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主 論 文 の 要 旨

Three-dimensional Cell Structure Assembly by On-chip
論文題目 Fabrication and Manipulation (オンチップ作製と操作
による三次元の細胞構造体アセンブリ)

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論 文 内 容 の 要 旨

Lost or damage of an organ due to disease or injury is a serious problem for human body. The typical therapy is to get an artificial organ or a transplant. Unfortunately, artificial organs and other implanted abiotic devices often fail over time. Transplantation of organs to replace the incurable ones, such as heart, liver, or kidney, is a highly successful therapy. A successful transplant requires a compatible and willing donor. However, the need for donor organs far exceeds the supply. This donor scarcity problem has resulted in new technique which is to build artificial organs by cells and organic materials, which is defined as tissue engineering. Traditionally, many useful and important in vitro methods for tissue engineering are contributed by two-dimensional (2D) cell structures. However, an increasing number of researchers have questioned the validity of studying cells in an environment that is so far removed from the in vivo state. Three-dimensional (3D) cell structure assembly in tissue engineering provides a promising way to build artificial organs. A specific 3D multi-cellular structure is often critical for proper cellular function. Besides, if an artificial organ is to assemble and work properly, specific cells must be directed to specific locations at a specific time, forming patterns of cells with a defined function. This is a fundamental feature of tissue and organ assembly in all living organisms. 3D cellular structures provide a similar environment for cells as in the real tissues, which enhance the cell proliferation and cell-cell interaction during building artificial tissues. The ability to build such 3D structures would be useful for tissue engineering.

The organization of this thesis is divided into four main parts: (A) Introduction, (B) Methods and materials, (C) Results and Discussion and (D) Conclusions and Future Works. Part A initiates our research about 3D cell structure assembly for tissue engineering and proposes the motivation of our work. Part B presents the multifunctional microfluidic devices, including the current fabrication methods and applications. The preparation of used cell samples is also presented in Part B. Part C highlights our works in the whole 3D cell

structure assembly, including the achievements in each parts of the research. Part D concludes our works and shares several prospects for next endeavors. In short, part A consists of chapter 1; part B consists of chapters 2; part C consists of chapters 3 until 6: chapter 3 to 6 focus on the development and application of multi-functional microfluidic devices for 3D cell structure assembly (chapter 3 and 4 present the fabrication of the cell embedded components and chapter 5 and 6 present the assembly of these components to 3D shaped),; part D consists of chapter 7.

In chapter 1, based on reviewing the current problems and needs for tissue engineering, especially the current construction methods and the microtechnologies, the motivation and requirements of 3D cell structure assembly for tissue engineering were pointed out. The current techniques for tissue engineering were discussed, especially the construction methods (Top-down and Bottom-up approaches) and the microtechnologies. Brief reviews on microtechnologies for tissue engineering are highlighted. The issues for construction 3D cell structures are pointed out, and our methods and motivation is proposed.

In chapter 2, we introduced the brief background of multi-functional microfluidic devices. The current applications in cell manipulation and assembly were also reviewed. We introduced the basic fabrication methods for building multi-functional microfluidic devices. Various function integrated microfluidic devices were referred. Some of our fabricated functional parts inside the microfluidic device were presented. We also introduced the structure of the target tissue which was blood vessel, and the management of cell lines. The multi-functional microfluidic system for on-chip 3D cell structure assembly was presented.

In chapter 3, we presented the fabrication and assembly of microstructures inside a microfluidic device based on photo-crosslinkable resin and optical tweezers, for improving the microfluidic system and conducting on-chip cell cultivation. A solution replacement method inside a microfluidic channel to improve the manipulation performance and apply the assembled microstructures for single cell cultivation was reported. Microstructures with arbitrary shape were fabricated by photo-crosslinkable resin inside a microfluidic channel. The manipulation speed of the rotational microstructure increased when the viscosity of solvent decreased. We demonstrated the availability of the microstructures for manipulation with higher speed inside the microfluidic device. A novel cell cage was fabricated and the long-term cultivation of a single cell was demonstrated inside the cage. The on-chip fabrication, assembly and solution replacement will contribute to the microfluidic devices to the on-chip cell cultivation.

In chapter 4, a novel method of fabricating movable microstructures embedding controllable particles inside microfluidic devices was presented. It was combined with micromanipulation using DEP and immobilization using photo-crosslinkable resin. Particle micromanipulation, including patterning position control and concentration control was demonstrated. Several microelectrodes were fabricated using ITO and Cr/Au. Cell traps generated by microelectrodes were demonstrated. Cell position control and transportation was performed. A cell trap matrix was fabricated and high speed cell patterning was per-

formed. Immovable microstructures embedding 3 lines of cells were fabricated in the dielectrophoretic microfluidic device. Furthermore, another novel dielectrophoretic microfluidic device with separated patterning and fabrication areas was proposed. Movable microstructures embedding controllable microbeads were fabricated inside this device. These movable microstructures were able to be used as components and then further assembled to construct large 3D structures.

In chapter 5, we presented a novel method of constructing 3D multilayered tubular structures based on axis translation of 2D fabricated microstructures inside 2-layered microfluidic devices. Two approaches for assembling these movable microstructures were presented. One was a manual assembly method based on micromanipulation system and the other one was a self-assembly method based on microfluidic channel. Several manual assembly ways were demonstrated and a tube-shaped microstructure with 17 layers was assembled by an efficient assembly method. A novel 2-layered microfluidic device with a micro well and a micro groove was fabricated by 2 PDMS layers, for conducting fluidic self-assembly of 2D microstructures. The self-assembly and the axis translation of the 2D microstructures were experimentally demonstrated. Improvements including a funneled structure and 3 micro grooves were done for the microfluidic device. The improved self-assembly result of constructing 3D multilayered tubular microstructures with higher efficiency was demonstrated. The model of the self-assembly mechanism was established experimentally demonstrated. The key parameter of the microfluidic channel was characterized, and the success rate of the assembly process was evaluated.

In chapter 6, a fluidic self-assembly method of cell embedded microstructures to construct vascular-like microtubes was presented. A novel 4-layer PDMS microfluidic device was fabricated, including fabrication area, self-assembly area and extraction area inside one channel. On-chip fabrication was conducted and cell embedded microstructures were directly fabricated by PEGDA in fabrication area. Self-assembly of fabricated microstructures was performed in the micro well. The assembled microtubes were extracted outside the channel to culture dishes by the NC micro valve. The opening function of the NC micro valve was evaluated. Fibroblasts (NIH/3T3) embedded vascular-like microtubes with more than 4 mm length were constructed inside this reusable microfluidic device. Vascular-like microtubes were constructed by the presented device, with high efficiency and a contamination-free environment. It will contribute the construction of functional 3D tissues.

In chapter 7, the summary of this thesis is presented and future works are prospected.