Studies of a Novel Hybrid Organoid Consisting of Functional Gel Particles and Hepatocytes for Liver Tissue Engineering

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論 文 名　：Studies of a Novel Hybrid Organoid Consisting of Functional Gel Particles and Hepatocytes for Liver Tissue Engineering
（機能性ゲル粒子と肝細胞からなる新規ハイブリッド型細胞組織体を用いた肝組織工学に関する研究）

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論　文　内　容　の　要　旨

At present, liver transplantation is the only radical treatment shown to improve survival in patients suffering serious liver failure. However, this approach is limited by availability of donor organs. Consequently, tissue engineering approaches, consisting of a scaffold, growth factors and cells, are under investigation as an alternative treatment. The scalable construction of liver tissue is therefore envisaged using this technology. Hepatocyte cell-cell interactions are essential for liver construction and maintenance of liver function. However, the high hepatocyte density required for liver construction usually results in a lack of oxygen due to the high metabolic activity of the hepatocytes. Moreover, angiogenesis during cell transplantation is always insufficient. To overcome these problems, we have developed a structure termed a hybrid organoid, consisting of growth factor (GF)-immobilized gel particles and hepatocytes. This technology allows structures to form with high cell-cell interactions and low oxygen consumption, owing to the existence of the gel particles. Furthermore, the GFs immobilized by heparin can enhance blood vessel formation *in vivo*. Moreover, the hybrid organoid structure can be formed quickly and simply, and can be applied for cell transplantation and as a bio-artificial liver. Enhanced cell viability and liver function in the hybrid organoid have been observed in both *in vitro* culture and *in vivo* transplantation.

In Chapter 1, we discuss the current situation of liver transplantation and our study concept.

In Chapter 2, we highlight several treatment approaches for liver failure. We also focus on recent research in liver tissue engineering, including materials, cell source, and creating liver tissue structures.

In Chapter 3, we describe the use of heparin-conjugated gel and evaluate the characteristics of this gel, including immobilization and GF-release profile. We then detail formation of the hybrid organoid using gel particles and hepatocytes, and evaluate angiogenesis within it. The results show that GFs were successfully immobilized into the heparin-conjugated gel. Furthermore, sustained release of GFs was observed, which likely contributed to the enhanced cell viability demonstrated in our study. To construct the hybrid organoid, cells and gel particles are combined by simple centrifugation. Furthermore, by including gel particles into the transplantation approach, sufficient vessel numbers are expected to form following induction by the heparin-conjugated gel.

In Chapter 4, we investigate liver function and cell viability of the hybrid organoid system in static *in vitro* culture. We then apply the hybrid organoid in a bioreactor, to represent a novel bio-artificial liver. The hybrid organoid comprised cells embedded in a collagen matrix, which were then used in static cultures.

Albumin concentration and ethoxyresorufin-O-deethylase (EROD) activity were also investigated during culture. The hybrid organoid elicited higher rates of both albumin secretion and EROD activity compared with controls. Moreover, culture of the hybrid organoid in a bioreactor enhanced both albumin secretion and ammonia metabolism. However, decreased liver function was observed in all cultures due to sub-optimal culture conditions. Aside from this, formation of the hybrid organoid in the selected bioreactor was simpler than in other bioreactors. Therefore, improvements in liver function using this bioreactor can be expected after optimization of the culture conditions. With further development, the hybrid organoid is expected to be a potential bio-artificial liver.

In Chapter 5, we detail construction of a novel transplantation method for the hybrid organoid. Hybrid organoid samples were transplanted subcutaneously into rats and evaluated histologically 7 days post-transplantation. Serum albumin concentration following hybrid organoid transplantation was determined in nagase analbuminemic rats (NARs) as an index of clinical treatment potential. During transplantation, larger cell clusters and higher cell viability were observed in the hybrid organoid transplantation group. Fetal liver cells were used as a model cell source in the transplantation studies. During transplantation of these model cells, larger cell clusters, higher cell viability and increased vessel formation were observed in the liver tissue of the hybrid organoid transplantation group. This indicated the hybrid organoid was also effective in supporting proliferative cells. Interestingly, glycogen storage and albumin synthesis liver functions were also confirmed in the samples. Moreover, the albumin secreted by the samples enhanced the albumin level in the NAR serum. Taken together, these findings support clinical application of the hybrid organoid system, making the hybrid organoid a novel candidate structure for cell transplantation.

Chapter 6 is the conclusion of this paper.