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APPLICATION OF THREE DIMENSIONAL CULTURE OF ADULT RAT HAPATOCYTES IN POLYURETHANE FOAM PORES FOR AN ARTIFICIAL LIVER SUPPORT SYSTEM

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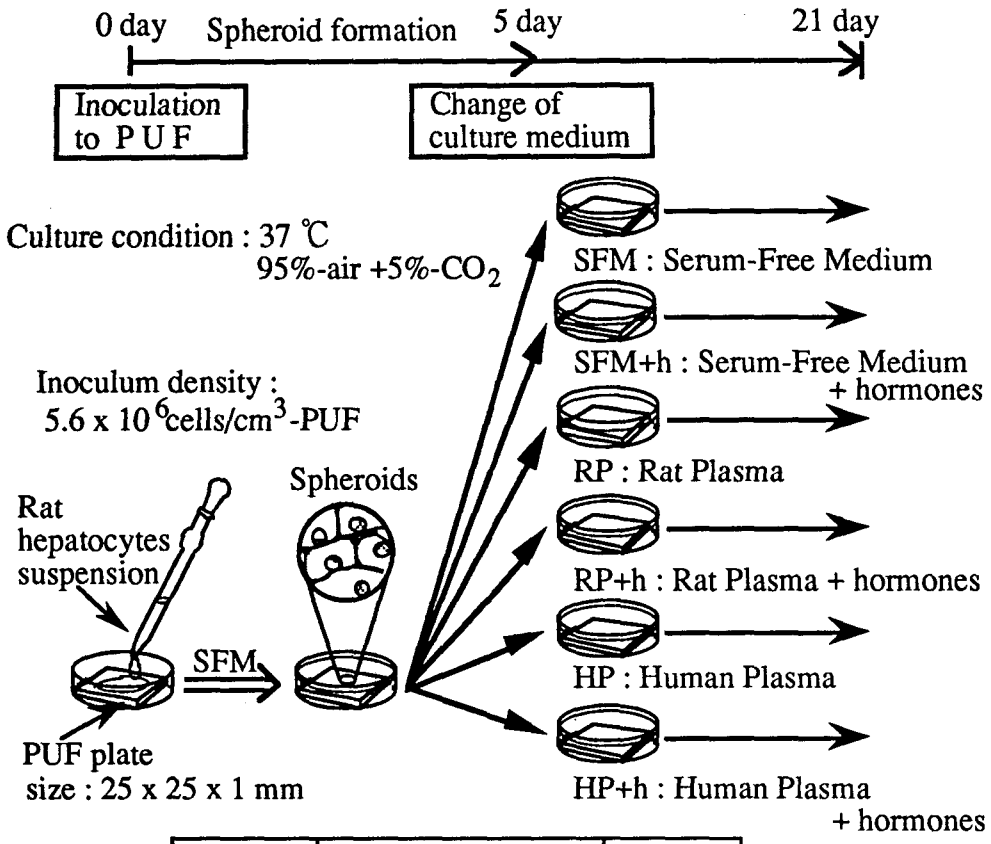
Spherical multicellular aggregates of adult rat hepatocytes (spheroid) which have tissue like structure, were formed and immobilized in the pores of polyurethane foam (PUF) which was used as a culture substratum. These hepatocyte/spheroids, about 100 μm in diameter, have maintained higher differentiated functions than those of hepatocyte/monolayer for about 3 weeks in serum-free medium. Then, we designed a prototype module of an artificial liver support system using a PUF/spheroid packed-bed, in which hepatocyte/spheroids were immobilized at high density. The urea synthesis activity of the artificial liver was maintained at least 10 days in 100% rat blood plasma. We start examining the performance of hybrid artificial liver in an *ex vivo* extracorporeal experiment with an acute hepatic failure rat.

