Design and evaluate of a multilevel laminar shear stress perfusion bioreactor

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Research

Keywords: bioreactor, shear stress, tissue engineering, online monitoring

Posted Date: March 26th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-18759/v1>

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Abstract

Background: Fluid shear stress affects cell viability and cell differentiation. This study aimed to build a bioreactor for tissue engineering in which the cultured cells are exposed to custom designed multilevel laminar shear stress.

Methods: The bioreactor was built based on a multi-layer plate configuration and equipped with an online monitoring system. The diameters of the diversion holes of each layer varied from 0.8mm to 1.4mm. Computational fluid dynamics and tracer liquid imaging velocity measurement were used to study the fluid field distribution. Finally, human umbilical cord mesenchymal stem cells (hUCMSCs) were cultivated in this bioreactor system.

Results: The actual flow field distribution was consistent with the simulation results, and the average relative error of the flow velocity was 10.52%. The variation in shear stress of each layer was less than 3.0×10^{-5} Pa, indicating that the flow microenvironment was uniform in each layer. Additionally, the shear stress increased with increasing diameters of the diversion holes. Cell culture experiments of Vero cells and hUCMSCs showed that the system continuously monitored morphological characteristics of the cells. The designed bioreactor achieved the desired proliferation, indicating that the bioreactor provided a suitable microenvironment for cell growth.

Conclusion: The fluid shear stress within the bioreactor can be specified as required, and the morphological characteristics of the cells can be monitored in real time. It achieved the proliferation of hUCMSCs. This bioreactor system will facilitate the study of tissue engineering.

Background

Tissue engineering technologies offer promising treatments for serious diseases ^[1–3]. Due to the challenging clinical requirement of providing large cell numbers, in vitro expansion and differentiation of cells are necessary ^[4]. Bioreactors are a well-established technology that provide a suitable microenvironment for cell cultivation in vitro and have received widespread attention ^[5].

The shear stress distribution is an important issue especially in bioreactor studies. Animal cells are very sensitive to mechanical stimuli because their cell membranes consist of only phospholipid bilayers and lack the protection of a cell wall ^[6]. Compared with a hydrostatic pressure, the production of nitric oxide (NO) and the mRNA expression of several mechanosensitive genes were higher at lower levels of shear stress in mesenchymal stem cells (MSCs) ^[7], indicating that these cells are more sensitive to shear stress than other kinds of mechanical stimuli. On the one hand, high levels of shear stress affect the growth of cells. Many studies in the literature have summarized the effects of high levels of shear stress on cell damage ^[8, 9]. On the other hand, specific ranges of shear stress influence the gene expression in cells, affecting the viability and differentiation of these cells such as human bone mesenchymal stem cells (hBMSCs) and human umbilical cord mesenchymal stem cells (hUCMSCs). Shear stress has an influence

similar to that of the leukemia inhibitory factor, which maintains and influences the gene expression of certain pluripotency markers^[10]. Compared with high levels of shear stress, a fluid shear stress of 10^{-5} Pa promotes MSC proliferation but reduces osteogenic expression^[11, 12]. While MSCs stimulated by 10^{-4} Pa shear stress shown stronger adherence ability and better survivability during cryopreservation than those cultivated in static state^[13]. In addition, a relatively larger shear stress may induce stem cell differentiation. MSCs have been reported to produce more endothelial markers at 1.5 Pa of shear stress, suggesting that shear stress can induce endothelial differentiation^[14]. A shear stress range of 1.5 to 0.15 Pa was shown to promote the hematopoietic and endothelial phenotypes of pluripotent stem cells^[15]. In another experiment, an average shear stress of 10^{-3} Pa was associated with the upregulation of mRNA expression of osteocalcin and alkaline phosphatase^[16]. In a cartilage differentiation experiment, a shear stress of 0.005 Pa stimulated the production of bone morphogenic protein-2 and led MSCs to produce more type I and type II collagen compared to the static group^[17]. Furthermore, rat MSCs were reported to differentiate into nerve cells and obtain the highest differentiation rate at a fluid shear stress of 0.03 Pa^[18]. Since the shear stress has such a significant impact on cellular behavior, a bioreactor with a designable flow field distribution and controllable shear stress is necessary for tissue engineering.

