Laser-Assisted Printing of Alginate and Cellular Tubes

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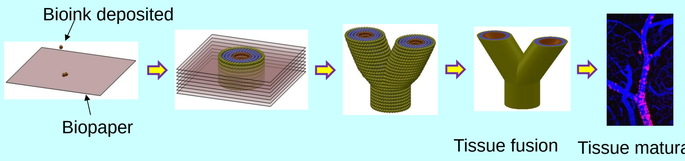
❑ BACKGROUND and OBJECTIVES

Background

Organ transplantation has always been a global issue due to the shortage of donor organs. Organ printing technology has emerged as a promising approach for the free-form fabrication of human tissue constructs and organs, relieving the pressure due to donor shortage.



Biofabrication of a vascular tree

3D printing-based organ printing can be realized using orifice-based and orifice-free approaches. Inkjetting is the main orifice-based approach. However, orifice-based printing may experience a great difficulty in printing viscous biological materials such as alginate, which may clog the nozzle during printing. Laser-induced forward transfer (LIFT) technique is able to print highly viscous materials without nozzle clogging.

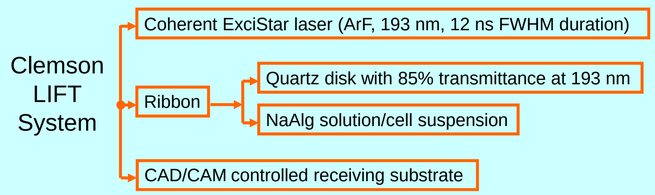
Challenges

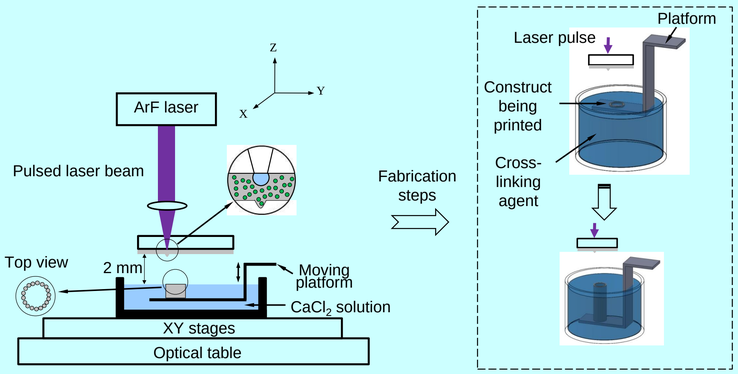
- How to print 3D tissue constructs automatically and efficiently using LIFT; and
- How to evaluate, model and quantify the LIFT-induced cell damage. Cell injury is unavoidable in bioprinting processes. Cell injury is sometimes reversible up to a certain point; however, exposure of a cell to a high magnitude and/or lasting external stress may cause irreversible cell injury even cell death.

Objectives

- To investigate the effects of sodium alginate (NaAlg) concentration and operating conditions on the printing quality during laser-assisted printing of alginate tubes; and
- To investigate the effects of operating conditions on the post-transfer cell viability and cell proliferation capacity.

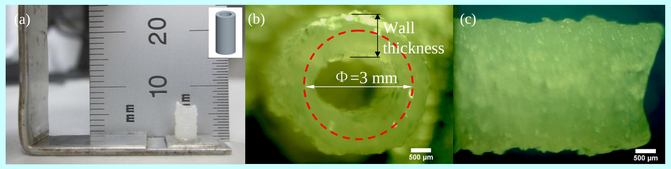
❑ MATERIALS AND EXPERIMENTAL SETUP





LIFT-based tube printing mechanism

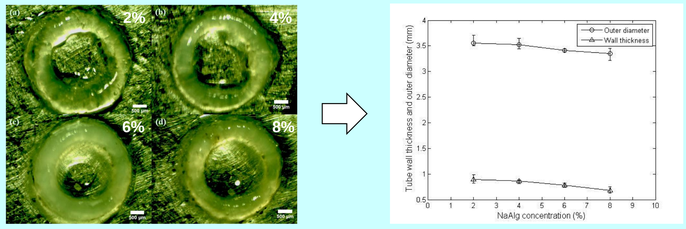
❑ REPRESENTATIVE ALGINATE TUBE



A representative alginate tube fabricated by laser-assisted printing (6 mm in length and 3 mm in diameter)

