Multi-Nozzle Biopolymer Deposition for Freeform Fabrication of Tissue Constructs

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Abstract

Advanced freeform fabrication techniques have been recently used for the construction of tissue scaffolds because of the process repeatability and capability of high accuracy in fabrication resolution at the macro and micro scales. Among many applicable tissue scaffolding materials, polymeric materials have unique properties in terms of the biocompatibility and degradation, and have thus been widely utilized in tissue engineering applications. Hydrogels, such as alginate, has been one of the most important polymer scaffolding materials because of its biocompatibility and internal structure similarity to that of the extracellular matrix of many tissues, and its relatively moderate processing. Three-dimensional deposition has been an entertaining freeform fabrication method of biopolymer and particularly hydrogel scaffolds because of its readiness to deposit fluids at ambient temperatures. This paper presents a recent development of biopolymer deposition based freeform fabrication for 3-dimensional tissue scaffolds. The system configuration of multi-nozzles used in the deposition of sodium alginate solutions and Poly-?-Caprolactone (PCL) are described. Studies on polymer deposition feasibility and structural formability are conducted, and the preliminary results are presented.

1. Introduction

Tissue engineering is considered to be the most innovative approach for tackling many diseases and body parts that need to be replaced [1-3]. Up to date, the ideal approach for perusing engineered tissue parts involves three subsequent procedures. First, the biological cells are identified and gathered in sufficient numbers. Second, the suitable biomaterial is identified and designed accordingly to host the gathered cells. Finally, the cells are seeded into the biomaterial for cell culturing in vitro or in vivo. Another approach of engineering tissue is to encourage cells in the host to populate in to the biomaterial structure upon implantation [4]. The biomaterial that is designed for housing the cells is referred to as scaffolds and is usually three dimensional. Biomaterials are used as scaffolds for their uniqueness of being biocompatible, which mean that they will not be rejected by the body upon implantation. These biomaterials are fabricated from a wide range of materials that could be inorganic synthetic such as metals, ceramics, and polymers or from organic materials such as proteins, chitosan, and alginate.

Scaffolds used in tissue engineering are also required to have specific properties that are vital for cell regeneration [5]. In order to fabricate scaffolds with such properties, a fabrication method
that maintains a high level of accuracy is necessary to maintain the consistency and repeatability in accordance to the initial design. Unlike the conventional fabrication techniques, solid freeform fabrication (SFF) has no restriction to on shape control and consistency. SFF are computerized fabrication techniques that can rapidly produce highly complex three-dimensional objects using data from CAD systems and computer medical imaging equipment such as MRI and CT Scans. The fabricated three-dimensional structures are built by reducing CAD designs of particular prototypes into a group of sliced 2-dimensional layers, to where the prototyping material is deposited to build the final structure in a layer-by-layer process. This makes SFF techniques very attractive for tissue engineering scaffold fabrication applications [6].

Hydrogels based on both natural and synthetic polymers have been of interest for encapsulation of cells and most recently such hydrogels have become especially attractive to the new field of tissue engineering as matrices [7, 8]. PCL has also proven to be a good candidate for scaffolds [9, 10]. The deposition of polymers can be controlled through various techniques for the fabrication process [11-16]. This paper presents three types of nozzle systems that can be used to deposit sodium alginate solutions and one to deposit PCL for the fabrication of three-dimensional structures. A comparison of the four systems is also presented, in addition to the operating parameters and performance of the systems.

2. System Configuration

We have developed a multi-nozzle biopolymer deposition system which is capable of extruding biopolymer solutions and living cells for freeform construction of 3D tissue scaffolds [16-18]. The deposition process is biocompatible and occurs at room temperature and low pressures to reduce damage to cells. Other SFF manufacturing methods utilize harsh solvents, high pressures or temperatures, or post-processing methods that are not suited for working with bioactive materials. By contrast, our system is capable of simultaneously, with the scaffold construction, depositing controlled amount of cells, growth factors, or other bioactive compounds with precise spatial position to form well-defined cell-seeded tissue constructs. This process may solve the problem of cell loading of preformed scaffolds which hitherto has been a significant barrier in tissue engineering.

