Three-Dimensional Dynamic Bioreactor Culture System Supports the Angiogenesis Directional of Human Umbilical Vein Endothelial Cells

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ARTICLE

Three-Dimensional Dynamic Bioreactor Culture System Supports the Angiogenesis Directional of Human Umbilical Vein Endothelial Cells

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Scaffold vascularisation is a prevalent challenge in order to develop a mature and functionalize engineering tissue construct. Vascularisation problem is one of the major hurdles in development of thick and complex engineered tissue. Bioreactors have been used to overcome complex interplay influencing tissue vascularisation. In this study we have designed and optimized our dynamic culture system for the angiogenesis development in a 3D environment. Testing with human umbilical vein endothelial cells shows that the cells are able to develop angiogenesis within two days of culture.

KEYWORDS: Bioreactor, Dynamic Culture, Three-Dimensional Environment, Angiogenesis.

1. INTRODUCTION

Currently available engineered tissues are limited on the thin and a-vascular ones such as skin, cartilage, and bladder.[1] The creation of thick tissue is still on the demanding target now.[2] Tissue engineering research relies on the increasing knowledge of endothelial cells organization in order to promote and orient the development of microvessel network inside a 3D scaffold.[3]

Scaffold vascularization found to be the most accepted strategy for the development of thick engineered tissue construct and artificial organ.[3, 4] The development of mature and functionalize microvessels inside the scaffold will facilitate the oxygen and nutrient transport to the construct as well as waste removal. Those problems often lead to the failure of the culture process or even to that of implants.[4]

Previously, we have developed a 3D culture system to guide HUVECs and orient the microvessel developments.[5] The development of a mature microvessel is of important in order to facilitate the fluid flow inside it. In this study we have further studied our *in vitro* model of microvessel patterning using polymer monofilaments embedded in fibrin in a dynamic bioreactor culture system.

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2. MATERIALS AND METHODS

2.1. Materials

Polycarbonate (Boedeker Plastics Inc., Texas, USA) frames were used fixed the 100-μm diameter monofilaments of poly(ethylene terephthalate) (Good Fellow, Devon, USA). This frame was designed to be fit into cell culture chamber (Fig. 1). Human umbilical vein endothelial cells (HUVECs) between passages 2 and 6 were used in all experiments and were purchased from Cambrex (Walkersville, MD USA).

2.2. Cell Adhesion and Angiogenesis Assay

In 6-well plates, fibrin gels were prepared to be used as the attachment bench, using 1 mL/well of fibrinogen solution (4.0 mg mL^{-1}) in HBSS and supplemented with 350 KIU mL^{-1} of aprotinin. After the polymerization process, sterile frames bearing the fibres were transferred to the top of this fibrin gel. Then, 100,000 cells were seeded directly.

2.3. Dynamic Bioreactor System

The bioreactor culture condition was tested on a flow rate of 80 mL/min and either with or no pulsation frequency of 140 beats/min. Temperature was set to 37 °C with pH of 7.4 and dissolved oxygen (DO) concentration was 12 mg/L he pulsation frequency. A schematic design of the bioreactor system is presented on Figure 2(a).

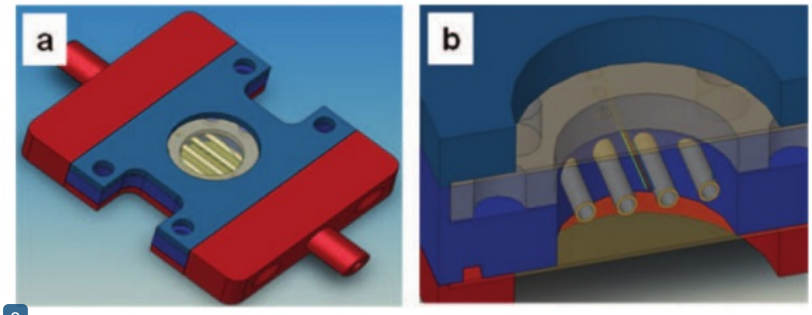

Fig. 1. Dynamic cell culture chamber. (a) A custom-made cell culture chamber. (b) Fibre frame fit into the cell culture chamber.

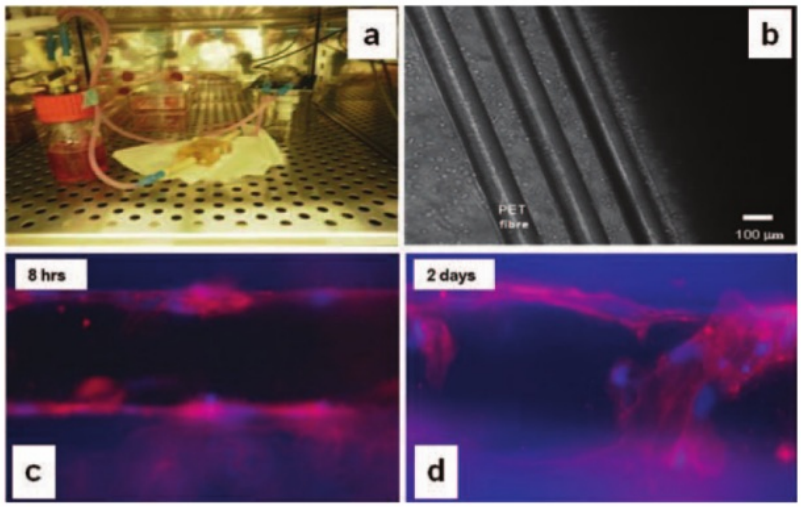

Fig. 2. Dynamic bioreactor culture system for angiogenesis assay. (a) Bioreactor system consists of micro-peristaltic pump, media bottle, and culture chamber. (b) Phase contrast images of cell-to-cell connection of HUVEC cultured in medium flow rate of 80 mL/min after 2 days. Fluorescence images after 8 hours (c) and 2 days of culture (d).

2.4. Angiogenesis Image and Analysis

To investigate microvessel formation between adjacent fibres, HUVECs were cultured in a 6 well plate then stained with SYTOX Green Nucleic Acid Stain (Invitrogen). Images of microvessels were taken with a Confocal Laser Scanning Microscope (Olympus Fluoview FV1000, Tokyo, Japan). Images were edited with Olympus FV10-ASW 2.0 viewer for 3D reconstruction.

3. RESULTS AND DISCUSSION

In this study, we have successfully developed a cell culture chamber for dynamic testing of Endothelial cells in a three dimensional environment, as presented in Figure 1. Dynamic cell culture experiment using human umbilical vein endothelial cell (HUVEC) seeded inside the chamber and connected on our bioreactor dynamic culture system shows a good cell proliferation and homogenous cell spreading, as presented on Figure 2. Our result is in a good agreement with others.[6,7]

Bioreactor system was able to maintain a more precise environment, as for example the flow rate can be modulated and controlled over time. This dynamic culture supports the formation of cell-to-cell connection after 2 days culture, as presented in a phase-contrast (Fig. 2(b)). Fluorescence image also prove the step-by-step development of angiogenesis in between the polymer fibres (Figs. 2(c)–(d)).

4. CONCLUSIONS AND RECOMMENDATION

This preliminary study revealed that engineering challenges for the development of a scale-able and automated processes are needed to give prove the potential of HUVECs angiogenesis formation inside a three dimensional environment. We recommend further angiogenesis study using other cell type, including tumor cells.

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