JASMAC Conference of the Japan Society of Microgravity Application

P03

オンチップ 3 次元組織灌流培養が可能な３D クリノスタットの開発

Development of a 3D clinostat for on-chip 3D tissue perfusion culture

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1. Introduction

The microgravity environment affects the musculoskeletal resulting in bone loss and muscle atrophy[1]. These musculoskeletal impacts not only increase the health risk of astronauts, but also pose an obstacle to the generalization of human spaceflight. To understand the effects of microgravity at the molecular or cellular level, the three-dimensional (3D) clinostat, a device that can simulate microgravity on Earth has been proposed to be an effective alternative for cell culture studies under microgravity on Earth[2,3]. However, since most 3D clinostats must be operated in a CO_2 incubator to maintain appropriate physicochemical conditions for cell culture other than simulated microgravity, it is required to be small, moisture resistant, and therefore high production cost. In addition, most of the cell cultures that have been performed under 3D clinostat are static cultures, and it is difficult to accurately reproduce the long-term effects of microgravity *in vivo*.

We have developed a low-cost system for long-term perfusion microfluidic culture under simulated microgravity. We designed and built 3D clinostats at low cost using 3D printing and open-source hardware. One 3D clinostat carries two silicone-based microfluidic chips with on-chip CO_2 incubation (OCI)[4] that enables cell culture without a CO_2 incubator, and with Braille-based microfluidic perfusion[5]. We evaluated gravitational acceleration, the trajectory, and the flowrate in microfluidic channels generated by Braille actuators under simulated microgravity.

2. Experimental

2.1 Device design

We developed a 3D clinostat shown in Fig. 1. All the structural members are 3D printed poly(lactic acid) (PLA). Two orthogonal axis that constrain the inner and outer frames are connected to stepper motors (PKP242D23A2 and PKP245D08A2, ORIENTAL MOTOR). The stepper motors are driven with two motor drivers (L6470, Akizuki Denshi) controlled by an Arduino Nano microcontroller board. A film heater (FH-10, AS ONE) is placed on the cell culture sample loading area, inside of the inner frame. Inner frame's stepper motor and film heater are powered through slip rings. Fig. 2A illustrates the microfluidic perfusion cell culture on a 3D clinostat without need of a CO_2 incubator. The OCI system consists of a pair of nested reservoirs filled with cell culture media and bicarbonate buffer insulated with gas permeable silicone walls. Heating the device at 37 °C brings the equilibrium in CO_2 level in the nested reservoir near 5% without any external feedback system[4]. As shown in Fig. 2B, peristaltic actions of three stranded Braille pins moves the liquid in a silicone elastomeric microchannel to generate flow. The layout of microchannels we used in this study is shown in Fig. 2C. A microfluidic chamber with side perfusion channel partitioned by pillars is for three-dimensional cell culture suspended in hydrogel perfused with a Braille micropump.

2.2 Measurement of flow rate under simulated microgravity

To monitor the gravitational acceleration at the chip loading area depending on the attitude of the inner frame, a motion tracking sensor MPU6050 was placed on the inner frame. The data from the sensor were transferred to a PC via serial communication with the same Arduino Nano controller and visualized with Excel.

To evaluate the flow generated by Braille under simulated microgravity or under 1G (i.e. with or without 3D clinostat drive), aqueous solution of blue food dye was introduced to the inlet of the microchannel primed with water. A parallel pair of two three-stranded Braille pin pumps are actuated as shown in Fig. 2B in opposite phases at a refresh rate of 4 s for 4 ~ 5 min. Color images of the top view of the microfluidic chips were acquired with a 12 megapixels smartphone camera and saturation (chroma) channels were extracted using the ImageJ image processing software to obtain the diffusion front of the blue dye inside of the microfluidic channel . The flowrate was calculated by the travel distance of the diffusion front of the dye divided by the time of travel and the crosssectional area of the microchannel.

