Direct Ink Writing Extruders for Biomedical Applications

Mr. Tony Fan, Wayne State University
Dr. Gene Yeau-Jian Liao, Wayne State University

GENE LIAO is currently Director of the Electric-drive Vehicle Engineering and Alternative Energy Technology programs and Professor at Wayne State University. He received a M.S. in mechanical engineering from Columbia University, and a doctor of engineering from University of Michigan, Ann Arbor. He has over 17 years of industrial practices in the automotive sector prior to becoming a faculty member. Dr. Liao has research and teaching interests in the areas of hybrid vehicles, energy storage, and advanced manufacturing.

Prof. C. P. Yeh, Wayne State University

Chih-Ping Yeh received his BS in Electrical and Electronic Engineering from TamKung University in Taiwan, MS and Ph.D. in Electrical Engineering from Texas A&M University in College Station, Texas. He is the Chair of the Division of Engineering Technology at Wayne State University in Detroit, Michigan.

Dr. Jimmy Ching-Ming Chen, Wayne State University

Assistant Professor 2015-present Division of Engineering Technology Wayne State University Ph.D 2006 Texas A&M University
Direct Ink Writing Extruders for Biomedical Applications

Abstract

There are many 3D printing processes using various printing materials for different applications. Among these printing methods, robocasting or direct ink writing (DIW) is suitable and mostly adopted for biology and biomedical applications. DIW is an additive manufacturing technique in which a filament of 'ink' is extruded from a nozzle. The ink is usually supplied through a syringe or container and does not need to be heated to a high temperature to extrude through the nozzle for printing. Therefore, cells and bacteria can survive during the printing process. The ink must have high viscosity or be gel-like to maintain the sturdy structure for the printed object before post-processing. Several professional DIW printers designed for biomedical and medical research are available in the market such as EnvisionTEC 3D-Bioplotter, however they are usually extremely expensive. Collaborating with the medical school, this project will design and build new extruding systems on a low-cost RepRap machine. One RepRap Prusa i3 printer is modified able to extrude independently two different hydro-gels dedicated to the stem cell research. The modification is expected to utilize other 3D printing methods to create parts. This is a team's Capstone Design Project with students involved to promote and extend the applications of 3D printing. Student working processes of design, hardware modification, as well as testing procedures will be observed and recorded. The project activities, the testing results, and the students’ learning experiences and outcomes will be present in this paper. Student working processes of design, hardware modification, as well as programing procedures are observed and evaluated for systematic course material development.

Introduction

Equipment and materials of additive manufacturing (AM) technology were developed in the 1980s, however not until the early 2010s that AM turned popular and 3D printing (3DP) became a modern term used in popular vernacular to encompass a wider variety of additive manufacturing techniques, and often referring to desktop sized devices and rapid-prototype usages. There are many 3D printing processes using various printing materials for different applications, such as ABS and PLA thermoplastics for fused deposition modeling (FDM), photopolymers for stereolithography (SLA), and various metal alloy powers for selective laser melting (SLM) or selective laser sintering (SLS). Among these printing methods, robocasting or direct ink writing (DIW) is an additive manufacturing technique in which a filament of 'ink' is extruded from a nozzle. The ink is usually supplied through a syringe or container and does not
need to be heated to a high temperature to extrude through the nozzle for printing. Therefore, cells and bacteria can survive during the printing process, making DIW suitable and widely adopted for biology and biomedical applications.

The DIW or robocasting technique was first developed around 1996 as a method to allow geometrically complex ceramic green bodies to be produced by additive manufacturing\(^1\). A 3D CAD model is divided up into layers during the printing process similar to other additive manufacturing techniques. The ink is extruded from the nozzle in a liquid-like state and hardens quickly, exploiting the rheological property of shear thinning. In other words, the ink must have high viscosity or be gel-like to maintain the sturdy structure for the printed object before post-processes if necessary. Depending on the ink composition, printing speed and printing environment, DIW can typically deal with moderate overhangs and large spanning regions many times the filament diameter in length, with the structure unsupported from below\(^2\). DIW has many technical applications\(^3\), using colloidal, polymeric, or semiconductor materials to fabricate 3D periodic structures in sensors\(^4\), microfluidic networks\(^5\), photonic-bandgap materials\(^6\), tissue-engineering scaffolds\(^7\), and drug-delivery devices\(^8\). We are particularly interested in the DIW application on stem cell research using the hydrogel material, which is an extension of tissue engineering.