Therefore, this paper aims to design a bioreactor system for adherent cell cultivation. Cell culture operations, such as disinfection and cell adhesion should be easy to perform in this system. Microenvironments parameters such as pH, dissolved oxygen and temperature should be automatically controlled, and cell culture should occur under a tunable laminar flow. The flow field distribution in the bioreactor should be tailored to specific research needs, and the fluid shear stress should also be controllable. Finally, an online monitoring system is required for conveniently observations of cell morphology.

Results

Computational fluid dynamics (CFD) modeling and simulation

The simulation results are shown in Fig. 1. As shown in Fig. 1A, the liquid enters the space between two plates from the edge, flows along the radial direction toward the center of the plate and gradually accelerates. As the liquid approaches the diversion holes, the direction of the liquid changes toward the diversion holes. Figure 1B shows the distribution of shear stress on the plates. This figure indicates that shear stresses were greater than 3.0×10^{-5} Pa in the high velocity zone, whereas the shear stresses in the other regions of the plate were less than 3.0×10^{-5} Pa and had a more homogeneous distribution. At the same time, as the diversion holes' size gradually decreased from the first layer to the seventh layer, the shear stress on the plate slightly decreased except the top layer (layer 1).

Tracer Liquid Imaging Velocity (TLIV) Measurements

In order to measure the actual flow velocity, we previously proposed the TLIV method [22]. Each tracer droplet was added at the plate edge. In the experiments for each plate, we injected droplets from three positions. As shown in Fig. 2A, the droplets initially moved along the radial direction, and gradually became a strip as the velocity increased. When the droplet approached the center of the plate, the droplets turned towards the diversion holes. The flow patterns in these experiments are similar to those in the simulations.

The velocity of the droplet was calculated based on trajectory images of the droplet and was compared with the velocity calculated in the CFD simulations. In each trajectory, the velocities of five points were picked out for comparison. The radial coordinates of these points were 34 mm, 30 mm, 26 mm, 22 mm and 18 mm, respectively. Fig. 2B shows the results of all 6 layers. As shown in the figure, the velocity and the difference between V_{TLIV} and V_{CFD} increased as the position moved from the plate periphery towards the plate center. The average relative error of all points between layers was 10.52%. The CFD simulation and TLIV measurement results indicated that the designed bioreactor provides steady laminar flow with controllable shear stress. Table 1 shows the comparison between V_{TLIV} and V_{CFD} of layer 1 from position a.

Table 1 Comparison of the TLIV and CFD simulation results of top 1 from position a.

	Coordinate			Velocity		
	<i>x</i> (mm)	<i>y</i> (mm)	<i>radial position</i> (mm)	V_{TLIV} (mm/s)	V_{CFD} (mm/s)	relative error
position1	0.213	-34.171	34.172	0.202	0.199	1.54%
position2	0.530	-29.797	29.802	0.257	0.228	12.65%
position3	0.520	-25.915	25.920	0.295	0.263	12.02%
position4	0.414	-22.006	22.010	0.360	0.319	12.79%
position5	0.305	-18.399	18.401	0.465	0.387	20.20%

Note: the center of the plate was set as the origin, the *radial position* was calculated from the *x, y* value in the cartesian coordinate system

Cell Culture Experiments In The Designed Bioreactor

During experiments, the monitoring curves of pH, dissolved oxygen and temperature respectively were shown in Fig. 3.

The images obtained from the online monitoring system during the experiments were shown in Fig. 4 and Fig. 5, respectively as Vero cells and hUCMSCs. According to the monitoring of cell morphology in different time, cell morphological changes during cultivation were apparent.

As shown in Fig. 4, immediately after the Vero cell suspension was added into the bioreactor, cells flowed across the plates and were potential to be deposited on the modified plates, while large number of cells were still unattached and suspended in the culture medium. In the first several hours unattached cells in the culture medium were constantly deposited and adhered to the plates. After cell adherence for about 24 h, cell morphology could be observed. From about 24 h to 48 h, as plenty of the blank areas on the

plate were filled with cells, the cells gradually proliferated. Then from about 48 h to 80 h, there was explosive growth of cell number, which indicated cells adapted to the microenvironment within the bioreactor and proliferated faster. After that, from about 84 h to 112 h, cells reached 90% confluence and cell proliferation was not obvious because the silicon plates were completely filled with Vero cells, causing that rare places were offered to enable cell proliferation.

