



Technical Paper

Identification of optimal printing conditions for laser printing of alginate tubular constructs



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ABSTRACT

Three-dimensional (3D) laser-assisted orifice-free printing technique has emerged as a promising approach for the fabrication of tissue constructs. For the better adoption of this technique, this study has investigated the effects of operating conditions, including the laser fluence and receiving substrate velocity, on the printing quality in terms of whether printed features are well-defined or not. Four main morphologies of printed lines before gelation have been identified during line printing: isolated droplets, discontinuous segments, well-defined lines, and over-printed lines. The 2125 mJ/cm² laser fluence and 100 mm/min substrate velocity conditions, corresponding to a 0.42 overlap ratio, have been identified as a combination of optimal printing conditions by using an 8% alginate solution under a 10 Hz laser repetition rate. Alginate straight and bifurcated Y-shaped tubes have been successfully printed by applying the identified optimal printing conditions.

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1. Introduction

Tissue substitutes, including transplantable autograft and allograft tissues, have been widely utilized to recover or repair acute injury, chronic diseases, or congenital defects. However, such practices are usually limited by various hurdles such as pathogen transfer, immune rejection, high cost, and especially the donor shortage. Fortunately, organ printing, a layer-by-layer bioprinting technique, has emerged to tackle some of these hurdles by fabricating three-dimensional (3D) tissues/organs using patient's cells based on the computer-aided model of patient-specific organs [1].

Comparing with scaffold-based tissue engineering approaches, 3D bioprinting such as jet-based laser printing [2] and inkjet printing [30] offers a potentially scalable technology to fabricate heterogeneous structures from various cell types. Generally, bioprinting can be implemented in two ways: orifice-based and orifice-free. Nozzle-based bioprinting including inkjet printing is a common orifice-based approach which has been successfully applied to print simple [31,32] as well as complex cellular tubes [3,4], and the droplet formation performance during the inkjet printing of cell-laden bioinks has been extensively investigated too [5]. Unfortunately, orifice-based printing may experience a great difficulty in printing highly viscous biological materials which results in

nozzle clogging during printing. For example, only sodium alginate with concentrations lower than 2% is recommended for inkjetting [6–8]. As such, orifice-free techniques are expected for the printing of viscous biomaterials and biological materials or bioinks with a high cell density, which are common constituents of many biological constructs.

Of various orifice-free printing approaches, laser-assisted printing/direct writing, developed based on modified laser-induced forward transfer (LIFT), has been utilized to print and pattern different materials including biomaterials and biological materials [2,9–16,33]. During a typical modified LIFT-based laser direct-write process [17], focused laser pulses are directed through a quartz ribbon which has a coating to be printed on the other side. These pulses are then absorbed by a sacrificial energy-absorbing layer or matrix of the coating, causing extremely localized heating and sublimation of a small portion of the coating. It leads to the formation of small vapor bubble(s), and the resulting bubble(s) further expand and may eject part of the coating material as a droplet for deposition or patterning. There are four main sequential steps during a typical laser printing process, namely, laser-matter interaction, bubble formation and expansion, jet/droplet formation, and jet/droplet landing.

Laser printing is usually implemented as matrix-assisted pulsed-laser evaporation direct-write (MAPLE DW) [17] as shown in Fig. 1. Once a laser pulse-induced bubble [18] is formed during laser printing, the bubble may lead to a forming jet. As the jet further develops, it may contact the receiving substrate, which can be a previously printed layer, before breaking up into droplet(s)

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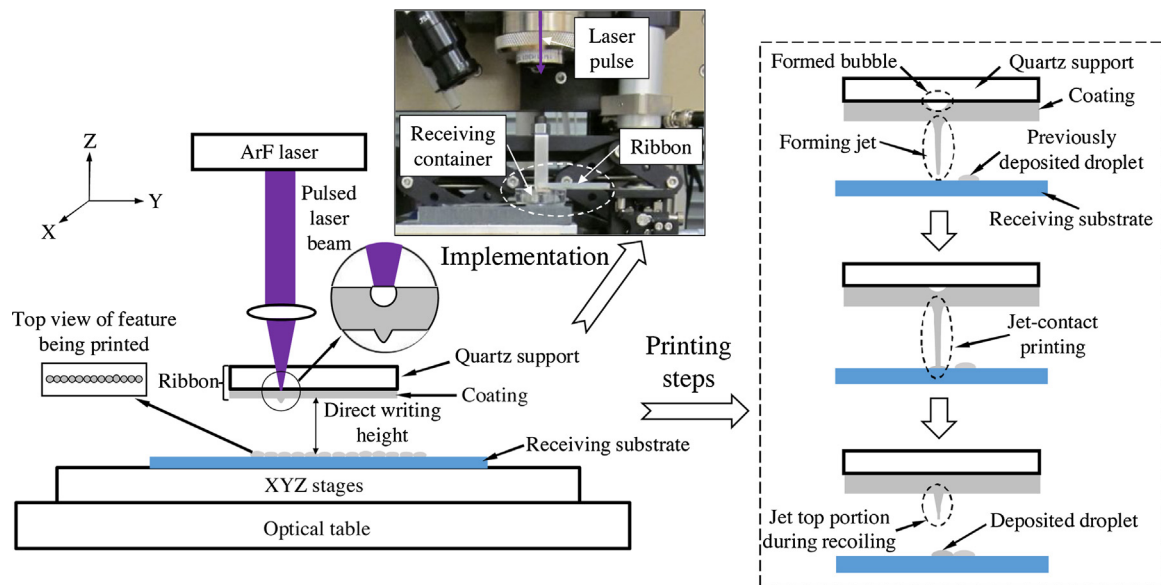


