論 文 の 内 容 の 要 旨

論文題目

ADDITIVE MANUFACTURING OF CELL FIBER BASED 3-D TISSUE CONSTRUCT

（細胞ファイバから構成される3次元組織の積層造形）

氏　　名　　聶 銘昊

1. Introduction

Additive manufacturing (a.k.a. 3-D printing) refers to the technology that manufacture 3-D objects by depositing materials/inks in a layer-by-layer fashion. With 3-D printing, manufacturers can make existing products more efficiently—and create ones that weren't possible before, such as printing human tissues/organs. Currently there are two major kinds of methods for the bioprinting of tissue/organ: cell-printing and tissue-printing. Cell-printing utilize cell-ink (cell polymer mixture) to rapidly fabricate macroscopic porous cellular constructs, yet the cell density it can achieve is low ($<2\times10^7$ cells/mL); tissue-printing utilize tissue-ink (cell aggregates) to achieve high cell density, yet the fabrication time is long (generally more than 1 week). To solve the problem, this thesis describes a bioprinter which is capable of printing high cell density multicellular microfiber (a.k.a. cell fiber) based macroscopic, porous tissue construct. To print cell fibers, a bioprinter with microfluidic printhead and syringe-vacuum substrate will be built up. Using this bioprinter, a one-step bioprinting of calcium alginate encapsulated cell-laden collagen with high cell density is expected to be achieved (Fig. 1).

2. The construction of the bioprinter for cell fiber printing

The system composition and actual assembly photo of the bioprinter is described. (Fig. 2) First, the microfluidic printhead is designed and fabricated using stereolithography-based approach; bench tests performed on the printhead shows that it is capable of generating core/shell hydrogel microfibers steadily,

with well-controlled fiber spinning velocity (Fig. 3). Second, the syringe-vacuum substrate is then conceived and implemented, by combining a commercially available filter membrane with a 3-D printed perforated chamber. The working principle of the vacuum substrate is then proposed and analyzed, with corresponding bench test experiments to verify the proposed working principle (Fig. 4).

3. Evaluation and capability demonstration of the bioprinter

The actual operation of the bioprinter is tested (Fig. 5). First, by analyzing the relationship in between the many tunable parameters, the stable protocols for printing alginate microfibers is probed by several printing trials. Then, cell-laden core/shell microfibers are printed using the stable protocols. After printing, different culture methods are tested for the maturation of cell fibers inside the printed constructs. As a result, we found out that the static culture method is unable to maturate the printed core/shell cell-laden fibers into cell fiber, while rotary culture method is capable of forming cell fibers upon culture.

4. Applications of the cell fiber printing technology

First, the cell fiber printing technology is used to create thick tissue construct based on HepG2 cell fibers. The thickness of the reconstructed tissue is approximately 2mm, consisting of 2 layers of rafting fibers and 10 layers of HepG2 cell fibers. As a result, the cells are alive with high cell density in the cell fibers, which shows that the printing technique is applicable for replicating in-vivo tissue like morphology. In addition, the cells in the printed construct showed enhanced albumin secretion function, comparing to the cells in traditional 2D cell culture. Then, the applicability of the HepG2 cell fiber based construct to be used as implantation graft is also tested. During the implantation and retrieval procedures, the good mechanical integrity of the construct makes it easier to handle. After 3 days of implantation, cells in the retrieved constructs are found to be partially viable; in addition, human albumin is detected in mouse blood samples. The results show that the cell fiber based construct a potentially useful short-term replacement tissue for the treatment of acute organ failures.

5. Conclusions

In this thesis, the technique for printing cell fibers is proposed and established. To enable the printing of cell fibers, a bioprinter is conceived and built up. The construction of the bioprinter covers detailed designs on its components as well as the practice of printer system assembly. Comparing to the existing bioprinting techniques, the cell fiber printing technique developed in this thesis is capable of rapidly printing densely cellularized tissue constructs. The cell fiber printing technique is then used to demonstrate the reconstruction of HepG2 cell fiber based tissue construct with in-vivo tissue-like morphology and function. In addition, as a proof-of-concept for using this technique for treating diseases such as acute liver failure, *in-vivo* implantation of the HepG2 cell fiber based tissue construct is also performed.