As shown in Fig. 5, similar to the situation of Vero cells, after hUCMSCs suspension was added into the bioreactor, cells flowed across the plates and were potential to be deposited on the modified plates, while a large number of cells were still unattached and suspended in the culture medium. In the first several hours unattached cells in the culture medium constantly were deposited and adhered to the plates. After cell adherence for about 24 h, the appearance of spindle-shaped cells could be seen, indicating that hUCMSCs had adhered and spread over the silicon plates. From about 24 h to 36 h, cell proliferation was not obvious while cell morphology constantly extended, indicating cells had adapted to the microenvironment of the bioreactor. From 36 h to 48 h, cells gradually proliferated and constantly filled the silicon plates. From 48 h to 72 h, cells proliferated swiftly and kept the morphology of spindle-shape. From 72 h to 92 h cells reached 90% confluence without obvious proliferation phenomenon because the silicon plates were completely filled.

Cell Count And Flow Cytometry Analysis

After cell cultivation, cells were collected. Figure 6 shows the number of cells after cell cultivation. The results showed that Vero cells and hUCMSCs proliferated respectively 2.28 times after 112 h and 3.39 times after 92 h culture within the bioreactor, while cell numbers were inhomogeneous in different layers. And the results of control groups were 3.64 times and 3.51 times, respectively for Vero cells and hUCMSCs.

The flow cytometry of hUCMSCs in control and experimental groups was conducted respectively, as Fig. 7 showed. The results showed the immunophenotype were consistent between the control group and the experimental group and cell differentiation did not occur.

Discussion

In tissue engineering, seeded cells expand or differentiate in vitro. The keys to the successful in vitro cultivation include providing a suitable microenvironment for cell growth and harvesting cells at the appropriate time. In this paper, we aimed to build a real-time monitoring and flow field-controllable bioreactor system.

On the one hand, for large-scale cell expansion, we expected suitable and uniform shear stress in different culture areas in the bioreactor. Stable and consistent flow fields have been reported to maintain cell viability and pluripotency^[24]. However, we found that predicting or simulating the flow field is difficult in most commercial bioreactors, let alone controlling the fluid shear stress. In the designed bioreactor, the

difference between the maximum and minimum shear stress on each plate was less than 3×10^{-5} Pa, and the shear stress in most culture areas was below 4×10^{-5} Pa, which is reported as a suitable shear stress for stem cell culture. This result indicates that the fluid microenvironment in this bioreactor achieved our design goal. On the other hand, as stated in the introduction, different levels of shear stress were used to study the effects of mechanical stimulation on cells. At a certain perfusion rate, in this bioreactor, the shear stress level of each plate depended on the diversion holes' size. Additionally, the relationship between the shear stress distribution and the diameters of the diversion holes was investigated in our previous study [25]. Thus, a series of different laminar shear stresses can be custom designed depending on the layout of the diversion holes. In the case of maintaining the same microenvironment, the experimenter can apply a variety of different but controllable shear stress to cells in just one experiment.

The simulation results may differ from the results obtained in an experiment. Measuring the actual flow velocity is a better approach for verifying the shear stress distribution. There are several methods for flow measurements in bioreactors, such as ultrasonic Doppler velocimetry [26], electrical resistance tomography [27] and magnetic resonance imaging (MRI) [28]. The most commonly used method is particle image velocity (PIV), which records particle trajectories with a high-speed camera and calculates the velocity with computer technology [29]. Since particles frequently enter or leave the imaging plane, measurement error always exists [30]. At the same time, reducing the particle size is necessary to improve the fluidity, although the particles may be more difficult to image [31]. Therefore, we proposed the TLIV method in our previous study [22]. This method improves the fluidity while maintaining good visibility. The relative difference between the TLIV and CFD results was 10.52%, indicating that the results of the TLIV and simulation were consistent. The relative error was slightly larger than that of the PIV experiment (8.8%) [32]. In addition to the difference between the ideal and actual situations, the TLIV measurement may also introduce limitations. As we stated in our previous study, the deformation and diffusion of the tracer liquid impacts the velocimetry performance.