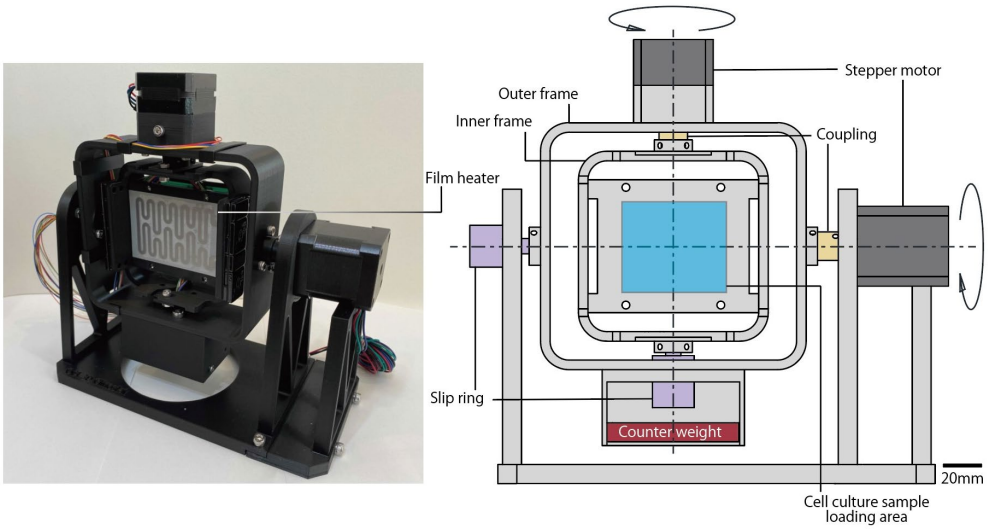

Fig. 1 A low-cost 3D clinostat compatible to on-chip perfusion cell culture. Two cell culture chips are fixed on the flat surface surrounded with the inner frame. A film heater was adhered to flat surface to maintain the surface temperature to 37 °C.

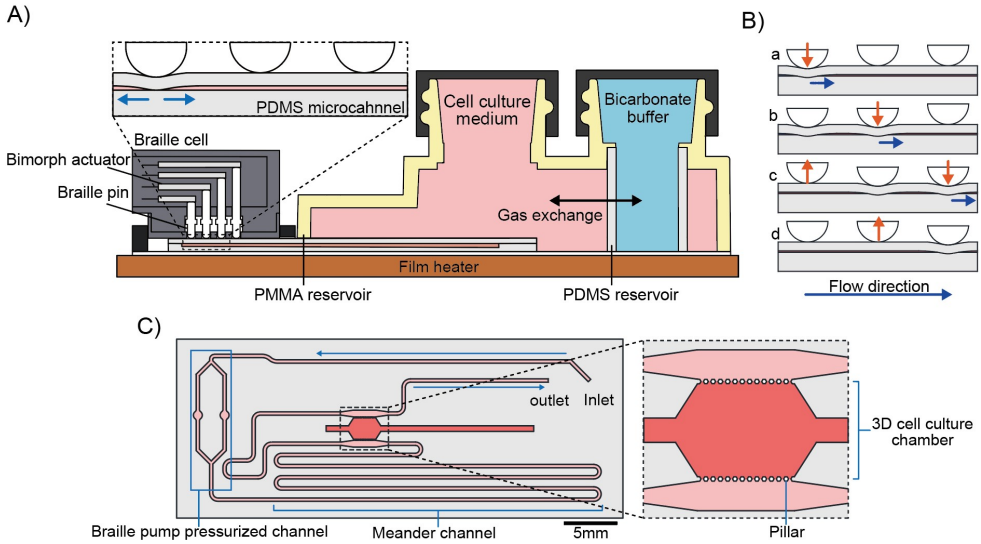

Fig. 2 Microfluidic perfusion culture system with on-chip incubation (OCI) and Braille micropumps. A) Illustrated cross-sectional view of the cell culture device. Bicarbonate buffer heated by a film heater supplies gas-phase CO2 to cell culture medium through poly(dimethyl siloxane) (PDMS) reservoir wall. B) Actuation patten of three-stranded Braille micropump. Pumping liquid by pressurizing the channel from the upstream from steps a. to d. C) Layout of microchannel used for 3D perfusion cell culture.