An information pipeline of multi-nozzle biopolymer deposition system for freeform fabrication of tissue constructs is presented in Figure 1. As shown in the figure, the data processing system processes the designed scaffold model and converts it into a layered process tool path. The motion control system is driven by the layered manufacturing technique; the material delivery system consists of multiple nozzles with different types and sizes, thus enabling the deposition of specified hydrogels with different viscosities for constructing 3D tissue scaffolds. Four types of the nozzles are used in the system: solenoid-actuated nozzles, piezoelectric glass capillary nozzles, pneumatic syringe nozzles, and spray nozzles, with size ranges varying from 30 µm to 500 µm. The system can continuously extrude hydrogel gels, or form hydrogels in single droplets with picoliter volumes. The multiple nozzle capability allows us to simultaneously deposit cells, growth factors, and scaffold materials, thus enabling the construction of heterogeneous scaffolds with bioactive compounds, or establishing functional gradient scaffolds with different mechanical/structural properties in different scaffold regions.
The multi-nozzle biopolymer deposition system consists of four different micro-nozzles: pneumatic microvalve, piezoelectric nozzle, solenoid valve, and precision extrusion deposition (PED) nozzle, as shown in Figure 2. A material delivery system was assembled to supply the nozzles with the appropriate biopolymer. The system consisted of an air pressure supply both positive and negative, a material container or reservoir, and a material delivery tube. Each nozzle system had its independent parameters adjusted as required such as the air pressure and biopolymer concentration.
As one example, Figure 3 shows a schematic diagram of pneumatic microvalve system. Pneumatic microvalve is a typical mechanical valve that opens and closes the valve via an applied air pressure and is regulated by a controller. The system could work in extrusion or droplet mode. In extrusion mode, the controller applies pressure to the open the valve by lifting the piston against the spring that lifts the needle from the needle seat. The biopolymer material is then extruded out of the nozzle tip under an applied pressure that is adjusted through the material delivery system. The extrusion is ended when the controller shuts the valve by placing the needle back to the needle seat. The pneumatic valve could perform in droplet mode by repeating the continuous mode in a cyclic manner. Multiple pneumatic valves were simultaneously operated for performing heterogeneous deposition in the development of the 3D alginate scaffolds.

<table>
<thead>
<tr>
<th>Piston</th>
<th>Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>Biopolymer</td>
</tr>
<tr>
<td>Nozzle Adapter</td>
<td>Needle</td>
</tr>
<tr>
<td>Nozzle Tip</td>
<td></td>
</tr>
</tbody>
</table>

In general, there are two modes of biopolymer deposition; the extrusion mode and the droplet mode. In the extrusion mode, the material is extruded out of the nozzle tip under an applied pressure. This mode can basically lay down the material in the form of line structures to create the desired model by moving the nozzle tip over a substrate in the designed path. This process can be repeated layer by layer to develop a freeform fabricated part. In the droplet mode, the material is deposited in the form of droplets that is controlled by using a frequency function and key parameters in the nozzle system settings. The droplet mode can form a structured layer by depositing multiple droplets at desired locations on a substrate. Similarly, this process can be repeated to fabricate a 3D structure. Figure 4 presents a schematic diagram of the extrusion and droplet mode for the deposition.

<table>
<thead>
<tr>
<th>Air Tank</th>
<th>Material Pressure Vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Sodium Alginate Aqueous Solution</td>
</tr>
<tr>
<td>100 ml Glass Bottle</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4: Biopolymer deposition: (a) extrusion mode, (b) droplet mode.
Each nozzle system is unique in its method of operation, which makes each system have its advantages and limitations over the others. All nozzle systems have a material delivery system; however, the detailed set-up for each system is different and the material delivery system parameters such as air pressure are not controlled by in-house software. Characteristics and comparison of the four nozzle systems with their advantages and limitations is shown in Table 1.

