PROCEEDINGS OF SPIE

[SPIDigitalLibrary.org/conference-proceedings-of-spie](https://www.spiedigitallibrary.org/conference-proceedings-of-spie)

Development of a robotic 3D bioprinting and microfluidic pumping system for tissue and organ engineering

Kowther Kahin, Zainab Khan, Malak Albagami, Sadaf Usman, Sarah Bahnshal, et al.

Kowther Kahin, Zainab Khan, Malak Albagami, Sadaf Usman, Sarah Bahnshal, Hibatallah Alwazani, M. A. Majid, Sakandar Rauf, Charlotte Hauser, "Development of a robotic 3D bioprinting and microfluidic pumping system for tissue and organ engineering," Proc. SPIE 10875, Microfluidics, BioMEMS, and Medical Microsystems XVII, 108750Q (4 March 2019); doi: 10.1117/12.2507237

SPIE.

Event: SPIE BiOS, 2019, San Francisco, California, United States

Development of a Robotic 3D Bioprinting and Microfluidic Pumping System for Tissue and Organ Engineering

Kowther Kahin^{1,2 #}, Zainab Khan^{1,2 #}, Malak Albagami^{1,2}, Sadaf Usman^{1,2}, Sarah Bahanshal^{1,2}, Hibatallah Alwazani^{1,2}, M. A. Majid², Sakandar Rauf¹, & Charlotte Hauser^{1*}

¹ Department of Biological and Environmental Science and Engineering, King Abdullah University for Science and Technology, Thuwal, 23955- 6900, Saudi Arabia

² Department of Electrical & Computer Engineering, Effat University, Jeddah, 21478, Saudi Arabia

*Corresponding author: Charlotte A. E. Hauser (charlotte.hauser@kaust.edu.sa)

# Both authors contributed equally to this work.

ABSTRACT

The technology of 3D bioprinting has gained significant interest in biomedical engineering, regenerative medicine, and the pharmaceutical industry. Providing a new scope in tissue and organ printing, 3D bioprinters are becoming commercialized for biological processes. However, the current technology is costly, ranging from USD\$9,000-\$30,000 and is limited to customized extrusion methods. Multiple microfluidic pump systems for bioink extrusion are commercially available at USD\$30,000. Additionally, the use of Cartesian systems for 3D printing restricts the user to three axes of movement and makes multi-material modeling a challenge. Consequently, it was proposed to design a cost effective robotic 3D bioprinting system, compatible with peptide bioinks which were developed at KAUST Laboratory for Nanomedicine. The components of the system included a programmable robotic arm, an extruder for bioprinting, and multiple microfluidic pumps. The extruder was designed using a coaxial nozzle made of three inlets and one outlet. The programmable microfluidic pumps transported the peptide bioink, phosphate buffer saline (PBS) and human skin fibroblast cells (in cell culture media solution) through the nozzle to extrude a peptide nanogel thread. Model cell structures were printed and monitored for a period of two weeks and subsequently found to be alive and healthy. The system was kept well under a budget of USD\$3,500. Future modifications of the current system will include adding a custom bioprinting arm to allow multi-material printing which can fully integrate and synchronize between the pumps and the robotic arm. This system will allow the production of a more advanced robotic arm-based 3D bioprinting system in the future.

Keywords: 3D Bioprinting, Bioink, Hydrogel, Ultrashort Peptides, Syringe pumps, Tissue Fabrication

1. INTRODUCTION

3D printing is progressively allowing the rapid production of three-dimensional objects using plastic, nylon and metal materials. While this in itself may begin a manufacturing revolution, the recent development of bioprinters is even more promising. With these bioprinters, living tissues are constructed artificially in a layer-upon-layer formation of living cells^[1]. Over a period of several hours, an organic object is formed by the merging of these thin layers. As the cells are extruded, the bioprinter also extrudes a hydrogel to enable cell support and protection.

3D bioprinters have gained much attention in recent research due to their advantages over traditional lab-grown organ development as well as other factors. One printing machine is capable of printing multiple print models without transformation of any key mechanical or electrical parts of the printer. The biggest advantage of 3D bioprinting is customization and flexibility. It allows increased productivity and efficiency while speeding up medical processes such as organ transplants^[2].

3D bioprinters are designed with several features that set them apart from conventional 3D printers. These features incorporate the biological processes of cell growth and tissue fabrication^[3]. Some bioprinters are designed to print cell structures on petri dishes or molds. This makes it easier to handle the structures in post-printing processes^[4]. High precision motors are required to ensure accurate printing of structures according to custom specifications. Most importantly, bioprinting must be conducted in a sterile environment to avoid cell contamination. Some commercial 3D bioprinters released into the market include GeSim's Bioscaffolder, Inkredible+, and NovoGen MMX^[5].

Although they are mostly in the experimental phase, 3D bioprinters have the potential to revolutionize medical practice. Bioprinters dispense cells from a print head that moves in three dimensions to be able to place the cells in the exact position required^[6]. However, bioprinters may be constructed in various configurations. Organovo, for instance, is one of the top ten 3D bioprinters that uses a robotic arm and performs most of the functions that a linear 3D bioprinter can perform^[5]. Its compact size gives it the privilege to reach places more easily than linear bioprinters. Overall, the field of bioprinting is continuously advancing and new methodologies are being tested for additional features and better print quality.

