Hepatic Cell Lines CultureD on Different Scaffolds and in Different Stages for Bioartificial Liver Systems

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Abstract: Because of a lack of livers being donated for transplantation, the use of bio-artificial livers (BALs) using hepatic cells is a valid alternative. There are two major problems: one is the source of potent hepatic cells for BALs and the other is that BAL's working period is still too short because of apoptosis of the hepatic cells. In order to prolong the working period of BALs, we previously generated an anti-apoptosis hepatic cell line, Hep-bcl2, which maintained cell viability and improved albumin productivity for longer periods. In this study we tested the effect of collagen on the proliferation and liver function, albumin synthesis, of the anti-apoptosis hepatic cell line Hep-bcl2 to improve the culture. Hep-bcl2 cultured on collagen showed increased proliferation, while albumin productivity was decreased. This result implies that a two-step culture condition should be developed: First, to expand the pre-culture population, Hep-bcl2 should be cultured on collagen-coated dishes because of the rapid proliferation. Second, for BAL culture, Hep-bcl2 should be cultured without collagen because of higher liver function and slow proliferation, which would avoid cell death due to over-growth.

Key words: bio-artificial liver, apoptosis, hepatoma, HepG2, *bcl-2*, Hep-bcl2, proliferation, albumin, type collagen, adhesion, scaffold

1. INTRODUCTION

Liver transplantation is the most effective remedy in serious liver disease, but currently there is a critical problem since the number of donors is much smaller than the number of patients in need of a transplant. This is why

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bioartificial liver (BAL) support systems composed of artificial materials and living liver cells were developed. If a temporary replacement for a failing liver could be achieved, liver regeneration occurred in patients with liver failure and many patients could be saved.

Although a variety of BAL systems have been investigated by many researchers, their working period is too short to allow a full liver recovery in patients [1]. The strategies for improving BAL systems could be classified into two categories. The first is the development of a BAL module such as hollow fibers, radial flow, and so on. The second is the improvement of cells used in BALs, their viability and liver-specific function. We focused on the second strategy and tried to generate novel human hepatic cell lines that do not undergo apoptosis and introduced the anti-apoptotic gene *bcl-2* into hepatoma HepG2 cells. The generated cell line was named Hep-bcl2 and it maintained higher viability for a longer period and had improved liver-specific functions such as albumin productivity and metabolic activity (CYP1A1) [2]. In order to achieve improved BALs, hepatic cells should be cultured under the best possible conditions. For this purpose, we focused on collagen, an effective scaffold, during the pre-culture stage before putting BAL's module.

Collagen is one of the most ubiquitous proteins in the animal world and occupies one third of total protein in human. Collagen constitutes connective tissue and functions as a matrix between a large number of cells forming animal body for tissue morphology, influencing cell adhesion and proliferation and enhancing the repair response after various damages [3]. Recently, hepatic cells cultured between layers of collagen gel, termed collagen sandwich [4], have been applied to BAL systems.

In this study, we cultured hepatic cell lines on 35 mm dish coated with type I collagen and measured the liver-specific function through proliferation and albumin synthesis and compared it with the cells cultured on uncoated dishes.

2. MATERIALS AND METHODS

2.1 Cell lines and culture conditions

The human hepatoma cell lines used were HepG2 and Hep-bcl2. The latter was established by transfection of HepG2 with the vector BCMG-bcl-2-neo. The medium was Dulbecco's modified Eagle medium (DMEM) containing 0.2% sodium bicarbonate, 10 mM HEPES, 2 mM L-glutamine, 0.06 mg/ml kanamycin, and 10% fetal bovine serum. The cells were grown in 35 mm culture dishes (Sumitomo Bakelite, Japan) at 37℃ in humidified air containing 5% CO_2.

2.2 Morphology

Morphology of HepG2 and Hep-bcl2 cells was observed under phase-contrast microscopy (Olympus, Japan).

2.3 Measurement of growth curves

HepG2 and Hep-bcl2 cells were seeded at $3*10^4$ and $4*10^4$ cells / 35 mm dish, respectively, on wells coated with collagen or on uncoated wells. Cells were maintained in DMEM medium and cell number was assessed using a hemocytometer. Viable and dead cell densities were determined by the trypan blue exclusion method.

