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Influence of hypothermic conditions on primary porcine hepatocyte-entrapped hollow fiber bioreactors

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Abstract

Transportation of bioartificial liver (BAL) device with viable cells and higher metabolic functions are necessary. The aim of this study is to evaluate hypothermic (4 °C) influence on the performance of hepatocyte-entrapped hollow fiber bioreactors, as well as the effects on cell function with or without medium supplement. Oxygen consumptions were stable at average of 15–25 mmHg per cartridge during 8 h normothermic incubation after cold-perfusion and cold-non-perfusion. All groups showed increase in glutamic oxaloacetic transaminase (GOT) and lactate dehydrogenase (LDH) level. Urea production and albumin synthesis were only slightly higher in cold-perfusion groups than that of the cold-non-perfusion groups and the control. There were no significant differences in the metabolic functions of bioreactors between each study groups during normothermic operation. Our study suggests that the use of a preliminary cold-storage step prior to normal culture condition or clinical therapy can prolong the transportation time without changing various functions of the BAL device.

Keywords: Porcine hepatocytes; Cold storage; Hypothermic; Bioartificial liver

1. Introduction

In recent years the development of bioartificial liver (BAL) devices has acquired special interest in clinical use, integrated into extracorporeal liver support systems, for patients with fulminant hepatic failure as a bridge to liver transplantation [1]. The bioreactor offers an incubator-independent dynamic cell culture design with a more physiologic culture environment or microfluid environment featuring avoidance of abrupt changes in substrate and product concentrations during medium changes. This has to be taken into account in static incubator cultures [2]. Several bioreactor designs have been introduced to act as liver support devices including hollow fiber membranes, microcarrier, packed-bed culture, spheroids and many others [3–9].

In order to prolong its use in actual clinical situations, some researchers cryopreserve the freshly isolated cells before delivering the cell-charged bioreactors to hospitals. Unfortunately, the metabolic activity and the plating efficiency of the primary hepatocytes are lost dramatically after cryopreservation [10–12]. Hence, a study of the biochemical functions of the bioreactor during the storage conditions is both important and necessary.

Liver cell bioreactors require the use of great amounts of high functionally and metabolically competent cells [13]. Methods of collagenase perfusion that successfully isolate hepatocytes from human liver samples have been described [14,15], but the availability of human liver tissue for cell harvesting purposes is limited. Alternative cell sources for bioreactors include human hepatoma cell lines and hepatocytes from other species [16,17]. Cell lines with an infinite

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life span could serve as an unlimited source of hepatic cells. However, the loss of specific liver functions as well as the potential risk of malignant transformation in these cells promotes the use of non-malignant cellular systems such as primary cultured hepatocytes [9,18–20]. Studies have shown that hepatocytes can survive for longer periods of time and maintain their specific functions when they are cold-stored at 4 °C or lower temperature [21–23] or co-culture with other cell types, such as non-parenchymal liver cells (NPCs) or bone marrow cells [24,25].

The use of hepatocytes from human donors is currently under investigation. Human liver cells are of greatest interest to pharmacological studies due to their unmatched potential to predict substrate metabolism in man [26–28]. The use of rodent hepatocytes facilitates preliminary studies in the development of bioartificial liver system, however, the limited number of cells isolated from each animal is a serious drawback for large-scale cell harvesting [29]. A large amount of functional cells is required for artificial liver support and, consequently, a larger animal is needed as a source of hepatocytes. Viable primary hepatocytes in the BAL system are generally accepted to constitute 10–20% of actual liver mass, corresponding to 2.5×10^{10} to 5×10^{10} hepatocytes. This is sufficient to support a human liver [30]. Thus, porcine is considered the best donor species of hepatocytes for use in bioartificial livers for clinical application [17].

In general, the single isolate or the spheroid form of cells has been employed for non-freezing preservation of hepatocytes for bioartificial liver use. It has not been reported, however, that the non-freezing preservation enhances the activity and metabolic functions of hepatocytes entrapped in bioreactors [21–23]. Here, the hepatocytes entrapped in bioreactors are preincubated at the hypothermic temperature with or without medium perfusion for 12 and 24 h before the normothermic culture (at 37 °C). During the experiments, biophysiological markers included pH, pO_2 and temperature changes are monitored as indirect parameter of medium loss due to condensation. Albumin secretion, urea synthesis, metabolism, morphological integrity and LDH leakage of cell cultures are stable at least until 8 h in culture, which is similar to clinical treatment (average 6–8 h). To identify the morphology of hepatocytes on the surface of hollow fibers after an 8 h normothermic operation, bioreactors were fixed in glutaraldehyde and dehydrated in an ascending series of ethanols for scanning electron microscope (SEM) study.