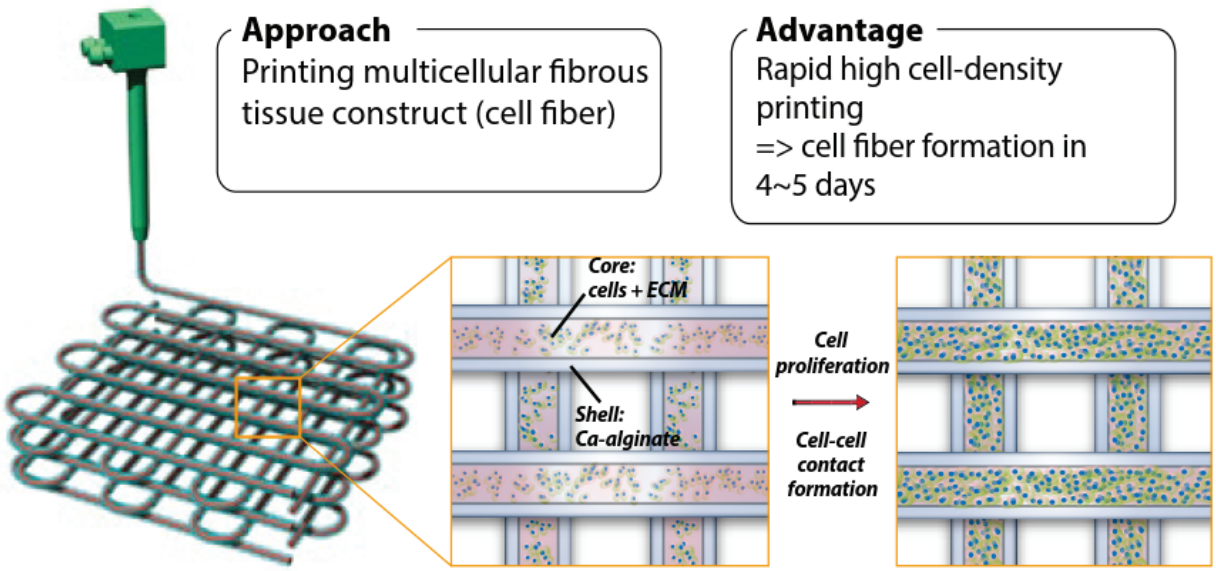

Fig. 1 Concept of this work.