2.4 Determination of albumin productivity

The albumin concentration in the culture supernatant was determined by ELISA. The 96-well plate used for ELISA was first coated with goat anti-human albumin polyclonal antibody and blocked with skim milk. Subsequently, the standard wells were incubated with purified human serum albumin. The rest of the wells were incubated with the experimental samples. Finally, the wells were incubated with horseradish peroxidase-conjugated rabbit anti-human albumin polyclonal antibody and added of citric acid buffer containing *o*-phenylenediamine. The absorbance was read at 490 nm.

3. RESULTS

3.1 Morphology

In order to investigate the morphology of the hepatic cell lines HepG2 and Hep-bcl2 cultured on 35 mm dishes coated with collagen and uncoated dishes, the cells were observed at 100x magnification using a phase-contrast microscope. Morphologically, HepG2 (Fig. 1A) and Hep-bcl2 cells (Fig. 1C) on collagen appeared fuzzily and their hand with adhesion plaque extended between the cells, forming tough adhesion. On uncoated dishes, both HepG2 (Fig. 1B) and Hep-bcl2 cells (Fig. 1D) clearly formed spherical clusters probably due to a weak attachment of the cells to the culture dish. The marked difference between the morphological shape of HepG2 and Hep-bcl2 cells were not appeared. These observations showed that collagen coated dishes seemed to provide a tough adhesion for the cells so the area of dish could be used fully.

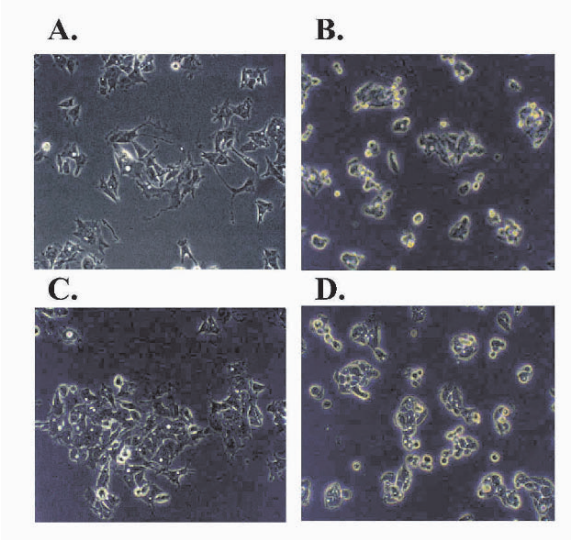

Figure 1. Phase-contrast microscopic observation of hepatic cell lines on collagen or on uncoated dishes. (A, B) HepG2; (C, D) Hep-bcl2. (A, C) The hepatic cell lines on collagen and (B, D) on uncoated. Cells were observed at 100x magnification.

3.2 Proliferation

To investigate whether culturing on collagen coated dishes altered the growth characteristics or not, the hepatic cell lines on the dishes coated with collagen and on the uncoated dishes were separately batch-cultured. As shown in Fig. 2A, viable cell density of HepG2 cells on uncoated dishes was not increased after 2 days because of a weak attachment of the cells to the culture dish, while HepG2 cells on collagen coated dishes showed an increase in viable cell number because of a tough adhesion induced by collagen. During the exponential growth phase, the growth rate of HepG2 cells on collagen was similar to that of untreated HepG2 cells. Untreated HepG2 cells started dying at day 8 because of a depletion of nutrients or growth factors, while HepG2 cells on collagen coated dishes seemed to have a prolonged life-span and higher maximum viable cell density. Hep-bcl2 cells also showed similar results (Fig. 2B). Proliferation of hepatic cell lines enhanced by collagen seemed to be dependent on cell adhesion immediately after seeding. These findings suggest that cells cultured on collagen might be useful at the precondition stage to rapidly expand the population of hepatic cells for BALs.