3. Results and Discussion

3.1 Measurement of gravitational acceleration

Fig.3A shows a typical time evolution of the gravity acceleration of each axis during the 3D clinostat driving. The cumulative gravitational acceleration after 30 minutes operation ranged from -2.9×10^{-3} to 8.4×10^{-3} [g], comparable to a typical value of a commercially available clinostat[6]. The three-dimensional trajectory of the gravitational acceleration was found to be driven by various solid angles as shown in Fig.3 B).

3.2 Measurement of flowrate under simulated microgravity

Fig.4 shows the average flow rates of the Braille micropump under simulated microgravity and 1G. The difference in flowrates under simulated microgravity was not significant. Braille actuators could be successfully operated on the 3D clinostat and perfusion culture could be performed under simulated microgravity without decrease of flowrates

4. Conclusion

We developed a 3D clinostat capable of reproducing simulated microgravity similar to that of commercially available 3D clinostats, and perfusion culture using a Braille micropump. By integrating the OCI system into the 3D clinostat, cell culture can be performed under simulated microgravity without need of a specialized 3D clinostat. The Braille micropump was so compact that it was easily integrated with the 3D clinostat, and the microflow generated by Braille was not affected by the simulated microgravity. The proposed system would have the potential to more accurately understand the effects of microgravity on living organisms.

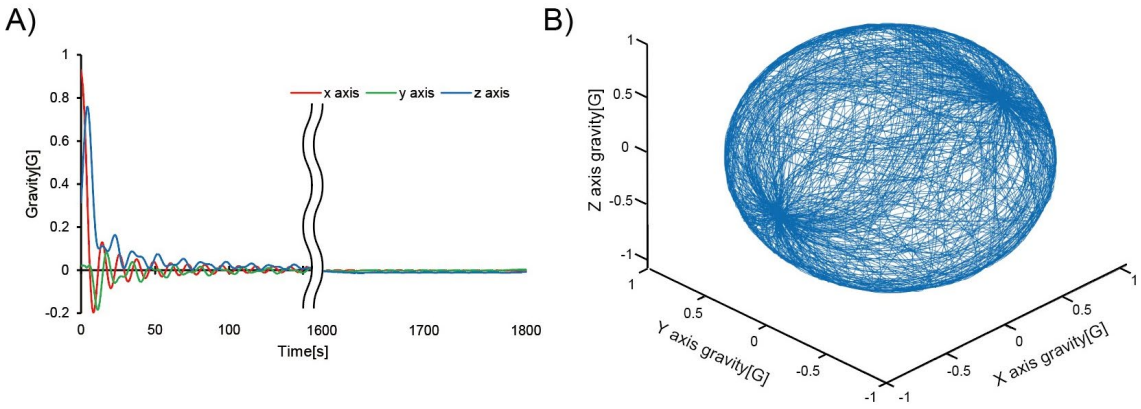

Fig. 3 A) Time averaged gravitational acceleration in each axis.
B) 3D trajectory of gravitational acceleration at the end of 30 minutes.

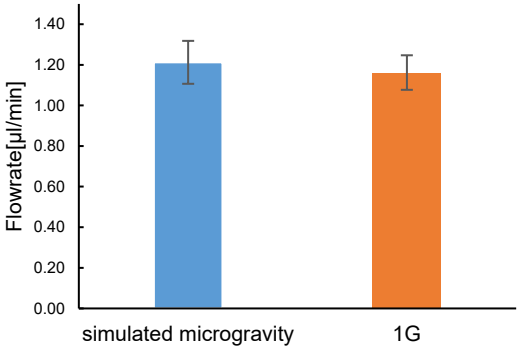

Fig. 4 Flow rate of the Braille micropump under simulated microgravity and 1G. Mean±SD. *N*=4 for each condition.

References

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