論 文 の 内 容 の 要 旨

論文題目　Liver tissue engineering based on three-dimensional scaffold fabrication and perfusion culture of hepatocyte progenitors
（三次元担体造形と肝前駆細胞灌流培養に基づいた肝組織再構築）

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The objective of this study was to develop an implantable liver tissue with a middle-scale volume using the principle of tissue engineering. The key obstacles in developing an implantable liver tissue includes differentiation of proliferative hepatocyte progenitors, design and fabrication of three-dimensional (3D) scaffold with an interconnected flow channel network, as well as optimal oxygen and nutrient transport.

We investigated the effects of various soluble factors on the differentiation and maturation of primary fetal porcine hepatotyes. 3D culture using biodegradable poly-L-lactic-acid (PLLA) scaffolds (0.1 cm^3) supplemented with hepatocyte growth factor (HGF) and sodium butyrate (Sb) remarkably enhanced various live-specific functions of fetal hepatocytes.

We designed a novel porous scaffold with a 3D flow-channel network and calculated the dimension of this scaffold based on oxygen consumption and shear stress. The scaffold (volume was 13 cm^3, porosity was 87%) with a pre-designed branching and joining 3D diameter-varying flow-channel network was successfully fabricated via selective laser sintering (SLS) technique by collaborative lab.

We evaluated its efficacy by perfusion culture of liver-derived cells, including Hep G2 cell line, primary fetal porcine hepatocytes. A novel cell-seeding technique based on avidin-biotin binding system (ABBS) for reconstruction of large tissues in vitro was also described. Results of perfusion culture demonstrated that such 3D flow channels and ABBS-based cell seeding are essential to the cells growth and function. A design of a large-scale porous scaffold with parallel channel array based on oxygen consumption and shear stress was proposed.

Oxygenation is the most important issue for high-density hepatocytes culture. Hemoglobin-based oxygen carrier has a potential capacity to enhance the oxygen transport in a physiological oxygen tension. We used a novel PEG-modified liposome encapsulated hemoglobin (LEH) oxygen carrier. Mathematical simulation and experimental results show that the efficacy of LEH in culturing primary rat hepatocytes in a flat-plat bioreactor.

This dissertation provides useful methodologies for engineering large-scale implantable human liver tissues.