1. INTRODUCTION

We have reported that PUF/spheroid culture system is effective for keeping several hepatic functions *in vitro* and Multi-Capillary (MC) PUF packed bed culture system including the hepatocyte/spheroids may be available for development of a hybrid artificial liver support system, which used for the medical care of acute hepatic failure [1, 2, 3]. As the next step we must examine the performance of MC-PUF packed bed culture system in *ex vivo* experiment using small animals. In this report, we have estimated the hepatic functions of spheroids in blood plasma, and tried to establish an extracorporeal circulation system for a rat.



Added hormones	insulin	10 mg/l
	dexamethasone	10^{-8}M
	glucagon	10^{-8}M
	epidermal growth factor	50 ng/ml
	aprotinin (protease inhibitor)	100KIU/ml

Fig.1 Method of PUF / spheroid stationary culture

2. MATERIALS AND METHODS

2.1. Cell and medium

The methods for isolation of adult rat hepatocytes and measurement of albumin, urea and ammonia were described in the previous paper [1, 2]. In this experiment, we used 6 kinds of culture media, which were serum-free medium which was described in the previous paper [1], rat plasma, human plasma, and

each medium supplemented with some hormones including 10 mg/l insulin, 10^{-8} M dexamethasone, 10^{-8} M glucagon, 50 ng/ml EGF, and 100 KIU/ml aprotinin (protease inhibitor) (Fig.1). The internal structure of the spheroids was observed by HE stained section of them.

2.2. Culture vessel

MC-PUF packed bed culture system used in this experiment was described in the previous paper [4]. Hepatocytes were inoculated in MC-PUF by a kind of centrifugal method [5] (Fig.2), and formed the spheroids in 5 days culture in serum-free medium. The initial cell density was 4.0×10^6 cells/cm^3-PUF. Medium exchange was performed every two day. At the measurement of urea synthesis, 1mM NH_4Cl was supplemented to the medium.

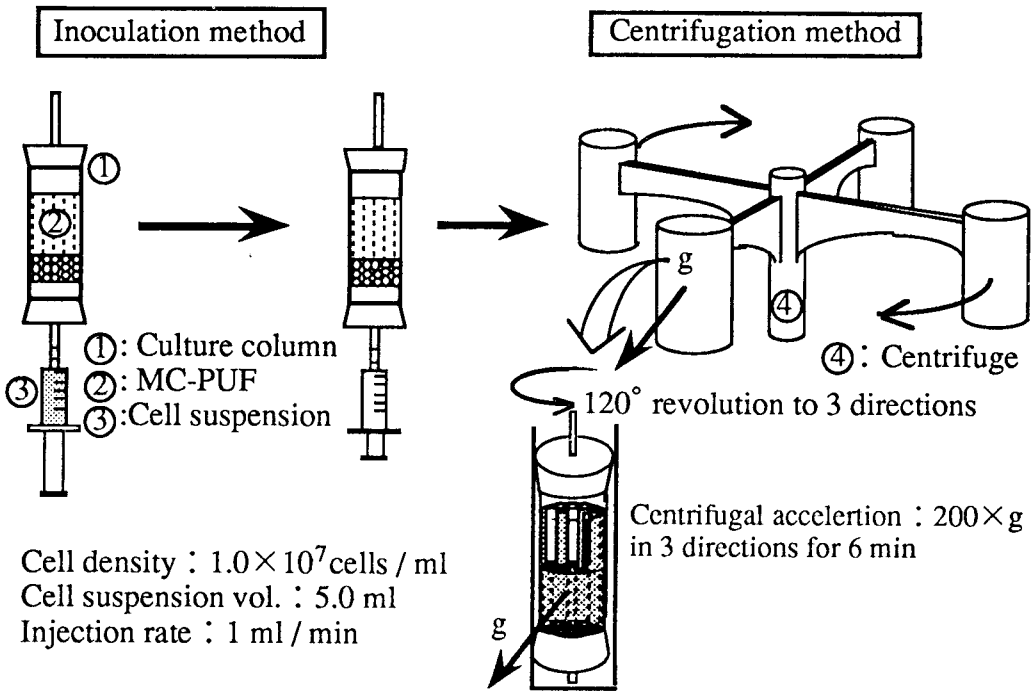


Fig. 2 Inoculation and centrifugation methods

2.3. Hybrid artificial liver support system

A hybrid artificial liver support system was composed of a rat extracorporeal circulation line and a hybrid artificial liver circulation line, which were linked via plasma separator (Fig.3, 4).

