主論文の要約

論文題目: Magnetically Guided Assembly of Porogen and Hydrogel Microfibers for Fabrication of Tissue-engineering Scaffolds （組織工学用スキャホールドの作製に用いたポロゲン及びハイドロゲルマイクロファイバーの磁気制御によるアセンブリ）

氏名: 胡程志

Scaffolds serving as artificial extracellular matrixes (ECMs) play a pivotal role in the process of tissue regeneration by providing optimal cellular environments for penetration, ingrowth, and vascularization. The requirements of scaffolds for tissue engineering are complex and specific to the structure and function of the tissue of interest. The scaffold fabrication technique needs to be developed appropriately to manufacture the scaffold with the desired characteristics such as the degradation rate, porosity, pore size, shape, distribution, and mechanical properties. In the previous study, the porous scaffolds have been studied by a variety of methods, such as gas foaming, fiber bonding, phase separation, solvent casting, particulate leaching, membrane lamination, high pressure processing, and hydrocarbon templating. In the thesis, I studied the use/development of magnetic sugar particles and segmented magnetic hydrogel microfibers to fabricate the biodegradable scaffolds. Many magnetic micromanipulation methods have been investigated for magnetically guided assembling the porogen and hydrogel microfibers. In sum the thesis can be divided into seven chapters.

In chapter 1, the general requirements for tissue engineering were presented. The current progress on the tissue engineering was addressed. The importance and the principles for fabricating tissue-engineered scaffolds were clarified. The state-of-art techniques for fabricating scaffolds were reviewed and discussed. Manipulation techniques in micro-nano scale were summarized from the aspects of principles, characteristics and applications.

Chapter 2 reported a magnetic steering method for controlled porogen fabrication of scaffold for blood vessel regeneration. The method described involves generating gradient magnetic field by a combination structure of Helmholtz coils and Maxwell coils to propel particularly prepared magnetic sugar particles (MSPs) moving in fluid environment at desired trajectory and forming a specified 3D shape as templates for particulate leaching. The movement properties of MSP were theoretically analyzed and the corresponding dynamic mechanics model was established. Further the magnetic field distributions inside the combined coils were calculated and optimal control parameters of coils configuration were obtained. Preliminary motion control experiment was also conducted to prove the feasibility of proposed method. The result demonstrated that MSP cluster can be manipulated with average speed of 0.25 mm/s for a cluster of 12 MSPs and 0.116 mm/s for a cluster of 37 MSPs with the proposed coil system, and could be used for improving interconnection of MSP dot patterns.

Stacks of sheet-like scaffold can be engineered to become artificial ECMs, suggesting a great potential for achieving complex 3-D tissue regeneration to support cell survival and growth. In the chapter 3, we proposed and investigated a combined particulate leaching of magnetic sugar particles (MSPs) and salt particles for the development of a sheet-like scaffold. MSPs were fabricated by encapsulating NdFeB particles inside sugar spheres and were controlled using magnetic fields as a porogen to control pore size, pore structure and pore density while fabricating the scaffold. We studied the influence of the strength of the magnetic fields in controlling the coating thickness of the unmagnetized MSPs during the fabrication of the sheet-like scaffolds. The experimental relationship between magnetic flux density and the thickness of the MSP layer was illustrated. Furthermore, we investigated the infiltration capacity of different concentrations of poly(L-lactide-co-ε-caprolactone) (PLCL) as a scaffold material on MSP clusters. Following polymer casting and removal of the sugar template, spherical pores were generated inside the scaffolds. Cultivation of NIH/3T3 fibroblasts on the fabricated scaffold proved that the proposed method can be applied in the cell sheet fabrication.

Chapter 4 reported two magnetically-guided porogen-assembly method using magnetic sugar particles (MSPs) as porogens for fabricating scaffold. A patterning device was utilized to align MSPs following designed templates and magnetic polyvinyl alcohol (PVA) was introduced to improve the layout of pores. The magnetic PVA was fabricated by mixing the NdFeB powders with PVA solution. The magnetic PVA can be magnetized and used for attracting the MSPs with specific pattern. After Poly(L-lactide-co-ε-caprolactone, PLCL) casting and removal of the sugar template, spherical pores were generated inside scaffold. The surface and inner morphologies of the scaffolds were evaluated with the aid of optical microscope and scanning electron microscope, respectively. The results showed controllable diameters with ranges of 105-150 micrometer, 250-300 micrometer and 425-500 micrometer and appropriate interconnection of pores, elegant pore wall morphology and high porosity were achieved in small-size scaffolds with the size of 8 mm in width and 10 mm in length. Tubular scaffolds were fabricated based on the sheet-like scaffold.

Alginate hydrogel has widespread applications in tissue engineering, cancer therapy, wound management and drug/cell/growth factor delivery due to its biocompatibility, hydrated environment and desirable viscoelastic properties. However, the lack of controllability is still an obstacle for utilizing it in the fabrication of 3D tissue constructs and accurate targeting in mass delivery. To support active drug delivery and the fabrication of 3D tailor-made cell-hydrogel constructs to mimic the real morphology of organs or tissues, magnetic hydrogel fibers are exceptional candidates. Magnetic hydrogel fiber blocks can be assembled, disassembled and reassembled easily to form complex tissue structures in a controllable way. Additionally, magnetic hydrogel fibers can have chemical or biological functionality. It is easy to apply the micro-fabrication and microfluidic technologies in this study to produce functional cell-incorporating hydrogel bulks.

In the chapter 5, we proposed a new method for achieving magnetic alginate hydrogel microfibers by dispersing magnetic nanoparticles in alginate solution and

solidifying the magnetic alginate into hydrogel fiber inside microfluidic devices. The microfluidic devices have multi-layered pneumatic microvalves with hemicylindrical channels to fully stop the fluids. In the experiments, the magnetic nanoparticles and the alginate solution were mixed and formed a uniform suspension. No aggregation of magnetic nanoparticles was found, which is crucial for flow control inside microfluidic devices. By regulating the flow rates of different solutions with the microvalves inside the microfluidic device, magnetic hydrogel fibers and nonmagnetic hydrogel fibers were fabricated with controlled sizes. The performance of the fabricated pneumatic microvalves was characterized, and the influence of the flow rate of the alginate solution on the thickness of the hydrogel microfibers was analyzed. The proposed method for fabricating magnetic hydrogel fiber holds great potential for engineering 3D tissue constructs with complex architectures and active drug release.

In the chapter 6, in order to fabricate arbitrary-shaped hydrogel scaffold with magnetic alginate hydrogel microfibers, many methods have been studied for 3D hydrogel microfiber manipulation. An improved method for exploring magnetic tweezers in patterning and aligning magnetic hydrogel fiber was addressed to fabricate large-scale engineered cell-hydrogel constructs. Magnetic hydrogel microfibers were fabricated based on a microfluidic device and a syringe pump system, respectively. The fabricated hydrogel fiber was made of alginate and with a diameter of 34 micrometer. MRI contrast media was added into the alginate solution to append magnetic material inside the fibers. The magnetic material inside the hydrogel fiber was regulated by the microfluidic device. Magnetic tweezers system based on solenoid electromagnet was utilized to evaluate the magnetic response of the magnetic hydrogel fiber. Evaluation results showed the hydrogel fiber can be maneuvered by the proposed system with a positioning resolution of sub-micro level. Other patterning device based on magnetic tape and micro pillar array were also investigated. The cultivation results of hydrogel fiber with C2C12 cells showed the potential for real applications of the proposed method in tissue engineering.

In the chapter 7, the thesis was summarized and some future perspectives were visualized.