2. Materials and methods

2.1. Hepatocyte isolation

Hepatocytes were harvested from 10 to 14 kg (11.9 ± 1.8 kg) white male pigs ($n = 5$) using a two-step committee approved in situ collagenase perfusion technique modified from the method described by Seglen [31] and Nyberg [29]. Briefly, animals were anesthetized intravenously with ketamine (1 mL/kg) and rompum (1 mL/kg). After portal vein cannulation, in vivo perfusion (100∼300 ml/min × 10 min) was performed using a calcium-free hydroxylethylpiperaineethanesulfonic acid (HEPES)-buffered solution (143 mM NaCl, 6.7 mM KCl, 10 mM HEPES, 100 mg% ethylene glycol-bis-aminoethyl ether (EGTA; Sigma, St. Louis, MO), pH 7.4). The resection liver was transferred into laminar flow before perfusion (100∼300 ml/min × 20 min) in harvest pot with a second HEPES-buffered solution (67 mM NaCl, 6.7 mM KCl, 4.8 mM $CaCl_2$, 100 mM HEPES, 1.0 g% fatty acid free bovine albumin (Sigma), pH 7.4) containing 0.05% collagenase D (Type II, 360 μ/mg, Worthington, Biochemical Co., Lakewood). The soft liver was rubbed and filtrated by a stainless filter (pore size: 104 μm). Hepatocyte pellets were flushed and pass through a stainless filterusing 500 mL of harvest medium (William's E medium (GIBCO), 2 mM L-glutamine (GIBCO), 15 mM HEPES, 1.5 mg/L insuline (I-6634, Sigma), 10,000 U/L penicillin G, 100 mg/L streptomycin sulfate), and collected into 600 ml of plastic blood bag (BB-T060CB, Terumo). The filtrate was centrifuged and washed twice by COBE machine (COBE 2291™, Cell processor) at 50 g for 2 min.

Each pig harvest yielded from 1.01×10^{10} to 2.7×10^{10} ($1.56 \pm 0.7 \times 10^{10}$) hepatocytes. Cell counts and viability were measured by trypan blue exclusion. Only hepatocyte suspensions with a viability > 80% were used (82.6∼87.0%, 84.5 ± 1.8%). All experiments were made in compliance with the Chung-Gung Memorial Hospital Guidelines on Animal Care.

2.2. Preparation of hollow fiber bioreactor

The hollow fibers in the cartridge (Spectrum Labs, Rancho Dominguez, CA) were made of mixed cellulose ester (ME) with a nominal molecular weight cut-off (MWCO) of 0.2 μm, and their inner diameter and surface area were 0.6 mm and 460 cm^2, respectively. The total volume of the inner space of the hollow fibers was 5 ml. The volume of the shell space between the hollow fibers and the cartridge housing was about 22 ml. Collected fresh porcine hepatocytes were suspended at a concentration of 2.7×10^7/ml in 10% serum contained William's E culture medium and seeded immediately by a syringe into the shell space of the hollow fiber cartridge at a density of six million cells per bioreactor. Both ends of shell space of the hollow fiber cartridge were then closed using two sterilized plastic caps. Afterwards, the cartridge was placed in a closed circuit as illustrated in Fig. 1. The hollow fiber bioreactor inlet and outlet pores were connected to the flow circuit via No. 14 silicone tubes (LS-14, Masterflex L/S, Cole-parmer Ins. Co., Vernon Hills, IL, USA). 500 mL of medium in a 1L glass bottle reservoir (GIBCO) was then pumped through the flow circuit via a digital variable-speed peristaltic pump with 3-rollers pump head (Model 7523-70, Masterflex L/S, Cole-parmer Ins. Co., Vernon Hills, IL, USA) at a flow rate of 6mL/min. The total volume of the circuit was