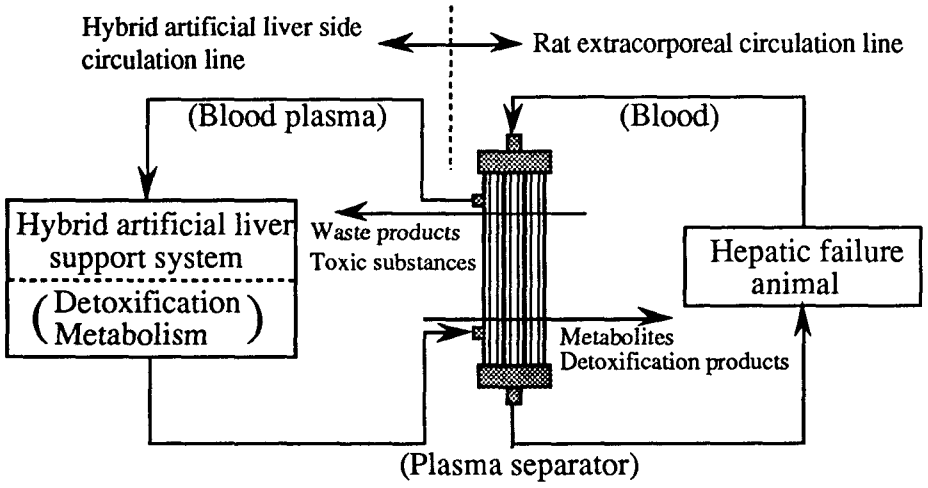


Fig.3 Outline of a hybrid artificial liver support

The rat extracorporeal circulation line was included a blood pressure monitoring system, automatically injecting line of anesthetic injection, transfusion, heparin and so on. The hybrid artificial liver circulation line was included gas exchange apparatus and MC-PUF module. At the initial stage, these lines were filled with 10 unit/ml heparin solved in the 100% rat blood plasma to prevent blood clotting in the line. Flow rate of blood in the rat extracorporeal circulation line and blood plasma in the hybrid artificial liver circulation line were about 0.5 and 3.0 ml/min, respectively. In the plasma separator, rat blood was flow into the lumen to minimize the total volume in the line. The total volume in the extracorporeal circulation line including a plasma separator was about 1.4 ml. Transfusion including rat blood plasma, glucose and isotonic sodium chloride solution.

Furthermore, we made a bypass line and incorporated into these lines to improve the mass transfer (Fig.4). The bypass line was lead out a piece of blood

plasma from the hybrid artificial liver circulation line and injected into the blood in the rat extracorporeal circulaton line, directly.