![Image of immunofluorescent staining](https://via.placeholder.com/150)

Figure 1. Immunofluorescent staining for MHC, MYL2, CD31, CD34, smMHC, and αSMA. The combination of the four factors, GHMT, induces abundant MHC and Myl2, and some expression of CD31 and smMHC 28 days after transduction. Nuclei were counter stained with DAPI\(^10\).

Stem cells are undifferentiated biological cells that can differentiate into specialized cells and
divide to produce more stem cells. Now stem cells can be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues able to be used in various medical regeneration therapies. Direct lineage reprogramming of one cell type to another to provide a reliable source of transplantable cells is an alternative to the differentiation of induced pluripotent stem cells (iPSCs). Recently the direct reprogramming of human dermal fibroblasts (HDFs) into cardiac progenitor cells (CPCs) using the QQ-protein and the techniques differentiating iPSCs into three cardiac lineages: cardiomyocytes, endothelial (vessel) cells, and smooth muscle cells by Wnt inhibition had been studied and represented⁹,10. Fluorescent immunostaining showed that cardiac markers (myosin heavy chain [MHC] and myosin light chain 2), endothelial cell markers (CD31 and CD34), smooth muscle cells (smooth muscle MHC [smMHC] and α-smooth muscle actin) were expressed in differentiated cells, as shown in Figure 1⁴,10. The cell differentiation pattern in the figure demonstrates a random distribution of the three lineages because the inhibition material was arbitrarily applied on iPSCs. If the locations of the inhibition material application can be guided and fixed, it will be possible to generate a segment of closed vessel tube structure surrounded by cardiomyocytes, which has great impacts to medical and surgical applications. The adoption of a dual extruder DIW 3D printer that can extrude two hydrogel (bioink) materials hosting both the iPSCs and the inhibition is then proposed. The task of the project is twofold, one is to study and explore a suitable hydrogel from many published research¹¹-¹⁴ that can host cells to live for several weeks while maintaining the physical dimensions, and the other is to build a reliable and low cost dual extruder DIW (robocasting) 3D printer, which is the main topic of this paper.

Figure 2. BioBot 1 (left) and 3D-Bioplotter (right).
Several professional DIW printers designed for biomedical and medical research are available in the market such as BioBot\textsuperscript{15} and EnvisionTEC 3D-Bioplotter\textsuperscript{16} shown in Figure 2, however they are usually extremely expensive. On the low cost printer side, Fab@Home\textsuperscript{17} was introduced as a multi-material DIW 3D printer and one of the first two open-source DIY 3D printers (the other one being the RepRap\textsuperscript{18}) led by students at Cornell University (Figure 3). The printer’s multiple syringe-based deposition method allowed for multi-material prints for direct fabrication of active batteries, actuators, and sensors; as well as esoteric materials for bioprinting and food printing. Although it allows a broad range of materials to be deposited, it is not convenient or popular for prototyping and other applications compared to printers depositing thermoplastics such as RepRap and most other consumer-scale 3D printers. In addition, Fab@Home did not emphasize self-duplication to promote itself and did not complete a controlling electronics family based on the current popular Arduino microcontroller. The project was closed in 2012 that the project’s goal for distributing and promoting DIY and consumer printers was achieved. It is currently difficult to obtain the parts and programs to build a Fab@Home machine and apply it to the stem cell research project aforementioned. This project designed and built new extruding systems to modify a low-cost RepRap Prusa i3 printer (Figure 3). The modification is expected to utilize other 3D printing methods to create parts. This is a team's Capstone Design Project with students involved to promote and extend the applications of 3D printing and apply it to biomedical engineering research.

Figure 3. Fab@Home (left) and RepRap Prusa i3 (right)

In this paper, a modified RepRap 3D printer with dual extruders performing DIW (robocasting) for course and student project development is demonstrated. The project is an application of instrumentation, mechatronics and CAD design that integrate a mount, actuators (stepper motors of the extruders) and the control unit (Arduino RUMBA). An undergraduate student (now in the
master program) was assigned to work part time (10 hours a week) on the hardware modification, coding, and testing. The working procedure and the time frame are recorded and evaluated for the development of curriculum. The project activities, the testing results, and the students’ learning experiences and outcomes will be present in this paper. The built platform will be used to develop teaching material for other 3D bioprinter functions in the future.

