 2017; Altmann *et al.*, 2021). Cultivation, expansion, stimulation of growth, and accurate cell functions of human cells using automated bioreactor systems could be performed by several approaches such as rotating Erlenmeyer flasks, wave bioreactors, rotating wall bioreactors, and stirred tank bioreactors (Kropp *et al.*, 2017). These techniques are capable of scaling up from experimental scale to manufacturing scale. The simple approach for generating bioreactors is a rotating wall vessel bioreactor (RWV), which was developed for improving and overcoming the limitation of 3D cell culture methods (Radtke and Herbst-Kralovetz, 2012). The RWV provides a 3D cell culture condition advantage by generating a flow of cell culture medium (Nishi *et al.*, 2013). However, the rotational axis of the RWV, which rotated only one axis is a limitation, and optimal culture conditions such as humidity, temperature, and CO_2, are needed (Kizilova, 2021). In this study, an in-house 3D rotational bioreactor, using local and low-price materials, was fabricated incorporate with a conventional cell culture CO_2 incubator, which is generally available in most of the biological and medical laboratories. The ultimate aim is to improve nutritional supply, for cells cultured in a 3D scaffold, by increasing the rotating direction with low price fabrication.

Materials and Methods

Fabrication of an In-house 3D Rotational Bioreactor

DC gear motors (ZGA37RG: Planetary gearbox, Zhejiang Zhengke Electromotor Co., Ltd, Zhejiang, China) were used to rotate each axis of the device. The rotation speed of each motor can be adjusted between 0-300 rpm, using a microcontroller (PIC16F887, Microchip Technology Inc., Arizona, USA). The micro-controller adjusted the device rotational speed, angle, and working duration via a human interface (input button and LCD display). The rotation angle was allowed to be set between 180° on the x-axis and 360° on the z-axis (Figure 1). The mechanical structures were fabricated using aluminum and stainless steel. The vessel holder was modified from PVC pipe with 2 points of M4 screw to fix the cell culture vessel in place as shown in Figure 2. The cell culture vessel holder was designed to apply to both 15 ml and 50 ml conical

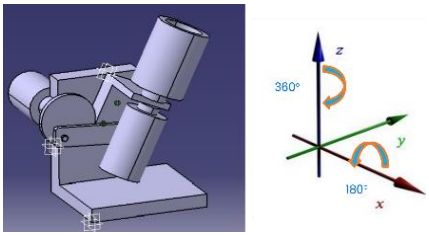

Figure 1. Rotation angle and direction

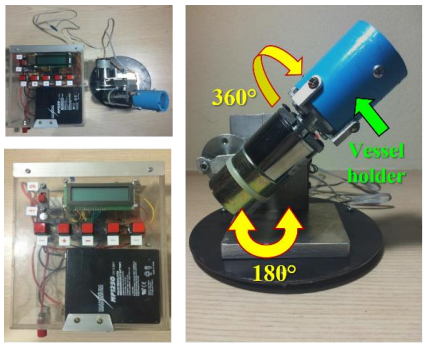

Figure 2. The in-house 3D rotational bioreactor

centrifuge tubes.

The Experimental Setup

Activated carbon powder (activated charcoal capsule, manufactured by Greater Pharma Manufacturing Co., Ltd., Nakhon Pathom, Thailand.) was used as an indicator for the bioreactor efficacy investigation. The water-insoluble property and the particle size of the carbon powder are the advantages. The carbon powder (260 mg) was mixed and diluted in deionized water (DI water). The carbon particles were counted under a microscope and counting chamber.

The cylindrical shape lyophilized pure chitosan porous scaffold (CS) (Wattanutchariya *et al.*, 2016; Thunsiri *et al.*, 2018) was used for generating 3D structure in this study (dimension: Ø 6.2 mm and 10 mm high). The experiment was started by immersing 10 pieces of CS into 5 ml of water containing 1.525 million carbon particles. The samples were tested separately in 3 rotating

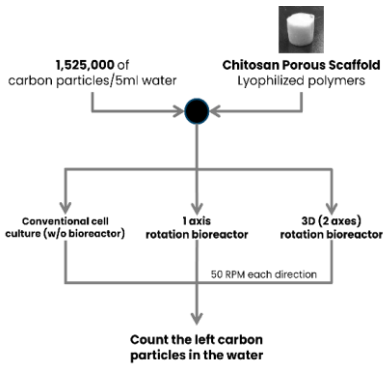

Figure 3. Experimental diagram

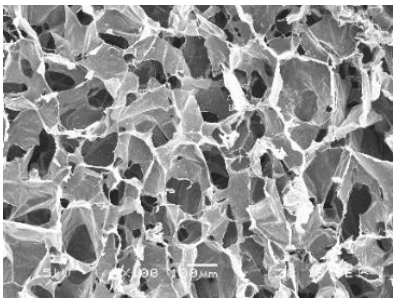

Figure 4. Scanning electron microscopy (SEM) image of the lyophilized CS scaffold