The online observation system allows the experimenter to determine the state and density of the cells in real time, allowing the appropriate timing to be met for harvesting or adjustments. The current methods for observation include X-ray phase contrast (XPC) imaging [33], ultrasound monitoring [34], resistance imaging techniques [35], optical coherence tomography (OCT) [36] scanning, etc. These methods focus on the macroscopic parameters of the tissue and the thickness of the extracellular matrix, with less concern for the morphological characteristics of the cells. The equipment used in these methods is complicated, which limits their application. Recently, microfluidic chips have been used in small number cell cultivation, and cells on chips can be observed under an optical microscope [18]. However, microfluidic chips are not suitable for large-scale cell culture and are difficult to monitor continuously. In this paper, we designed a simple monitoring system with LED lights and an optical camera. As shown in Fig. 4 and Fig. 5, the cell morphology during cell culture in vitro can be clearly seen, and this monitoring method can work continuously. The imaging principle of the designed imaging system was based on reflection, which was different from the phase contrast microscopy, so the images obtained were also different. A lot of

improvements would be performed in future. In addition, an image recognition program to determine cell density and to perform morphological analysis can be developed in our further research.

The designed bioreactor achieved the desired proliferation in this experiment, indicating that it provides a suitable microenvironment for the growth of hUCMSCs.

However, the results exposed the issue that we did not achieved a higher cell proliferation rate in the currently designed bioreactor, and the cell proliferation efficiency for Vero cells was even less than that in control group (petri dish) in this experiment. The main reason is the cell seeding efficiency in this bioreactor. When we conducted the hUCMSCs experiment, the seeding method was simply optimized to enhance the cell seeding efficiency, and a comparable result with the control group was obtained. But even after the optimization, the cell seeding efficiency in the currently designed bioreactor was about 64% of the control group. The main reason we summarized was that the diameter of culture chamber was 150 mm, the area was $17.7 \times 10^3 \text{ mm}^2$, while that of each silicon plate (authentic cell growth region) was only 100 mm, the effective attachable area except the drainage column was only $7.7 \times 10^3 \text{ mm}^2$, which formed invalid volume causing the deficiency of cell seeding and proliferation. The following are the details of the experiments of cell seeding processes. In the Vero cell experiment, cell suspension was pumped in into the bioreactor and kept static for 24 h, and we conducted a special experiment whose steps were the same to test the seeding efficiency, which showed that only about 30% of cells attached to the silicon plates with this seeding method. In the hUCMSCs cell experiment, we improved the method of cell seeding: after 900 mL cell suspension was pumped entirely into the culture chamber, we extracted about 100 mL medium, return it back to the culture chamber to increase the cell suspension fluidity, and kept the bioreactor static for about 2 min to facilitate cell adherence. We repeated the above steps three times. By this method, the results showed that in this experiment the seeding efficiency could be improved to about 48.8% while that of control group was 76.3%. To solve the existing problems, the further research would focus on reducing the diameter of culture chamber, which increased relatively the effective volume of cell culture.

The reason why we harvest most cells in layer 1 was that, cell suspension above layer 1 was the most, for we should ensure the cell suspension submerged the glass of the observation system, while the thickness of the cell suspension in the other layers was only 4 mm. From Fig. 1, larger FSS were observed in the third, the fourth and the fifth silicon layers. These difference in FSS was designed. It may be the reason we harvested less cells in these layers. But the current results could not fully prove this relation, more detailed experiments are needed.

This study also included some other limitations. First, to create a uniform and controllable flow field, a multi-layer plate configuration was used. The space utilization in this structure was less optimal than that of a three-dimensional porous scaffold. This drawback can be mitigated by reducing the interval space between plates. As stated above, the uniformity of imaging was affected by the light source system, which would be optimized in the future research. In addition, more accurate experiments on shear flow effects will be performed in further studies based on this bioreactor.