**Project Description**

The project is to achieve a single task depositing two kinds of hydrogels at the same time to build a ‘colorful’ 3D object by modifying the extruding system of a RepRap Prusa i3 3D printer. The modified printer is expected to possess the following features:
1. The hydrogels are loaded in syringes which can be easily mounted on the extruding system.
2. Rotational motions from stepper motors are converted into linear motion by some mechanical parts to push the syringes in order to extrude the hydrogels.
3. The modified printer can create 3D ‘colorful’ objects in which two different hydrogels embed in each other to form certain patterns according to the 3D models input.

The 3D bioprinter system has the following major components:
1. The RepRap Prusa i3 3D printer with the RUMBA controller.
2. An acrylic frame and syringe mounts as well as designed 3D printed thermoplastic parts.
3. Two Nema 17 bipolar stepper motors with holding torque 45 N·cm, 200 steps/rev, and rated current 2A with resistance 1.1ohms.
4. Metal parts including 608ZZ bearings, SCS8UU linear ball bearings, 5mm×8mm couplers, 8mm in diameter linear motion shafts, and 8mm lead screws with flange nuts, as shown in Figure 4.

**Design and Make the DIW Extruder Frame**
1. The acrylic frame
   A frame carrying the motors, the syringe pushing parts, and the syringe mounts was designed and made of transparent acrylic boards, for being consistent to the original printer’s frame. The frame parts were designed by using CAD software and cut by laser cutter. The CAD file of the design is show in Figure 5.

![Figure 5. The CAD design of the acrylic frame.](image)

2. 3D printed syringe pushing parts
   The original naïve design of the extrusion system was mounting the motors on top of the syringes, away from the x-axis shafts. However, it gave the entire extruder a center of mass that was too high, resulting in serious vibration during printing. Trying smaller and lighter motors did not improve the situation, in addition to the smaller motors not producing enough torque to push the syringes. Therefore, in the new design the stepper motors were placed low...
and close to the x-axis shafts to avoid large torque and thus vibrations during printing. On the other hand, the Prusa i3 is an x-z head arrangement for the extruder motion and has a gantry structure which limits the dimension of the extruder if the original designed printable space in the z direction is desired. It was apparent that the new low motor location was under the gantry structure, which reduced the already limited printable space in the z direction, therefore it was necessary to arrange the locations of the syringe pushing parts away from the gantry structure. The major piece of the syringe pushing parts is shown in Figure 6.

![Figure 6. The major piece of the syringe pushing parts.](image)

### Software

1. **Building ‘colorful’ 3D models for printing**

   STL (STereoLithography) is the most popular and widely used file format for rapid prototyping, 3D printing and computer-aided manufacturing which was originally for stereolithography created by 3D Systems. STL files describe only the surface geometry of a 3D object without any color, texture or other common CAD model attributes. New file formats were introduced to support for colors such as VRML and AMF. VRML stands for Virtual Reality Modeling Language (“verma”) supporting 3D printers with more than one extruder with plastics of different colors, or with full-color binder jetting technology, and AMF, Additive Manufacturing File (AMF) format, is an XML-based open standard supporting for color and able to be compressed to about half the size of a compressed STL. There are many software able to create and slice 3D models with multiple colors or textures in of VRML and AMF formats for printing, including 123D Design\(^\text{19}\) and Simplify3D\(^\text{20}\). Figure 7 demonstrates a simple dual colored 3D model for testing. However, STL files are so standard and widely used for 3D printing that the current popular method of printing a multiple colored 3D object is loading individual STL models of parts with different colors (two for most printers) of this object in popular 3D printing software such as Cura\(^\text{21}\) and
Repetier\textsuperscript{22} and using these software to control the extruders to deposit different materials for these individual models. We mainly used this model merging method in our testing.

![Figure 7. The testing model with two colors.](image)

2. Printing flow controlling parameters

Most of the consumer 3D printers including RepRap machines are designed for depositing thermoplastics, and thus the filament sizes are standard, 3mm and 1.75mm in diameter. Currently the latter is dominating the market. The default setting of all printing control/slicer software is mostly for the 1.75mm-sized filament. In addition, the popular nozzle size for thermoplastics ranges from 0.3mm to 0.5mm. Since our hydrogel is loaded in a 10ml syringe and extruded through a needle, the printing parameters of flow rate need to be modified to create the correct dimensions. Theoretically, the diameter parameter has to be changed to the syringe inner diameter, 12mm, while the nozzle size was changed to the used needle gauge 20 inner diameter, 0.6mm. The values were not fixed and needed to be further adjusted due to other factors such as the hydrogel viscosity.