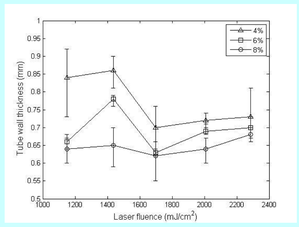
Effects of NaAlg Concentration and Operating Conditions

The tubular structures printed were designed as 1mm in height and 3 mm in diameter

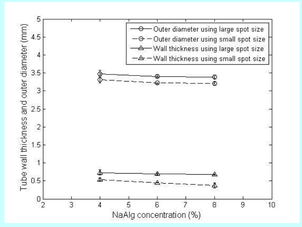


Tube outer diameter and wall thickness as functions of NaAlg concentrations (laser fluence=1437 ± 28 mJ/cm²)

Tube wall thickness and tube outer diameter decrease with NaAlg concentration



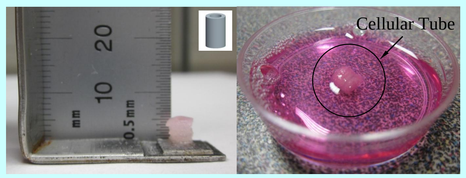
Tube wall thickness as functions of laser fluence using 4%, 6% and 8% NaAlg solutions



Tube outer diameter and wall thickness as functions of NaAlg concentration using different spot sizes

- Tube wall thickness first increases, then decreases, and then increases a little bit with laser fluence;
- Tube outer diameter decreases using a smaller laser spot size; and
- Tube wall thickness decreases using a smaller laser spot size, and this sensitivity increases with the NaAlg concentration.

❑ REPRESENTATIVE CELLULAR TUBE

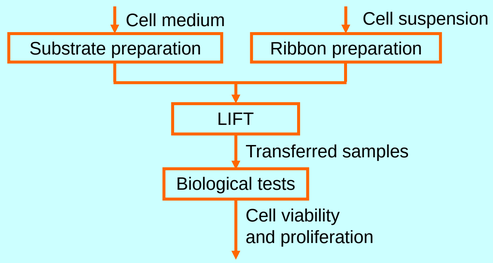


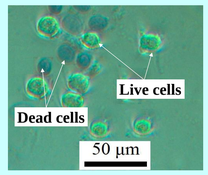
A representative cellular tube fabricated by laser-assisted printing (5 mm in length and 3 mm in diameter)

- Cell viability: 65% after printing, 80% after 24 h incubation
- Bioink used included 2% NaAlg and 5×10^6 ml^{-1} NIH3T3 cells)

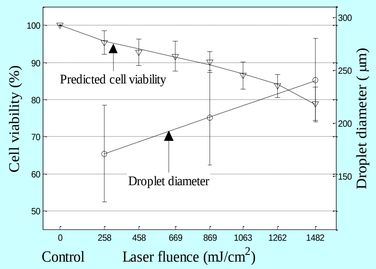
❑ PROCESS-INDUCED CELL INJURY

Materials and Method





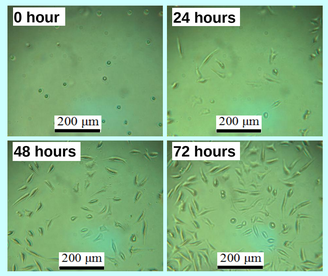
Post-transfer human colon cancer cells (HT-29) stained with trypan blue



Post-transfer HT-29 cell viability and droplet diameter as functions of laser fluence

- Post-transfer HT-29 cell (1×10^7/ml) viability decreases with the increase of laser fluence; and
- Cell droplet diameter increases with the laser fluence.

Experimental Results



Cell proliferation under 258 mJ/cm² laser fluence

- Post-transfer HT-29 cells can proliferate to confluency; and
- HT-29 cells suspended in medium immediately after transfer. Living cells adhered to well bottom and grew well after 24 hours. Some round shape cells after 24 hours or more might be dead or quiescent.

❑ CONCLUSIONS AND FUTURE WORK

Conclusions

- Highly viscous materials can be laser printed into well-defined tubular and cellular structures, and laser fluence should be carefully selected to print smaller diameter and thin walled tubes;
- Post-transfer HT-29 cell viability decreases as the laser fluence increases from 258 to 1500 mJ/cm²; and post-transfer cells can proliferate after incubation.

Future work

- Understand the jet formation process using imaging analysis;
- Automate the printing process to achieve a higher efficiency;
- Print 3D cellular constructs; and
- Develop mathematical models accounting for process-induced cell injury.

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