conditions including conventional cell culture (without bioreactor), 1 axis rotation, and 3D (2 axes) rotation bioreactor with 50 RPM rotation speed in each direction. For 1 axis rotation protocol, the vessel was fixed at 0° on the x-axis (parallel to the y-axis) and rotated 360° on the y-axis. In the 3D rotation protocol, the rotation on the x-axis is available from 0°-180° while the vessel was rotated 360° during the x-axis rotation. After rotating for 5 min, CS was removed and 1 ml of water containing carbon particles was collected and counted for the carbon particle number as presented in the experiment diagram (Figure 3). Low particle number represents high efficacy of the device to supply the solution to the middle of the 3D structure while high carbon particle number represents low efficacy. The carbon particle floated through the porous structure of CS and was trapped inside the intricate shape of the lyophilized CS scaffold as shown in Figure 4.

Results and Discussion

At the end of the experiment, 1 ml of water containing carbon particles from each rotating condition was collected. The carbon particles were

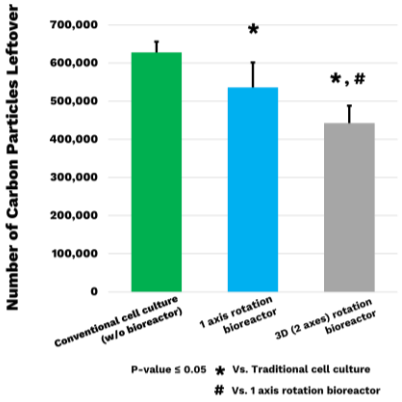

Figure 5. The number of carbon particles leftover of each rotating protocol

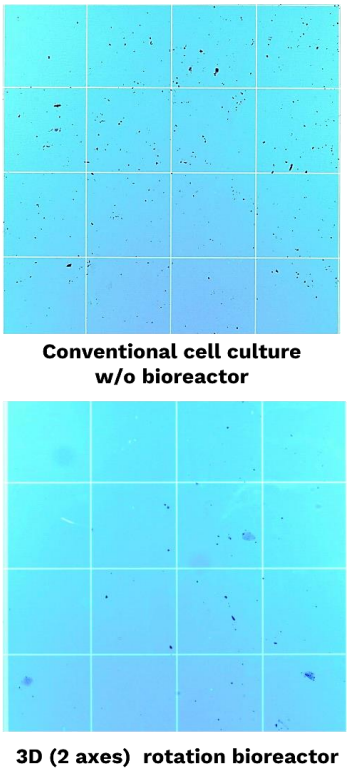

Figure 6. The microscopic images of water containing carbon particles in the counting chamber

counted and calculated back to particle number per 5 ml of the solution. The highest number of carbon particles leftover in the solution was 627,500± 28,504 particles/5 ml in the conventional 2D cell culture. There were greater than 700,000 particles were trapped inside 10 pieces of CS due to their absorption ability (Jaifu *et al.*, 2019; Thunsiri *et al.*, 2020; Wattanutchariya *et al.*, 2020) without any movement. Furthermore, in 1 axis rotation

protocol, the carbon particles leftover in the solution were significantly less than a conventional 2D cell culture (535,700±65,839 particles/5 ml). Meanwhile, the 3D (2 axes) rotation protocol presented the lowest carbon particles (442,500±45,586 particles/ 5 ml) and significantly lower than the other protocols as shown in Figure 5. The comparison of carbon particles leftover in the solution between the conventional cell culture and 3D (2 axes) rotation bioreactor in the counting chamber under the microscope was shown in Figure 6, where the tiny black dots are carbon particles. The black dots scatter the plane of conventional cell culture with higher density than the 3D rotation protocol. Furthermore, the scaffolds from the conventional cell culture, 1 axis rotation, and 3D rotation bioreactor were cross-sectional sliced and the images were collected under microscope. The results in Figure 7 showed the microscopic images of carbon particles in the CS scaffold of the conventional cell culture and 3D rotation bioreactor. The carbon particles were found as trapped inside and sedimented at the bottom of the porous structure. The carbon particle could be spread from the outside through the structure due to their interconnected pores (Chung *et al.*, 2002; Wattanutchariya and Thunsiri, 2015; Thunsiri *et al.*, 2016; 2018).

Figure 7. The microscopic images of carbon particles in the CS scaffold

Conclusions

The in-house 3D rotational bioreactor showed the potential to increase the number of carbon particles, which represents the ability to increase the nutrition supply to the center part of the 3D scaffold. An increasing rotation axis enhanced the number of carbon particles compared with the conventional 2D cell culture technique. Even the CS scaffold provided high absorption ability, which allowed carbon particles to penetrate to the center of the 3D scaffold, the results also showed that an in-house 3D rotational bioreactor significantly enhanced the performance. Moreover, the fabrication cost was ten times lower than the cheapest commercially available RWV. For future study, the experiment with human cells should be investigated with various rotational speeds. Finally, the microcontroller will be upgraded, and the appearance of the device should be modified and improved.

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