**Experiment Results**

The modified DIW extrusion system is shown in Figure 8, and the printed testing model using lotion and toothpaste is shown in Figure 9. The extrusion system worked well, and currently the two major problems of this projects are (1) the hydrogel for the stem cell research is not ready and it depends on the work schedule of the medical school; (2) The two needles on the syringes are not usually level in height, so the lower one could scratch the printed material. The reason is the needle mounting position to the syringe is not fixed. We are planning to use a screw or knob to solve this problem.
Figure 8. The modified DIW extrusion system on a RepRap Prusa i3.

Figure 9. The printed testing model.

<table>
<thead>
<tr>
<th>Time</th>
<th>Work/Tasks</th>
</tr>
</thead>
<tbody>
<tr>
<td>June-July 2016</td>
<td>Meeting with people from medical school.</td>
</tr>
<tr>
<td>August 2016</td>
<td>Group meeting for the direction of the system design.</td>
</tr>
<tr>
<td>September 2016</td>
<td>CAD design and make for the first frame.</td>
</tr>
<tr>
<td>October 2016</td>
<td>Test for the first frame, and design and make for the second frame.</td>
</tr>
<tr>
<td>November 2016</td>
<td>3D printed the thermoplastic parts, and assembled the system</td>
</tr>
<tr>
<td>December 2016</td>
<td>Wired the electronics and fine-tuned the printer.</td>
</tr>
<tr>
<td>January 2016</td>
<td>Model building and test printing.</td>
</tr>
</tbody>
</table>

Table 1. Student’s work schedule record.
Student Learning Activities and Evaluation for Course Implementation

The objective of this project is to evaluate the work load and time frame of implementation of a similar or equivalent project on the topic of 3D printing systems in student senior project and final project of courses. We planned to recruit two to three students to work part time (10 hours a week) to evaluate the project, but only one undergraduate student (now in the Master’s program) completed the project. His work and activities are briefly summarized in Table 1. The major engineering learning and research activities are:

- **Task-oriented parts design using CAD software.** The student had to design the extruding system parts using the CAD software they learned in the prerequisites. To represent a working model satisfying the needs of bio-medical applications for medical school, he needed to communicate with medical school researchers. The student was required to take meeting minutes and collect designing ideas, and present them to all the engineering faculty after meeting with the researchers from medical school. The models designed by the student on the CAD software were reviewed by the faculty before physically making the parts. The student gained many design experiences in this back and forth process.

- **Physically making and assembling the frame parts.** The student used a laser cutter (assisted by our technician) to cut acrylic parts from boards. He also utilized another thermoplastic 3D printer to make parts with special 3D shapes. The cut and printed parts, motors (selected during design) and other hardware were assembled first, then the whole DIW extrusion system was mounted on the 3D printer. The extrusion functions were examined after the control board was connected. If the extrusion system could not accomplish the designed task the student had to start over from the CAD model design. In this procedure the parts and the assembled system were reviewed by faculty. The student obtained manufacturing and hands-on experiences preparing these parts.

- **3D printer control board wiring and fine-tuning.** The student had to connect the two extrusion stepper motors to the control board. Since it is a DIW printer, the extruder nozzle heaters and thermistors needed to be disabled via Arduino coding. The student had to demonstrate that he could manually control the printer extrusion system to move in x, y, and z directions and push both the syringes filled with gels. In the first design, the student adopted two stepper motors with linear shafts and mounted them high to push the syringes directly. Two problems occurred in this design: The motor mounting positions were so high that the whole extrusion system suffered serious vibration when printing, and the motors could not provide enough torque to push the high-viscosity gel in the syringes. The student had to start over and make the second design, presented in the previous pages. The student gained the ability to wire electronics and control machines, as well as system integration.
and troubleshooting/problem solving experiences in this process.

- “Two-colored” 3D model building and test printing. The student built simple dual colored 3D models and converted them into 3D printable formats. As introduced above, both color-supporting formats (such as VRML and AMF) and conventional single color STL format with two combined models were considered, and eventually the student chose the latter method to print. During test prints, the student had to modify printing parameters and adjust the syringe positions to print the object well. He had to demonstrate to the faculty that the printed object matched the designed 3D model. The student gained experience in controlling 3D printers and product development.