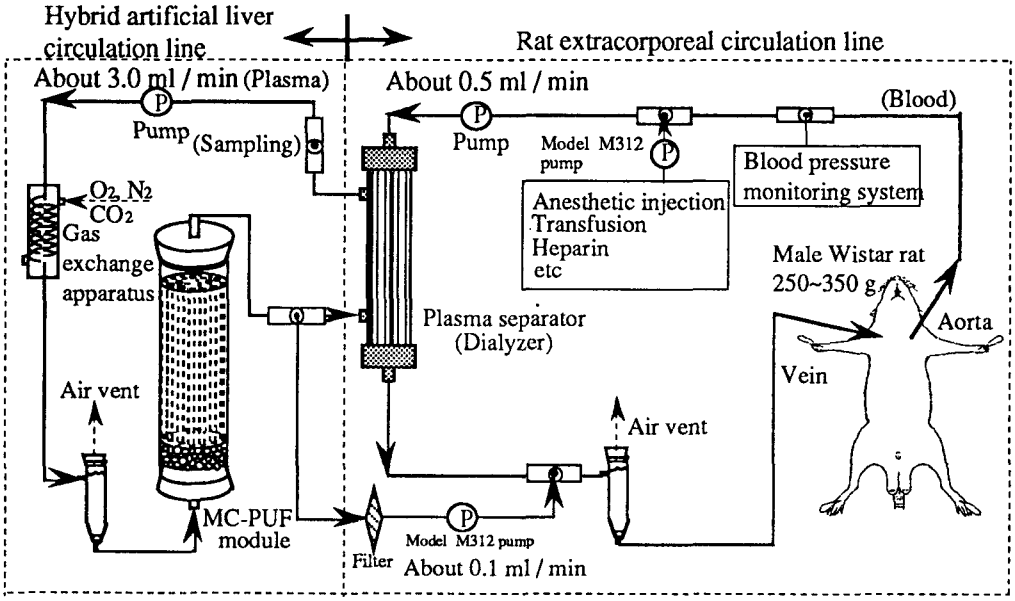


Fig.4 Hybrid artificial liver support system

3. RESULTS AND DISCUSSION

When the spheroids composed of rat hepatocytes were cultured in 100% rat plasma, the internal structure of the spheroid was broken down on 16th day. In the rat plasma supplemented with epidermal growth factor, insulin, glucagon, dexamethasone, and aprotinin, the structure of the spheroid was maintained for at least 20 days in PUF stationary culture (Fig.5).

The urea synthesis rate per hepatocyte nuclei of the spheroid in PUF stationary culture was maintained in both rat plasma (0.28 μmol/10^6 nuclei/hr) and rat plasma with hormone (0.25 μmol/10^6 nuclei/hr), but the rate decreased in human plasma after 10 days culture. This decrease of urea synthesis rate was suppressed by supplying hormones to human plasma (Fig.6). Furthermore the fact that urea synthesis rate in plasma was higher than that in serum-free medium indicates that some factors stimulating the urea synthesis might exist in plasma.

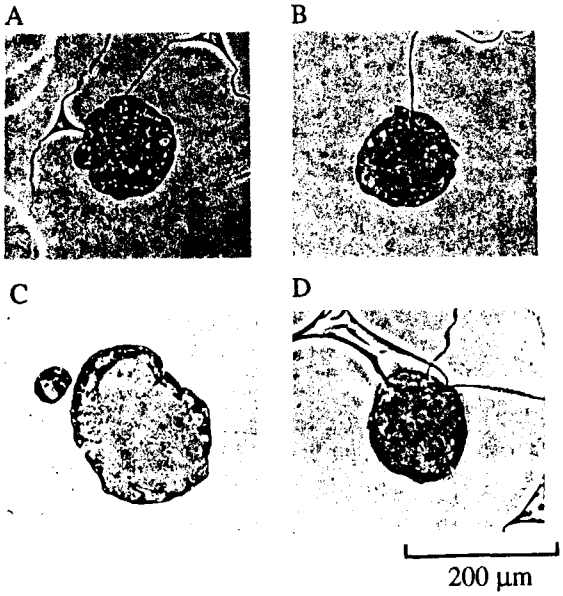


Fig.5 Time course of internal structual change of spheroid(HE stain)

A : Rat plasma only on 9th day
B : Rat plasma with hormones on 9th day
C : Rat plasma only on 16th day
D : Rat plasma with hormones on 16th day

