Radial Flow Type Bioreactor for Bioartificial Liver Assist System Using PTFE Non-Woven Fabric Coated with Poly-amino Acid Urethane Copolymer

Azizi Miskon[1,2], Tetsuji Yamaoka[1], Hiroshi Uyama[2], Makoto Kodama[3]

[1]Department of Biomedical Engineering, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, Japan.
[2]Department of Chemical Engineering, Osaka University. Japan.
[3]Department of Biological Function and System Engineering, Kyushu Institute of Technology, Japan.

Introduction

Recently, the research on the hybrid bioartificial liver using the porcine hepatocytes has been developed but their clinical usage is limited by various difficulties. An efficient bioreactor with radial flow system equipped with silicon oxygenator was developed. In this bioreactor system, the radial flow system supplies the nutrient medium efficiently and the adhesion and culture of porcine hepatocytes is greatly improved by using polytetrafluoroethylene (PTFE) non-woven fabrics coated with poly-amino acid urethane copolymer (PAU).

1. Materials and Methods

1.1 Isolation of Hepatocytes

Hepatocytes were isolated from a lobe (about 84 g) of liver of slaughtered adult pig by perfusion method using dispase and collagenase by our method. The total amount of over 5.26×10^9 hepatocytes were routinely obtained from a lobe. Hepatocytes of more than 90% viability, determined by trypan blue exclusion method, were used for the experiments.

1.2 Perfusion Culture in Radial Flow Bioreactor System

Perfusion culture experiments were performed in a radial flow bioreactor system at 37°C. The culture medium was composed of WE medium supplemented with 5 %(v/v) fetal bovine serum (Sigma, USA), 0.01 μmol/l insulin(Wako Pure Chemical Industries, Ltd., Japan), 0.2 μmol/l dexamethasone (Wako Pure Chemical Industries, Ltd., Japan), 5 μg/l epidermal growth factor (Wako Pure Chemical Industries, Ltd., Japan), 10^5U/l penicillin(Sigma, USA), 0.1 g/l streptomycin (Sigma, USA) and 1.5 mmol/l L-ascorbic acid phosphate (Wako Pure Chemical Industries, Ltd., Japan).

The radial flow bioreactor consists of a PTFE non-woven fabric coated with poly- amino acid urethane copolymer (PAU) and hollow fiber. Hepatocytes suspension (2.0×10^9 in 50 ml) was inoculated into a medium-preparative tank, and then the medium was perfused to the bioreactor from the medium-preparative tank at a flow rate of 17 ml/min for 10 min. Subsequently, the medium was circulated at 84.2 ml/min during the culture experiments. A mixed gas containing air, oxygen and carbon dioxide was introduced into the medium-preparative tank through a control equipment to maintain pH at 7.3 and DO at 313 μmol/l. The medium exchange was performed every day throughout the culture period.

1.3 Measurement

To assess the ammonium metabolism of the cultured hepatocytes, 1 mmol/l NH_4Cl was supplemented in the medium after medium exchange. Ammonium concentration was measured at 0, 3, and 6 h after ammonium-loading using a commercially available kit (AMICHEK™ meter; Arkray Factory Inc., Japan). The medium sample is taken during before and after the changing of the medium for albumin secretion measurement. The amount of albumin secretion was measured by enzyme-linked immunoabsorbent assay, (ELISA).

2.3 Scanning Electron Micrograph (SEM)

Hepatocytes attached on the PTFE non-woven fabric coated with PAU were fixed with 4% glutaraldehyde, and dehydrated with graded ethanol (50, 60, 70,80,90,95 and 99.5%). The specimens after critical point drying with carbon dioxide (Drier EMITECH K-850; Meiwa Shoji Co., Ltd., Japan) were coated with palladium by sputtering (plasma multi coater PMC-5000, Meiwa Shoji Co., Ltd., Japan) and then were subjected to SEM observation (SM-300, Topcon Co., Japan).

3. Results and discussion

The ammonium metabolizing activity of the hepatocytes was found to be kept for 1 week at the high value in the bioreactor system as shown in Figure 1.

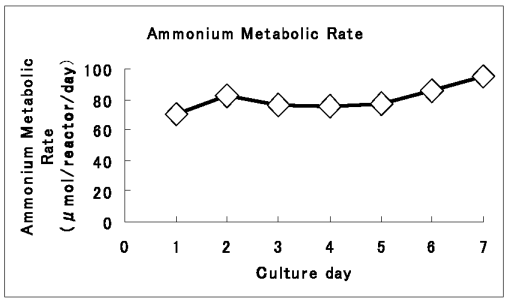

Figure1. Mean hepatocytes ammonium metabolic rate of the hepatocytes cultured in radial flow bioreactor for 7 days

PAU is the block copolymer consists of a small amount of a small amount of poly(γ-methyl-L-glutamate) (PMLG) and the polyurethane. The urethane segments are hydrophobic and then strongly interact with the other hydrophobic materials such as PTFE, and the PMLG segments with the α-helix structure possess the cytocompatibility. Therefore, PAU can be easily coated onto the PTFE fiber and acts as an artificial extracellular matrix with the high cytocompatibility as shown in Figure 2.

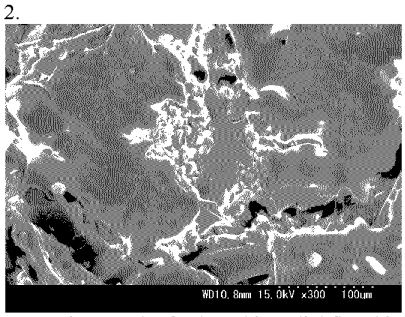

Figure2. SEM micrograph of cultured in radial flow bioreactor (x300)

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