- The student was required to submit the work log, the CAD and STL files and other materials and records in the project folder online, and prepare for publication and oral presentation. Professional development experiences were obtained for the student during these works.

It took about seven months for one student to design the parts and modify the printer, including several weeks planning and discussion in the beginning. Although the time spent was more than a semester, we still concluded that it is a suitable project with proper work load to implement in a course or a student project on mechatronics and 3D printing in a single semester. The reasons are: (1) the student was also working on another project concurrently; (2) there were lengthy communications with the medical school for the collaboration and the hydrogel production was delayed; (3) there are many options for the modification and it turned out to be a trial and error process.

**Assessment**

The assessment methods of this project are:
A1. Ability of using CAD software
A2. Complexity and functionality of the mechanical parts design
A3. Ability of using 3D model building software
A5. Basic electronic knowledge: Motor, control board (Arduino), and wiring
A6. Ability of using 3D printing software and test the DIW system
A7. Project writing report and oral representation

This project is basically an implementation to the existing courses Instrumentation and Mechatronics as a term final project, and potentially can be extended as students’ senior project.
Thus, these assessment methods can be applied to the course objectives of these two courses:

<table>
<thead>
<tr>
<th>Course Learning Objective</th>
<th>Student Outcome</th>
<th>Assessment Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Perform and explain laboratory tests based on specified procedures of engineering systems</td>
<td>a, c</td>
<td>A6</td>
</tr>
<tr>
<td>3. Correlate results from experiments with predicted values</td>
<td>c</td>
<td>A2, A6</td>
</tr>
<tr>
<td>4. Analyze and interpret test data and write technical reports</td>
<td>c, g</td>
<td>A7</td>
</tr>
<tr>
<td>5. Work in teams and do oral presentation</td>
<td>e, g</td>
<td>A4, A7</td>
</tr>
<tr>
<td>6. Design and develop experiments and instrumentation using measurement techniques</td>
<td>a, d, f, M1</td>
<td>A1-A6</td>
</tr>
</tbody>
</table>

Where the student outcomes, in terms of the capabilities defined by ABET, are:

General engineering technology (Bachelor):

a. an ability to select and apply the knowledge, techniques, skills, and modern tools of the discipline to broadly-defined engineering technology activities;

b. an ability to conduct standard tests and measurements; to conduct, analyze, and interpret experiments; and to apply experimental results to improve processes;

d. an ability to design systems, components, or processes for broadly-defined engineering technology problems appropriate to program educational objectives;

e. an ability to function effectively as a member or leader on a technical team;

f. an ability to identify, analyze, and solve broadly-defined engineering technology problems;

g. an ability to apply written, oral, and graphical communication in both technical and non-technical environments; and an ability to identify and use appropriate technical literature;

Mechanical engineering technology:

a. (M1) geometric dimensioning and tolerancing; computer aided drafting and design; and a basic knowledge and familiarity with industry codes, specifications, and standards;

In addition, if students are from electrical engineering technology and bioengineering technology backgrounds, the corresponding outcomes are:

Electrical engineering technology:

c. (E1) the ability to analyze, design, and implement control systems, instrumentation systems, communications systems, computer systems, or power systems;

d. (E2) the ability to apply project management techniques to electrical/electronic(s) systems.

Bioengineering technology:

c. (B1) the ability to analyze, design, and implement bioengineering systems.
Conclusion

This project is designed for senior students who have taken courses such as Electrical Machines and Power Systems, Micro and Programmable Controllers, Design of Machine Elements, Computer-Aided Design and Manufacturing, and Control Systems as prerequisites. The major objective of this project is to evaluate the work load and time frame of implementation a similar or equivalent project on the topic of 3D printing systems in student senior project and final project of Instrumentation/Mechatronics courses. It took about seven months for one student to design the parts and modify the printer, including several weeks planning and discussion in the beginning. Although the time spent was more than a semester, we concluded that it is a suitable type of project with proper work load to implement in a course or a student project on mechatronics and 3D printing in a single semester. The assessment methods of this project cover mostly the course objectives of Instrumentation/Mechatronics, which contribute to student outcomes of general engineering technology for Bachelor degrees as well as mechanical, electrical, and bio-engineering technologies. In addition, students will benefit from hands-on practice and strategy analysis, and most important of all, prepare for a potential career in the future advanced manufacturing industry.

Acknowledgements

References

15. https://www.biobots.io/biobot-1/
22. https://www.repetier.com/