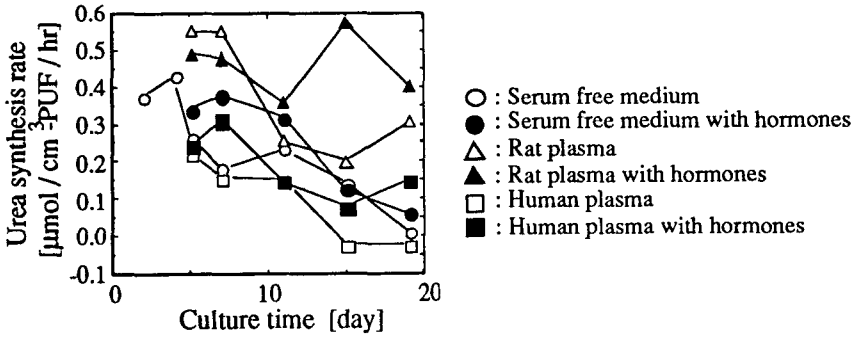


Fig.6 Time course of urea synthesis rate in various culture media

In MC-PUF packed bed culture, the higher urea synthesis rate in rat plasma with hormones (0.23 μmol/10^6 nuclei/hr), was kept stable for 10 days than that in serum free medium (0.04 μmol/10^6 nuclei/hr) (Fig.7).

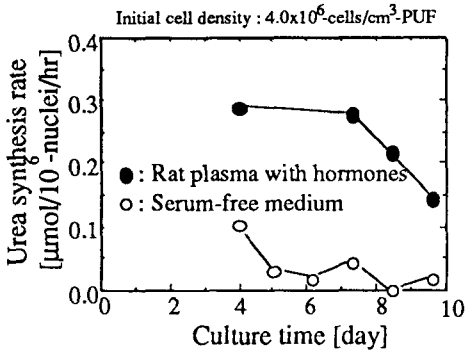


Fig.7 Time course of urea synthesis rate in various culture media of MC-PUF packed-bed culture

Furthermore, we have examined the performance of MC-PUF packed-bed culture system as a hybrid artificial liver support system by the extracorporeal circulation system including a D-galactosamine induced acute hepatic failure rat. The ammonia concentration of plasma in hybrid artificial liver circulation line, which was a toxic causal substance for hepatic coma, decreased at normal level for 3 hours circulation (Fig.8).

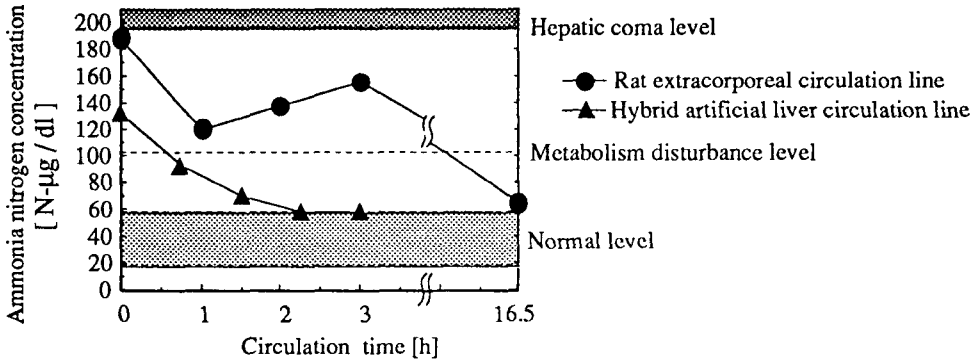


Fig. 8 Change in ammonia nitrogen concentration during the hepatic failure rat extracorporeal circulation including the hybrid artificial liver module

4. CONCLUSIONS

4.1 The morphological structure and hepatic function of the rat hepatocyte/spheroids were maintained excellently in rat and human plasma supplemented with some hormones.

4.2 We developed the rat extracorporeal circulation system of about 1.4 ml in volume including blood pressure monitoring system and plasma separator.

4.3 The ammonia concentration of plasma in hybrid artificial liver circulation line decreased at normal level for 3 hours circulation.

5. REFERENCES

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