Conclusions

In this paper, we constructed a multi-layer plate bioreactor with a controllable flow field that could be monitored in real time. CFD and TLIV results showed that the bioreactor produced a consistent shear stress on each plate, and the shear stresses of different layers could be controlled by the diameter of the diversion holes, providing a suitable shear stress level for cell growth. In the cell culture experiment, the monitoring system obtain continuous real-time morphological images. This research also demonstrated that the bioreactor is suitable for hUCMSCs culture and can be used in tissue engineering.

In later research, we will continue to improve our bioreactor system and develop an image recognition program to determine the cell number and to perform morphological analysis. This bioreactor will be used to design different fluid shear stress to study the proliferation and differentiation characteristics of cells under different microenvironment, especially the shear stresses. Moreover, different strains of cells will also be studied in the bioreactor. In this future work, we aim to describe the mechanism of how shear stress influences in vitro cell cultivation in tissue engineering.

Methods

Overview and flow circuit of the designed bioreactor system

Figure 8 shows an overview of the bioreactor system and the perfusion circuit. The bioreactor vessel was made of stainless-steel and placed inside the incubator (37°C) during experiments. The bioreactor was equipped with sensors for respectively measuring pH, dissolved oxygen and temperature. The above parameters were controlled by BioBundles of Applikon™ (Holland) ^[19] and recorded by a computer automatically. The culture medium (pink part in Fig. 8A) was driven by a peristaltic pump and flowed in the clockwise direction between the culture chamber (the right side of bioreactor) and the control chamber (the left side of bioreactor), as shown in Fig. 8C. These two chambers were disconnected at the bottom. And the sensors were placed in the control chamber, for we expected the cell culture area in the culture chamber to be as large as possible. Dissolved oxygen and pH were adjusted by N₂ and CO₂, respectively. The cell suspension bottle was connected to the outlet at the bottom of the bioreactor via a four-way valve, and the medium storage bottle was also connected to the perfusion circuit via this valve. A real-time camera system was equipped on top of the bioreactor vessel, and the image data were recorded automatically by the computer.

Bioreactor Construction

Multi-layer parallel plate bioreactors have been previously used to develop an artificial liver ^[20] and to culture heart cells ^[21]. In this study, we used a similar configuration that provided a stable fluid environment for cell culture. Circular parallel plates and the drainage column were installed in the center of culture chamber. We designed six circumferential homogeneously distributed diversion holes in each

layer. Streamline diagram of the circulation are shown in Fig. 8C. During self-circulation process the medium firstly enters the control chamber on the left side of the bioreactor from the inlet and then flows upwards. In ascension process, bubbles in the medium gradually rupture to prevent damage to the cells in the culture chamber. Subsequently, the medium enters the culture chamber on the right side. In this chamber, the medium flows in the space between two adjacent layers, primarily in the radial direction, into the central drainage column through the diversion holes, and finally out of the bioreactor. This flow field design provides laminar flow in the bioreactor, preventing turbulence from damaging the cell growth.

In this study, 7 layers of plates were used for the experiment. To create different laminar velocities, the diameters of the diversion holes of the layers were 0.8, 0.9, 1.0, 1.1, 1.2, 1.3 and 1.4 mm, in ascending order from bottom to top, and the diameters of the other diversion holes were 1.0 mm. The other parameters are listed in Table 2.

Table 2
Symbols in Fig. 8C

Symbol	The parameters represent	The dimension in this study (mm)
D1	diameter of the central drainage column	15
D2	diameter of the parallel circular plate	100
D3	diameter of the culture chamber	150
H1	thickness of parallel plate	1
H2	height of the interstitial space between adjacent layers	4

The inner diameter, outer diameter and thickness of the silicon plates were 15 mm, 100 mm and 1 mm, respectively. To enable quick and easy assembly and disassembly, we designed a 3D printing scaffold, as shown in Fig. 9C. The silicon plates were placed in scaffold layer by layer. During cell culture, the scaffold and plates were fixed to the central drainage column. The silicon plates were reflective, which enabled diffuse reflection imaging, as shown in Fig. 9D. And silicon plates could be sterilized without degeneration in high pressure and temperature about 120°C.