Table 1: Characteristics and comparison of the four nozzle systems

<table>
<thead>
<tr>
<th>Features</th>
<th>Microvalve Nozzle System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pressurized Mini Extruder</td>
</tr>
<tr>
<td>Deposition Mode</td>
<td>Continuous</td>
</tr>
<tr>
<td>Operation/Control</td>
<td>Rotating screw gear via motor</td>
</tr>
<tr>
<td>Key Process Parameters</td>
<td>Pressure and Speed</td>
</tr>
<tr>
<td>Temperature</td>
<td>Temperature</td>
</tr>
<tr>
<td>Material</td>
<td>Material</td>
</tr>
<tr>
<td>Nozzle diameter</td>
<td>Nozzle diameter</td>
</tr>
<tr>
<td>Deposition speed</td>
<td>Deposition speed</td>
</tr>
<tr>
<td>Operating Range limitations</td>
<td>Screw speed &lt; 1rps</td>
</tr>
<tr>
<td>Temperature &lt; 150°C</td>
<td>H: (1-1200 Hz)</td>
</tr>
<tr>
<td>D: 7 ~ 10 mil</td>
<td>D: (30, 50, 70 µm)</td>
</tr>
<tr>
<td>Structure Formation</td>
<td>Physical solidification</td>
</tr>
<tr>
<td>Formation</td>
<td>Chemical reaction</td>
</tr>
<tr>
<td>Advantages</td>
<td>Fast solidification</td>
</tr>
<tr>
<td></td>
<td>No solvents</td>
</tr>
<tr>
<td></td>
<td>Strong structure</td>
</tr>
<tr>
<td></td>
<td>Sterile environment</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Temperature</td>
</tr>
<tr>
<td></td>
<td>Low melting material</td>
</tr>
</tbody>
</table>

3. Preliminary Results

3.1 Preliminary data on biopolymer deposition

We have conducted some preliminary studies on using the multi-nozzle biopolymer deposition system to construct 3D biopolymer based tissue scaffolds. For example, shown in Figure 5 are, several 3D hydrogel scaffolds (10 layers, calcium alginate), extruded as a 3% (w/v) alginate filament within a cross-linking solution (Figure 5a) and simple alginate geometrical pattern (Figure 5b). Depending upon the size of the syringe nozzle, the pressures used, and the type of deposition method (extrusion), we were able to create alginate filaments within the 30-40 micron range (Figure 5c) for 3% (w/v) sodium alginate solution with a 5% (w/v) calcium chloride cross-linking solution, at 0.50 psi.
The deposition flow rates were calculated by weighing the solution mass on a balance at a time interval to obtain the mass flow rate, which was then divided by the solution density. Some of the results are shown in Figures 6 through 8.

![Figure 5: Preliminary data for alginate hydrogel deposition](image)

![Figure 6: Pressure versus flow rate for 3% (w/v) sodium alginate solution using the pneumatic nozzle system.](image)

![Figure 7: Frequency vs. flow rate for 0.4 % (w/v) sodium alginate aqueous solution with 50 microns nozzle diameter using the piezoelectric nozzle system](image)
Figure 8: Frequency vs. flow rate for 0.4 % (w/v) sodium alginate aqueous solution at 3 mills using the solenoid nozzle system.

3.2 Multi-nozzle heterogeneous printing

Feasibility of printing different materials through multi-nozzle heterogeneous are demonstrated in Figure 9 to Figure 11. For example, the materials being simultaneously deposited in Figure 9 contain different alginate solutions at concentrations in the range of 0.1% - 0.4% (w/v), with the yellow material also containing microspheres.

Figure 9: Multi-nozzle, heterogeneous material deposition

Example of deposition geometry with complex pattern is presented in following figure:

Figure 10: Deposition pattern with complex geometry
In addition, we have experimented with creating a hybrid PCL/alginate scaffold. In doing so, we deposited PCL first, and then precisely depositing an alginate solution into the PCL scaffold as an alternative method for creating hybrid scaffold structures. Alginate was deposited into the desired pores on the scaffold (Figure 11a) or deposited in a simple fractal branching pattern (Figure 11b). [Note: the spreading of color is due to the diffusion of the water-soluble dye].

![Figure 11a, b: Construction of hybrid PCL/alginate scaffold](image)

### 3.3 Construction of 3-dimensional structures

The pneumatic microvalve nozzle was used to construct 3-dimensional (3D) tissue scaffolds. The diameter of the nozzle tip was 250 µm and the velocity and pressure were 10 mm/s and 16 psi. Experiments were set to investigate the effect of the Early Deposition Termination (EDT) on the geometrical formation of the 3D scaffolds. The first EDT tested was at 0 mm/s, which means that the valve was closed at the very end of the formation of every straight line. The results were analyzed using an optical digital camera that viewed that excess material was deposited at the curved line structures at the edge perimeter of each layer. It was observed that the diameter of the curved sections (310 µm) were much larger than the straight lines (270 µm) shown in Figure 12a. As a result, the 3D alginate structure was thicker around the edges and caused concaving of the scaffold after 20 layers as shown in Figure 12b.

