D119

HGF 固定化培養基材の開発と肝組織工学構築に向けた試み

(九大院工) ○(学)侯 詠徳*,(九大工) (学)白木川 奈菜,(九大院工) (正)武井 孝行,(正)境 慎司,
(正)井嶋 博之,(正)川上 幸衛

1. Introduction

Hepatocyte growth factor (HGF) has been considered to act as a hepatotropic factor for liver regeneration and expression of hepatocyte functions. However, the use of HGF in tissue engineering field was inhibited due to the high cost. Therefore, we aimed to develop a new HGF/heparin-immobilized collagen gel system by using a high affinity of heparin with HGF in this study. Furthermore, we tried to estimate the effectiveness of it for hepatocyte culture *in vitro* and transplantation.

2. Materials and methods

In vitro culture: Hepatocytes were mixed well with HGF/heparin-immobilized collagen sol (2.07 g/l collagen, 3.63 mg/l heparin, 9.1 ng/ml HGF). Hepatocytes-embedded HGF/heparin-immobilized collagen gels were obtained by incubating at 37°C for 30 min. Then, the hepatocytes were cultured in 220 µl of D-HDM. The cultured-medium was collected for subsequent analysis of albumin synthesis.

Transplantation: Hepatocytes (1.25×10^6 cells/ml-substratum) were inoculated into polyurethane foam (PUF, 1mm ×1cm). The hepatocytes formed spheroid during 3 days of culture. Then, 220 µl heparin-immobilized collagen sol was poured into PUF. Hepatocytes spheroids-embedded heparin-immobilized collagen gel-filled PUF were obtained by incubating at 37°C for 30 min. This sample was transplanted into a rat subcutaneously, and retrieved it after 6 days of transplantation. Hematoxylin and eosin stain (HE) was performed for checking the histological observation.

3. Results and Discussion:

In vitro culture: Albumin synthesis of hepatocyte was up to 30 µg/well/day and maintained for more than 16 days in HGF/heparin-immobilized gel (Fig.1), which is superior to the other conditions. However, there are no morphological differences among all the conditions. The hepatocytes maintained their original round shape even in the long-term culture in all conditions. In other words, hepatocyte functions were affected by the immobilized HGF-heparin complexes in 3D gel culture.

Transplantation: Partial hepatectomy pretreatment has a benefit for remaining and regeneration of hepatocyte in the transplanted sample (Fig. 2). We can also find that blood vessel from surrounding tissues penetrated into this transplanted sample. It is necessary to supply oxygen for hepatocytes inside the sample.

In the future work, we will try to find optima condition by using higher density of inoculum hepatocyte and higher strength of collagen gel for transplantation. We believe that HGF/heparin-immobilized collagen system has the effectiveness not only for albumin synthesis of hepatocytes *in vitro*, but also angiogenesis enhancement and hepatocyte regeneration *in vivo*.

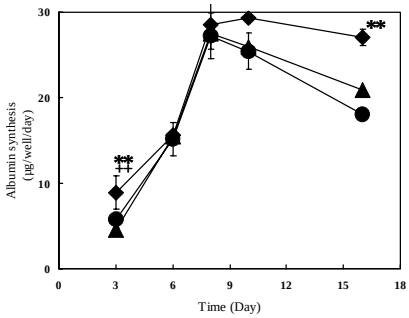

Fig. 1.
Albumin synthesis of hepatocytes cultured in various types of 3D collagen gels. Diamonds: HGF/heparin-immobilized collagen gel; triangles: HGF-immobilized collagen gel; circles: collagen gel.

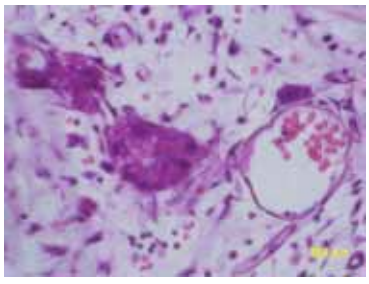

Fig. 2.
Histological observation of hepatocytes spheroids-embedded heparin-immobilized collagen gel-filled PUF with HE stain method.

*E-mail: b89611034@ntu.edu.tw