Computational Fluid Dynamics (CFD) Modeling And Simulation

The geometric model of the bioreactor was created using SolidWorks™ (2018, USA). Meshing and flow field calculations were processed using commercial CFD software (ANSYS™ 15.0, USA). The simulation was performed with the following settings: non-structural tetrahedral meshing; 300 K isothermal; an incompressible, Newtonian fluid with a dynamic viscosity of 0.0025 kg/(m·s) and a density of

1007.4 kg/m³; and 9.8 m/s² acceleration due to gravity. The inlet velocity was set at 0.000138 m/ s (i.e. a flow rate of 1.828 mL/s), and the outlet of the central drainage column was set as the outflow. A laminar flow model was used in the calculation and energy transfer was ignored. The fluid shear stress (FSS) and fluid velocity were simulated with the CFX unit of ANSYS.

Tracer liquid imaging velocity (TLIV) measurements

This technique was performed by adding tinted droplets to the fluid, recording these flowing droplets by camera, and calculating the speed of the droplet centroid, which represents the fluid velocity. Figure 10 shows the experimental setup of the TLIV measurement, which included a camera, a glass bioreactor with multi-layer plates, a tracer liquid injector, peristaltic pumps and a reservoir (not shown in Fig. 10). For better lighting, a glass bioreactor of the same size was used in the measurement. In the case of constant liquid flow, the velocity distribution in the bioreactor is independent of fluid viscosity ^[22], so pure water was used instead of the medium for better imaging. In this experiment, water was pumped from the reservoir to the bioreactor at the same speed as in the CFD simulation and flowed along the path shown in Fig. 8C. The tracer droplet was injected to the outer edge between two plates, and flowed toward the diversion holes in the water and the camera recorded trajectory images of the tracer droplet.

The relative error between the velocities obtained from CFD and TLIV was calculated as follows:

$$relative\ error = \frac{V_{TLIV} - V_{CFD}}{V_{CFD}} \times 100\% \quad (1)$$

Online Image System

To monitor the cell culture in the bioreactor, an online camera system was designed. The camera system includes an industrial lens with 0.7-4.5x magnification (T168, SunTime™, Taiwan), a CMOS image camera with 10 million pixels, 4–40 frames per second and 1/2.3 imaging area (D1000E, SunTime™, Taiwan), and LED ring lights, as shown in Fig. 9. The light radiated from the LED, passed through the observation window in the bioreactor, and then reached the plate and diffusely reflected. The schematic diagram of the optical path is also shown in Fig. 9A. The observation window was designed as follows: a waterproof cabin was located on the top of the bioreactor, and an optically transparent glass was mounted at the bottom of the cabin (as shown in Fig. 9B). Except the inlets and outlets, the bioreactor was isolated from the external environment by a sealed design.

Cell Sources

The Vero cells and hUCMSCs were purchased from Sciencecell™ (Carlsbad, CA, USA) and cultured in T75 cell culture flasks (Corning™, USA) containing α -MEM medium (HyClone™, Logan, Utah) which was supplemented respectively with 10% fetal bovine serum (FBS) from Gibco™ (USA) and Excell™ (USA), and a 1% penicillin-streptomycin (PS) solution (Gibco™, USA) in 5% CO₂ at 37 °C. The sixth passage of Vero cells and the fifth passage of hUCMSCs were used in corresponding experiments.

Preparation Before Experiments

The flow chart of the cell culture experiment is shown in Fig. 11.

To be modified, silicon plates were added to 20 mg/mL poly-L-lysine and kept soaked for about 1 h. The cleaned bioreactor system was connected by silicone tubes according to the flow circuit. The modified silicon plates with the drainage column were installed inside the bioreactor, which constructed a contact bioreactor system. The gas tightness of bioreactor system was tested, which made sure the whole system was completely isolated from the external environment. Subsequently the prepared bioreactor system was entirely sterilized by high pressure steam in autoclave and then was exposed to ultraviolet for about 30 min.

Cell Seeding And Circulation

900 mL culture medium with 1×10^7 cells were prepared in the cell suspension bottle connected to the outlet of the bioreactor with silicone tube, while this outlet for circulation was also used as inlet in cell seeding. After cell seeding the bioreactor kept static for about 24 h in incubator to facilitate cell adherence on silicon plates. For the culture and control chamber were disconnected at the bottom, the cell suspension solution was kept in the culture chamber during seeding and adherence processes, with the purpose to enhance the chance of cell attachment on silicon plates. During these processes, there was no medium existing in the control chamber, which resulted in that the pH, dissolved oxygen sensors and the corresponding controllers did not work.