Fig. 1. Laser printing schematic and jet-contact printing steps.

depending on the direct writing height, and the top portion of the jet may recoil back to the coating of the ribbon. Such a jet-contact printing scenario usually leads to a good feature resolution. Alternatively, the jet may break up into droplet(s) first and then land onto the substrate. Once deposited, a droplet with a landing velocity may impact [19] and further spread over the substrate. Ideally, successively printed droplets should gel/solidify and coalesce into an integral, continuous feature. Such a feature is continuously deposited at the same elevation with the help of support material as needed, and once a layer is formed, the receiving substrate moves downwards by a distance of layer thickness inside a container until a construct is printed as described by Yan et al. [16].

In addition to being utilized to print various cells [9,10,12,13,33], laser printing has been pioneered to make two-dimensional (2D) and 3D constructs [16,20,21]. Of various laser printed 3D printed constructs, their surface finish and dimensional accuracy are still to be further improved by optimizing laser printing conditions for any given material being printed. As a logical step to solve this printing quality challenge, laser line printing should be first carefully investigated since printed lines are basic building blocks to form each layer, eventually leading to a layer-by-layer fabricated construct.

Line quality during laser printing has been a research subject of numerous studies. For example, the printing of conductive nanoparticles has been investigated by varying the laser pulse energy and donor–receiver substrate separation to analyze their effects on transferred droplets [22]; the influence of droplet spacing on the morphology of printed lines has been researched during the laser printing of glycerol solution [23]. Unfortunately, there is still no work to study the laser line printing of viscoelastic solutions/suspensions, which represent most common bioinks. The fluid viscoelasticity usually significantly influences the jet/droplet formation process as well as the droplet spreading process after landing. Furthermore, further research on applying optimized printing conditions to laser print 3D constructs is still missing.

The objective of this study is to experimentally investigate operating conditions on the line printing quality in terms of whether printed features are well-defined or not during the laser printing of viscoelastic alginate solution and further apply identified optimal conditions for the fabrication of 3D alginate tubular constructs. In particular, laser fluence and substrate velocity have been selected as two operating conditions of interest herein. Alginate, especially

sodium alginate, has been widely used as a constituent of bioinks in bioprinting, and it can be chemically modified for better cell adhesion capacity and biodegradability. As such, sodium alginate has been chosen to prepare the viscoelastic bioink to be printed in this study. While sodium alginate solution has been printed herein, the resulting knowledge may be applicable to the printing of other bioinks.

2. Experiment setup and design

2.1. Experiment setup

Laser printing was implemented as MAPLE DW to investigate the effects of laser fluence and substrate velocity on the printing quality and further fabricate 3D alginate tubular constructs. The MAPLE DW apparatus consisted of an ExciStar argon fluoride (ArF) 193 nm, 12 ns full-width half-maximum excimer laser (Coherent, Santa Clara, CA), a laser beam deliver system, an alginate coated ribbon, and a moving substrate controlled by XYZ stages (Aerotech, Pittsburg, PA). In particular, the printing setup is illustrated in Fig. 1.

In this study the laser spot size was controlled as 150 μm in diameter, the laser repetition rate was 10 Hz, and ultraviolet (UV) fused silica quartz (Edmund Optics, Barrington, NJ) with 85% transmittance for 193 nm laser was used as the quartz support for the ribbon. The coating was prepared using a film applicator (MTI, Richmond, CA), resulting in a ribbon coating with a thickness of approximately 50 μm . The laser fluence was measured using a FieldMax laser power/energy meter (Coherent, Santa Clara, CA), the ribbon motion was controlled by computer-controlled motion stages (Thorlabs, Newton, NJ) to continuously expose new coating materials for laser printing, and printed features were examined using an optical microscope and analyzed using ImageJ (National Institute of Health, Bethesda, Maryland). Herein the direct writing height, defined as the distance between the ribbon and the receiving substrate, was set at 2 mm based on the preliminary testing to achieve a jet-contact printing condition, which usually leads to a good printing quality while avoiding an undesirable contact between the coating material and the cross-linking solution. Such a 2.0 mm direct writing height was also adopted in a previous laser printing study [16].

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