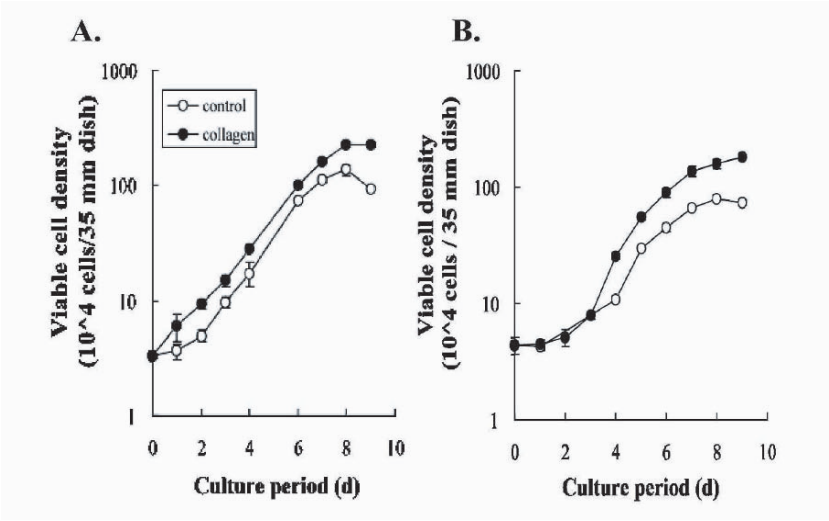

Figure 2. Proliferation of hepatic cell lines on collagen or untreated. (A) 3 * 10^4 cells HepG2 were batch-cultured in 35mm dishes coated with collagen closed circle and on uncoated dishes (open circle for 9 days. (B) Similarly, 4 * 10^4 cells Hep-bcl2 was batch-cultured. Viable cell densities were determined by the trypan blue exclusion method.

3.3 Albumin productivity

In order to estimate liver-specific functions of the hepatic cell line, human serum albumin concentrations in the culture supernatant were determined (Fig. 3). 0.44 and 0.34 μg/ml albumin was secreted by 3 days into the supernatant of Hep-bcl2 on collagen coated dishes and on uncoated dishes, respectively. The hepatic cell lines on uncoated dishes presented with increased albumin production compared to cells grown on collagen coated dishes. These results suggest that cells cultured without collagen might be useful to maintain liver-specific functions of hepatic cell lines in BALs. Albumin productivity per cell and per culture period (day) was calculated and shown in Table 1. The productivity per Hep-bcl2 cell was five times higher than that of HepG2 cells. In both cell lines, albumin productivity on untreated dishes was three times higher than that of cultures on collagen coated dishes.

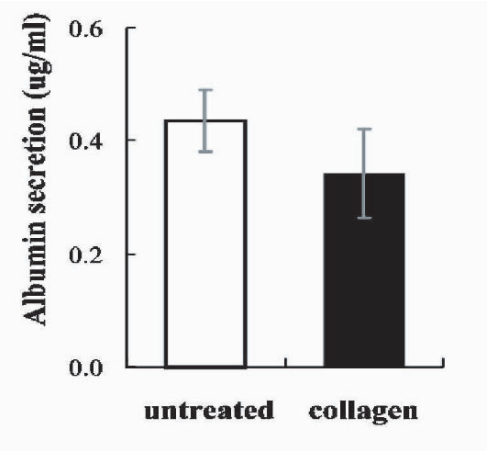

Figure 3. Albumin concentration of culture supernatants of Hep-bcl2 on collagen (closed bar) or uncoated dishes (open bar) at day3.

Table 1 Calculated albumin productivity (pg/cell/day).

	Uncoated	coated with collagen
HepG2	1.69 ± 0.14	0.55 ± 0.15
Hep-bcl2	9.75 ± 1.22	2.87 ± 0.66

4. DISCUSSION

These results suggest that a two-step culture strategy for BAL systems would be effective. At the first pre-culture stage for expansion of the population of cells, Hep-bcl2 cells should be cultured on collagen-coated dishes because of the rapid proliferation. At the second stage within the BAL, Hep-bcl2 cells should be cultured without collagen because of higher liver function and slower proliferation that could reduce cell death due to over-growth.

REFERENCES

1. Park, J. K. *et al*., 2005, Bioartificial Liver Systems; culture status and future perspective, J. Biosci. Bioeng. **99(4)**: 311-319.
2. Terada, S. *et al*., 2003, Generation of a novel apoptosis-resistant hepatoma cell line, J. Biosci. Bioeng. **95(2)**: 146-151
3. Pachenc,e J. M. *et al*., 1996, Collagen-based device for soft tissue repair, J. Biomed Mater. Res. **33(1)**: 35-40.
4. Dun, n J. C. *et al*., 1991, Long-term in vitro function of adult hepatocytes in a collagen sandwich configuration, **7(3)**: 237-245.