論文の内容の要旨

Abstract of Dissertation

Thesis title: Liver tissue engineering based on the integration of 3D scaffold fabrication and cellular aggregate assembly

(三次元造形担体内への細胞凝集体充填に基づく肝組織構築)

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As the central organ in drug metabolism and toxicity, liver failure often results in a fatal threat to human life, which raised up the demands for liver tissue construct of a clinically relevant size (for instance, about 500 cm^3, which is the limited required volume of implantable liver with the cell density and functionalities being equal to those of the real tissue). General introduction of liver and the clinical treatment, previous technologies as well as this thesis approach to engineer large implantable liver tissue equivalents is presented in Ch. 1. Towards constructing an implantable liver tissue, both decellularization and artificial liver construction are promising approaches. However, the former method requires organ to organ operations and the donor shortage and low cell density issues cannot be ignored. On the contrary, the more practical artificial liver construction *in vitro* has recently been the focus of much attention in the field of bioengineering using “bottom up” route—cellular aggregate assembly, and “top down” route—three-dimensional (3D) scaffold fabrication. Whereas this approach still faces the unsolved problems of insufficient oxygenation in aggregate assembly and low resolution design of scaffolds. Therefore, the two major issues in approaching to implantable liver tissue equivalents can be pointed out as: (1) improvement of mass transfer in the engineered liver tissues to achieve high per-volume-based functionality; (2) scaling up the engineered liver tissue to a clinically significant size. My research goal is to establish the engineering methodology towards implantable liver tissue equivalent construction, by integration of both bottom-up and top-down technologies. This is a novel approach resolving mass transfer deficiencies simultaneous from micro and macro scales, meanwhile addressing the adequate cell number. Specifically, the research purposes are divided into 3 parts as follows:

(1) To improve the oxygenation and nutrient supply in cellular aggregate assembly by utilizing their interstitial gaps, which intends to solve the problems in local (within culture chamber) mass transfer;

(2) To investigate on the cellular aggregate dimension with improved design;

(3) To optimize the scaling up towards a clinically significant mass by fine designed 3D scaffold.

Firstly, cellular aggregate assembly was improved by applying "spacing tissue elements" concept in Ch. 2, namely creating some spacing between the packed aggregates for securing better mass transfer by immobilizing them with biodegradable single poly-L-lactic acid (PLLA) fibers in small perfusion bioreactor. To achieve this, a novel culture method whereby aggregates were constructed using a polydimethylsiloxane (PDMS)-based honeycomb microwell arrays. Primary rat hepatocyte aggregates around 100 μm in diameter coated with human umbilical vein endothelial cells (HUVECs) were spontaneously and quickly formed after 12 h of incubation. Then, recovered endothelialized rat hepatocyte aggregates were mixed with PLLA fibers in suspension, and packed into a PDMS-based bioreactor. 7 days of perfusion culture was successfully achieved with more than 73.8% cells retained in the bioreactor. As expected, the fibers enhanced aggregate retention and functions, which were evidenced from the improved albumin production and more spherical morphology compared with fibre-free packing. The results showed the advantages of using PDMS-based microwells to form heterotypic aggregates and also demonstrates the feasibility of spacing tissue elements for improving oxygen and nutrient supply to tissue engineering based on modular assembly.

During the perfusion culture, I found the cellular aggregate disintegration gradually happen. The broken aggregate parts may result in floating debris that hinders the perfusion to some areas. Thus, to maintain the homogeneous flow with sufficient mass transfer, stable cellular modules with enough mechanical strength are necessary. To circumvent this obstacle of cellular aggregate disintegration, I designed a module with a hollow structure in Ch. 3, and fabricated it using biodegradable polyglycolic acid (PGA). The module was designed to be a cylinder with a hollow central canal 500 μm in diameter and an outer diameter varying from 1100 to 1300 μm. Hep G2 cells, which were considered as proliferative progenitors, were found to be well immobilized on the PGA module after collagen coating. Given that 200 μm is the limitation of

oxygen diffusion *in vivo*, PGA hollow modules were able to sufficiently secure good mass transfer to the cultured cells. Hep G2 cells were found to maintain higher cell viability (suggested by calcein staining results) along with higher hepatic function (suggested by cellular albumin production and glucose consumption results) when cultured in the module with both the inner canal and transverse pores on the side wall. As possessing the advantages of cell affinity, maintaining hepatic cell functions and stable in culture medium, PGA hollow modules with loaded cells are expected to replace pure cellular aggregates in the future for further scale up.

To further produce a large liver tissue equivalent by scaling up the "spacing cellular aggregates" along with improved macro-circulation of medium for better oxygen and nutrient supply, a 3D scaffold was designed with flow channels homogeneously delivering medium to the whole construct in Ch. 4. The scaffold was designed based on the real liver structure, which comprised 43 culture chambers assembled on three layers. 12 chambers are posited on the 1st and the 3rd layer, and 19 chambers on the 2nd layer, producing a construct with symmetrical outer shape. Then, the construct of 43 culture chambers was arranged again in the same way of pattern to finally achieve a structure 500 cm^3 in volume. Culture chambers were designed with a hexagonal section about 0.27 cm^3 in volume. Interconnected flow-channels were designed to deliver culture medium independently to each of the chambers. The diameter of each flow channel was calculated based on Murray's law and Hagen-Poiseuille's equation to ensure the same pressure drop in the flow to each chamber. The scaffold was fabricated using nylon-12 due to its high mechanical properties via selective laser sintering (SLS) technique by a collaborative lab.

So far here, the insufficient mass transfer situation was intended to improve from both micro- (within culture chamber, local scale) and macro- (out of culture chamber, at higher hierarchical) scale. In Ch. 5, to address another problem of clinically significant mass of the constructed liver tissue, the loosely packed aggregates were perfusion cultured in the designed 3D scaffold. Endothelialized hepatic aggregates were obtained by co-culture of Hep G2 and TMNK-1 cells, which are the immortalized human liver sinusoidal endothelial cells, using PDMS microwells 200 μm in diameter. Formed aggregates were collected and mixed with a PLLA fiber suspension and inoculated to the scaffold. After 10 days of perfusion, the results of increased albumin production, glucose consumption and high cell viability demonstrated that present 3D scaffold consisting of chambers for cell culture and independent flow channels for medium delivery was essential for proper cell growth and function. The first attempt on approach to implantable liver tissue equivalent by integration of "bottom-up" and "top-down" concepts was

proved successful according to above mentioned results. With fully packing aggregates in the culture chamber and further scaling up the scaffold to second hierarchic, the present design and methodology are expected to finally realize an implantable liver of 500 cm^3 with well reserved per-volume-based functionality.

Finally, in Ch. 6, I present the conclusions and future outlook of the thesis. This dissertation provides efficient methodologies arranging the implantable liver tissue equivalents simultaneously from micro and macro scale. The integrated approach provides the engineered liver tissue with improved mass transfer in bottom-up aspect, meanwhile the scalable feature of the scaffold design guarantee the possibility of approaching to a clinically relevant size with well revered per-volume-based functionalities in top-down aspect. As expected, a liver tissue construct 11.61 cm^3 in volume has been successfully engineered with better maintained cellular viability and hepatic function, suggesting the promising future of further developing the scalable scaffold towards a construct 500 cm^3 sized in the future. The developed methodology of integration of “bottom-up” and “top-down” concepts will contribute to tissue engineering promising approach to construction of implantable tissue organ equivalents.