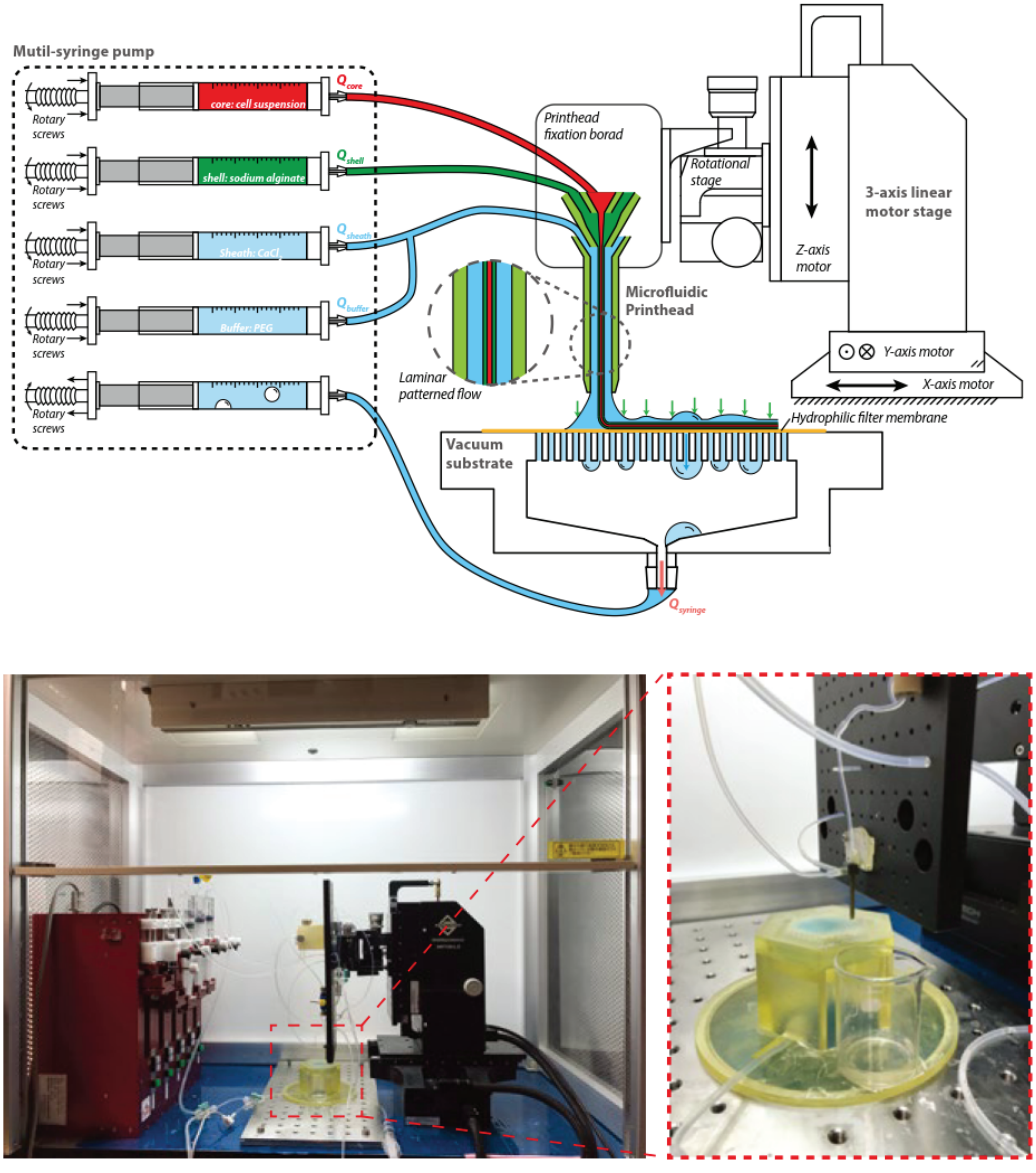

Fig. 2 Schematic and actual photo of the bioprinter for cell fiber printing.

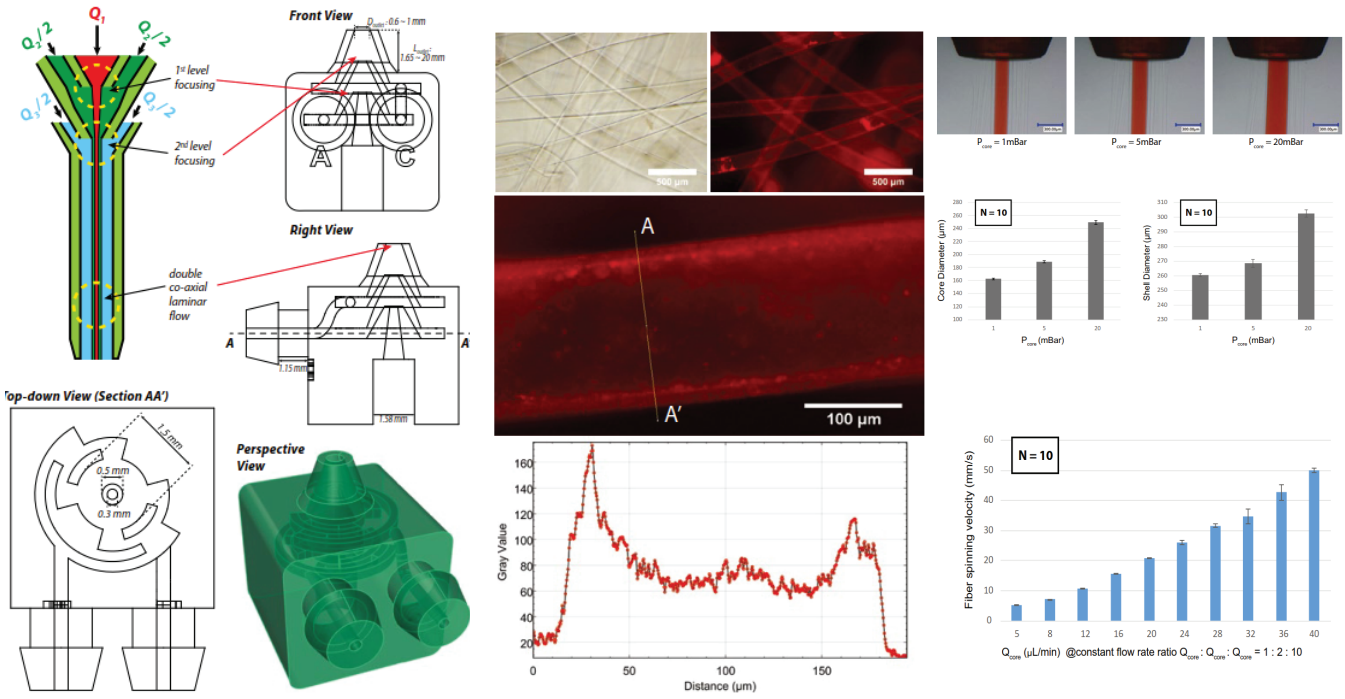

Fig. 3 Design and tests of the printhead.

