Three-Dimensional Cell Seeding and Culture in Novel Radial-Flow Perfusion Bioreactor

Tetsuji Yamaoka and Tatsuya Kitagawa

Department of Biomedical Engineering,
National Cardiovascular Center Research Institute,
5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan

INTRODUCTION

Tissue engineering has been developed as a new approach in regenerating tissues and organs using cells and scaffolds. An important element in successful tissue engineering *in vitro* is a suitable bioreactor system (Freed and Vunjak-Novakovic 1997). In general, some types of bioreactors have been used for cell culture on polymer scaffolds, namely spinner flasks, rotating vessels, and perfusion system. Although these systems improve the limited oxygen diffusion to the cells on the scaffolds (Navarro et al. 2001; Radisic et al. 2003), cell pre-seeding under a static condition is required.

The purpose of the present study was to create a new bioreactor in which the full process of tissue regeneration, from cell seeding to cell growth, can be performed in successive steps. The effect of the media flow rate, and the culture time on the efficiency of cell seeding and cell growth were investigated.

EXPERIMENTAL

Scaffolds

Poly-L-lactic acid (PLLA, Mw=130,000) was dissolved in 1,4-dioxane at 2 %, and the solutions were transferred to the polypropylene mold. Then the constructs were slowly frozen and lyophilized for 24 h to form porous tubular scaffolds with an inner diameter of 1.0 mm, outer diameter of 3.0 mm, and length of 20 mm (Figure 1).

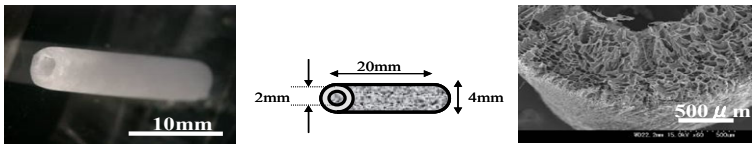

Figure 1. Porous tubular scaffold made of PLLA

Medium perfusion systems

The perfusion flow system was composed of a culture flask, a peristaltic pump with

silicon tubing, and injection ports of cell suspensions and culture medium. The tubular scaffold was jointed with silicon tubing. The culture medium was pumped continuously at various flow rates from the central lumen toward the periphery of the tubular scaffolds (Figure 2A) and re-circulated back to the culture flask. The amount of culture medium was 30 mL for each scaffold. A medium change was performed every day to supply fresh oxygen and nutrients. The residual oxygen concentration was monitored during the cultivation period by a residual oxygen monitor (B-505; Iijima Electronics Corp., Aichi, Japan).

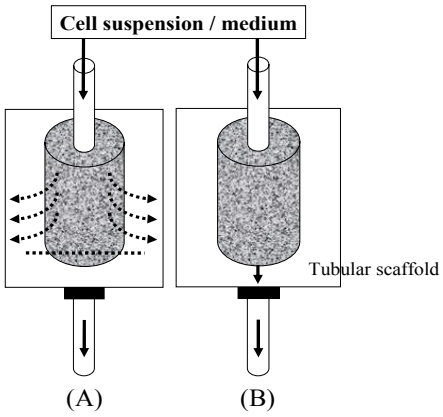

Figure 2. Perfusion system for seeding and culture of (A) smooth muscle cells and (B) endothelial cells.

Cell seeding and cultivation

The scaffolds were sterilized and immersed in DMEM culture medium before use. The NIH/3T3 cell suspension (2.0×10^6 cells in 1.0 ml medium) was delivered through the injection port of the perfusion system. The perfusion seeding was performed at flow rates of 1.1, 4.0, 7.2, and 11.0 mL/cm^2·min at 37°C under a 5% CO_2 condition for 24 h. In the static seeding, the lumens of the PLLA scaffolds were filled with the cell suspension (2×10^6 cells/100 μL), and the constructs were incubated for 24 h. After cultivation, the cells were dissolved with lysis buffer and the numbers of adherent cells in the scaffolds were determined using an LDH Cytotoxicity Detection Kit. The cell distribution was evaluated under an optical microscope after Giemsa staining.

After static or perfusion cell seeding, the cell/scaffold constructs were cultured in the perfusion bioreactor systems at various flow rates at 37°C under a 5% CO_2 condition for a given period of time. The number of cells that washed out from the scaffolds was measured with a hemocytometer at specified time intervals. After cultivation, the cells within the scaffold were dissolved with lysis buffer and counted using an LDH Cytotoxicity Detection Kit. The cell distributions were evaluated by Image-Pro® Plus Version 4.5 software as described in the previous section.

RESULTS AND DISCUSSION

The developed perfusion reactor is expected to improve cell proliferation, especially at the central part of the 3-D scaffolds. In order to evaluate the proliferation efficiency, NIH3T3 cells were seeded over an entire scaffold at a flow rate of 1.1 mL/cm^2·min. The

concentration of oxygen was continuously monitored and the culture medium was changed daily to avoid a shortage of oxygen and nutrients. Under the perfusion condition, the cell number increased with the increasing flow rate and reached 35 times that for the originally seeded cells at the optimum perfusion rate (4.0 mL/cm^2·min). The perfusion system improved the cell growth in a similar fashion irrespective of the cell localization in the scaffold.

At the flow rate of 4.0 mL/cm^2·min, the seeded cells proliferated well over the 5-day culture period at all regions of the scaffold. A slight and slow movement of the cells towards the peripheral side during the culture period was observed. This result might have been due to a difference in the total flow area. That is, the total flow area at the luminal surface of the scaffold is less than at the outer surface, resulting in a higher velocity in this region. In contrast, only the cells around the luminal surface proliferated under the static condition, indicating that the static culture could supply oxygen and nutrients only to the surface area of the scaffolds.

When the flow rate increased to 7.2 or 11.0 mL/cm^2·min, the number of adherent cells rapidly decreased and fell below the number of initially seeded cells. About 1% of the seeded cells were washed out in the static culture and in the perfusion culture at the flow rate of 1.1 mL/cm^2·min for 5 days but at the high flow rates (7.2 and 11.0 mL/cm^2·min), the numbers of washed-out cells were comparable to the number of adherent cells.

In conclusion, cell seeding under a radial-flow perfusion condition of 1.1 mL/cm2•min was effective and the perfusion cell seeding resulted in a uniform distribution of cells throughout the scaffold. The optimal flow rate for cell growth was 4.0 mL/cm2•min and at this optimal rate, the increase in seeded cell was 7.3-fold greater than that by static culture. The perfusion seeding/culture system was a much more effective strategy than the conventional system in which cells are seeded under a static condition and cultured in a bioreactor such as a spinner flask.

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