![Figure 12: (a) Excess material deposition during closing of the valve in curved portions of the scaffold at 0 mm EDT; (b) Concave 3D alginate scaffold at 0 mm](image)
The second EDT tested was at 1 mm/s so that the valve would close 1 mm prior to the end point of the straight line formation. The image in Figure 13a reveals that the diameter of the curved portion of the alginate strand is almost equal to the straight strands. As a result, the 3D alginate structure is fabricated uniformly up to 40 layers without geometrical defects as shown in Figure 13b. Figure 14a and 14b shows the third and last EDT experiment for the valve close 2 mm prior to the end point of the straight line formation. It was observed through the images in Figure 14 that the lines formed were not placed as was originally designed and as a result constructed an irregularly shaped alginate 3D scaffold. This resulted from the relatively large EDT (2 mm/s) that caused dragging of the alginate strands every time the valve was closed. The EDT at this parameter forced the nozzle tip without any deposition for a particular distance. The alginate strand was attached to the nozzle tip because of the calcium chloride crosslinking solution during the time where the nozzle was traveling without any deposition. An analytical model for the EDT is currently under development.

Figure 13: (a) Moderate material deposition during closing of the valve in curved portions of the scaffold at 1 mm EDT; (b) Uniformly shaped 3D alginate scaffold at 1 mm

Figure 14: (a) Irregular material deposition placement of the scaffold at 2 mm EDT; (b) Irregularly shaped 3D alginate scaffold at 2 mm
The distance recorded between the struts was found to be 920 µm and the length of the square pore was found to be 650 µm for the scaffolds fabricated with EDT at 1 mm. The struts showed uniform geometry at a diameter of 270 µm as shown in Figure 16.

![Figure 16: (a) Close up image of pore for 3D alginate scaffold at 1 mm EDT; (b) Overall image of 3D alginate scaffold at 1 mm EDT](image)

3.4 Preliminary results on deposition of cell-hydrogel threads

Preliminary cell deposition/extrusion studies were conducted by extruding alginate hydrogel mixed with human endothelial cells (Figure 17) and fibroblast cells (Figure 18) at a cell concentration: 750,000 cells/ml with sodium alginate: 1.5% (w/v), nozzle: pneumatic 200 µm at pressure: 2 psi, deposition speed: 10 mm/s, and calcium chloride: 5% (w/v) (Figure 17). As shown in the figures, cells were able to be deposited with alginate solution to form cell-embedded hydrogel threads. The survivability of the cells after the deposition under the given process condition was averagely above 85% and the cells were cultured and proliferated in seven days after the deposition. Detailed experiments for the effect of the process parameters on the scaffold cellular biological behavior are currently conducted.

![Figure 17. Endothelial cell with alginate hydrogel](image)
4. Conclusion

A multi-nozzle system was integrated into a 3D motion system for solid freeform fabrication of tissue engineering applications. The free form solid freeform fabrication system also accommodated a material delivery system of various arrangements to accustom each nozzle system. The four nozzle systems were the pneumatic microvalve, piezoelectric nozzle, solenoid microvalve, and the PED. The former three were capable of depositing sodium alginate aqueous solutions of various viscosity ranges.

The pneumatic nozzle system is a robust system that is fairly simple to operate with few parameters to deal with. It can operate at relatively high pressures that allow it to deposit biopolymers with viscosities over 2000 cP. The piezoelectric nozzle system was capable of depositing flow rates as low as 0.001 µl/s in the form of microdroplets, which classifies it as the system with the lowest capable flow rate deposition out of the four. Precise deposition could be achieved using this system; however, there is an operating window in which the deposition could only occur in. This operating window can be extrapolated by changing the voltage timing profile, which needs to be adjusted for every new concentration of the same biopolymer. The solenoid microvalve could deposit sodium alginate at various parameters in droplet mode. Microdroplets cannot be formed below a certain pressure or frequency values for every concentration. The biopolymer depositions depend on the parameters that are adjusted for system. The flow rates could be controlled by these parameters and can therefore control the amount of material that is desired to be for a structure at specific locations given by the freeform fabrication design model.

On-going research is conducted to establish the process model and to investigate the effect of the process parameters on the polymer deposition and structure formation of 3D tissue scaffolds. Biological studies are also carrying on for understanding cell survivability under the deposition and the cellular behavior of cell proliferation, migration, and cell-tissue formation after the deposition.
Acknowledgement:
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References