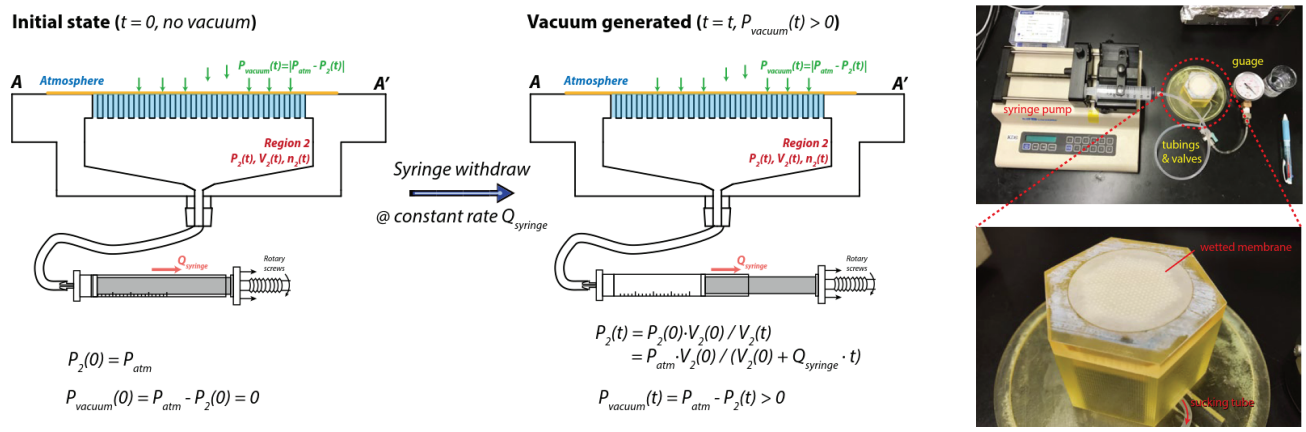

Fig. 4 Design and tests of the syringe-vacuum substrate.

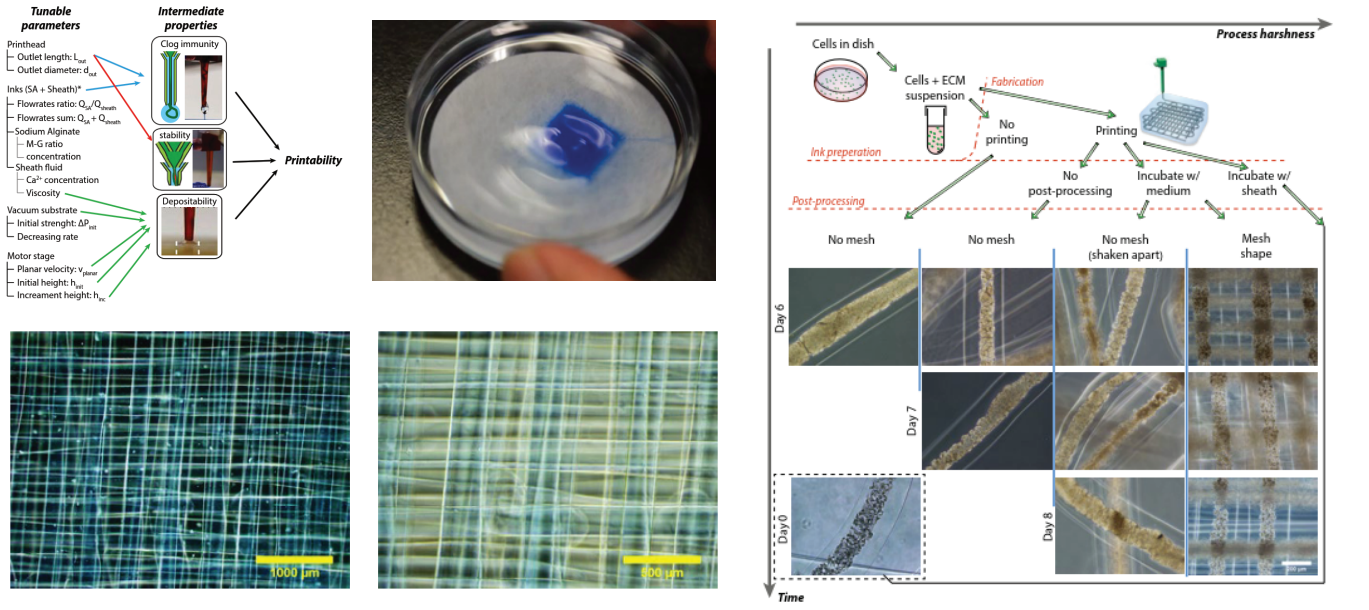

Fig. 5 Demonstration of alginate fiber printing and cell fiber printing.