After cell adherence, 600 mL fresh medium was added into the culture chamber via the same outlet. Subsequently to initiate the self-circulation the medium was transferred from the culture chamber to the control chamber, which formed a self-circulating system realizing the control of medium parameters and renewal of medium ingredients. The circulation provided constant and stable flow shear stresses for cell growth on seven parallel silicon plates, which was same with the results of CFD simulations.

Abbreviations

CFD: computational fluid dynamics; TLIV: tracer liquid imaging velocity; hUCMSCs: human umbilical cord mesenchymal stem cells; hBMSCs: human bone marrow mesenchymal stem cells; CMOS: complementary metal oxide semiconductor; LED: light emitting diode; FSS: fluid shear stress; V_{TLIV} :

velocity from TLIV measurement results; V_{CFD} : velocity from CFD simulation results; FBS: fetal bovine serum; PS: penicillin-streptomycin; MRI: magnetic resonance imaging; PIV: particle image velocity; XPC: X-ray phase contrast; OCT: optical coherence tomography; HLA-DR: human leukocyte antigen DR.

Declaration

Acknowledgments

Not applicable.

Authors' Contributions

Songrong Sun performed the experiments and drafted the manuscript. Yihong Gong and Shaoxiong Huang carried out the cell culture of hUCMSCs and provided the guidance on tissue engineering. Xuejia Li and Xiaoyan Wang carried out some statistical analysis and drew the figures. Yuxi Luo designed and supervised the project. All authors have read and approved the final manuscript.

Funding

This work was supported by the Guangzhou Science & Technology Program, China under Grant No. 201604046028, by the Guangdong Natural Science Foundation, China under Grant No. 2018A030313885.

Availability of data and material

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Results of computational fluid dynamics (CFD). A: Streamline and velocity distribution between two adjacent plates (top1 represents the results between the uppermost layer plate and the second plate, and

top2 represents the result between the second and third plates, etc). B: Shear stress distribution on the plates, where layer1 represents the fluid shear stress on the upper surface of the top plate.

Figure 2

Measurements of tracer liquid imaging velocity (TLIV). A: The centroid trajectories of three tracer droplets (position a faced the direction towards the diversion holes, and position b and c were approximately 10 degrees different from position a in circular coordinate system). B: Comparison of the TLIV results and CFD simulation results for 6 layers, where the velocities of five points were picked out for comparison and the average relative error of all points between layers was 10.52%, indicated that the designed bioreactor provides steady laminar flow with controllable shear stress.

Figure 3

The monitoring parameters (pH, dissolved oxygen and temperature) of Vero cells (A) and hUCMSCs (B) during corresponding experiments lasting for 112h and 92h respectively.

Figure 4

Online observation of Vero cell morphology during the cell culture in vitro in the designed bioreactor lasting for 112h. Morphologies of cells (in start, 24h, 88h and 112h) were respectively enlarged.

Figure 5

Online observation of hUCMSCs morphology during the cell culture in vitro in the designed bioreactor lasting for 92h. Morpgologies of hUCMSCs (in start, 18h, 72h and 92h) were respectively enlarged.

Figure 6

The results of cell expansion in vitro in the designed bioreactor, respectively of Vero cells (A) and hUCMSCs (B).

Figure 7

Flow cytometry results in control group (A) and experimental group (B) showed hUCMSCs expressed CD73, CD90, and CD105, did not express CD34, CD45, CD11b, CD19, and had low levels of human leukocyte antigens (HLA)-DR.

Figure 8

Overview of the bioreactor system and the perfusion circuit. A: Schematic diagram of the bioreactor perfusion circuit. B: Photograph of the bioreactor. C: Schematic diagram of the bioreactor structure and streamline diagram.

Figure 9

Online image system and scaffold. A: Optical path of the camera system. B: Profile of the camera system. C: 3D printing scaffold. D: Silicon plate.

Figure 10

Diagram of the velocity measurement device in TLIV, where 1-4 represents camera, injector, parallel circular plates and peristaltic pump respectively.

Figure 11

The flow chart of the cell culture experiment.