Bioinks are pumped into the extruder either pneumatically or mechanically^[7]. Pneumatic-driven systems using air pressure allow for printing with several types of bioinks of various viscosities. However, these systems risk delays due to the volume of compressed gas. On the other hand, mechanical-driven systems using screws or pistons allow for better control of bioink flow rate. They are fragile, however, and can cause cell death if not designed properly^[3]. Different bioinks are made in the form of hydrogels, collagen, or gelatin to name a few^[8].

Self-assembling ultrashort peptides containing 3 to 7 amino acids have been demonstrated to serve as natural but synthetic 3D scaffolds which encapsulate $\geq 99.9\%$ of water and compete well with existing bioinks and hydrogels^[9-13]. This makes them a good candidate for many organ fabrication and tissue engineering applications^[10]. Their ability to take form and easily be modified chemically give them an advantage for 3D bioprinting^[11-13].

However, there are several factors that need to be taken into consideration as areas for improvement. Firstly, the cost of 3D bioprinters needs to be reduced for them to flourish in the market^[5]. Current commercial bioprinters range from USD\$9,000-\$30,000. Quality of printing is a major concern and current methods need to be enhanced to achieve better results^[14]. Better synchronization between different parts of a 3D bioprinting system would allow for a smoother user experience. There is also a potential for multi-material printing to enable printing of more complex structures^[15].

A recent breakthrough has been made in the development of a multi-material 3D bioprinter through continuous injection of different bioinks without the need of mechanical switching between bioinks^[16]. More development in such areas will allow 3D bioprinters to excel in the medicine industry.

This paper proposes a cost-effective multiple syringe pump system to reduce the total cost of a 3D bioprinter. Currently, commercial multi-syringe pumps such as the New Era Pump Systems can cost up to USD\$30,000. A screw-driven syringe pump system was developed, with capacity of up to three pumps containing different solutions. Unlike previous methodologies, the new syringe pumps were designed to be compatible with a 3D Bioprinting Robotic Arm to maximize efficiency, cost effectiveness, agility, and quality.

2. METHODOLOGY

The aim of this research was to develop a complete Robotic 3D Bioprinting system with integrated syringe pumps, at a fraction of the cost. This required a multi-phase process. Phase 1 entails the conversion of a conventional open-source robotic arm into a 3D bioprinter compatible with peptide bioinks. Phase 2, 3, and 4 cover the design and building of the multiple syringe pump system. Phase 5 covers the synchronization of the system with the 3D bioprinting robotic arm while Phase 6 explains the bioprinting process as a whole.

2.1 Conversion of Robotic Arm to 3D Bioprinter

The conversion of a conventional 3D printing robotic arm into a 3D bioprinter involved the removal of the nozzle and installation of an extruder that would be compatible with peptide bioinks. The following method was selected to achieve this. Firstly, the conventional extrusion unit was dismantled and removed from the robotic arm. This included the extruder head, the heating block, the extruder motor, and the filament feeder. The thermistors of the printing bed and extruder unit were not needed as the peptide bioinks were to be printed at room temperature without additional heating. A coaxial nozzle was designed to fit three inlets and one outlet (Fig. 1a).

An optimal design was achieved and consequently tested for leakage of bioink, pressure build-up, and nozzle clogging. The new nozzle allowed for the merging of peptide solutions and PBS buffer with cell solutions to form the peptide hydrogel. The nozzle was secured to the head of the robotic arm using a custom designed acrylic holder (Fig. 1b). The

three inlets of the nozzle were connected to the microfluidic tubing for the passage of biological solutions. The solutions were to be pumped into the tubing using the custom designed syringe pump system.

Fig. 1. (a) Coaxial Nozzle installed on Robotic Arm with custom designed holder. (b) Close-up of coaxial nozzle with three inlets and one outlet

2.2 Design of Multi Syringe Pump System

The process of building the syringe pump system required electrical and mechanical design. Different models and circuits were tested to reach the optimum design for the above-mentioned purposes. SolidWorks and OpenScad design software were used to model custom parts for the syringe pump. Test models of the designs were 3D printed with a conventional linear printer through Cura software. Accurate measurements were crucial in producing fully aligned parts.

Fig. 2(a) shows the SolidWorks^[17] assembly with all the designed parts of a single syringe pump model. The final prototype was rigorously tested for stable structure and smooth movement. Some parts of the final prototype were inspired by the open-source syringe pump library^[18]. Fig. 2(b) shows the final 3D printed prototype of the mechanical design.

Fig. 2. (a) 3D model assembly of single syringe pump in SolidWorks Design Software^[17] (b) 3D printed syringe pump assembled with Nema 17 motor and 1mL syringe

Likewise, the electrical circuit was designed and modified to be as efficient and cost-effective as possible. A Nema 17 stepper motor was operated by a motor driver. Arduino Mega 2560 was used to control the circuit. Step and direction pins of the driver were connected to the digital output of the microcontroller. Fig. 3 displays the circuit diagram of the triple syringe pump system. The code is uploaded onto the Arduino. The command was sent via a Graphical User Interface with three elements: the motor displacement, speed and direction.

Fig. 3. Circuit diagram of three syringe pumps system

2.3 Syringe Pump Resolution Calculations

The amount of volume that is needed to pump bioinks to the 3D bioprinter is around 10 - 60 $\mu\text{L}/\text{min}$. Calculations were done to determine the required volume. The parameters that were taken into consideration were the syringe diameter, resolution of the stepper motor and its driver, and displacement of the lead screw per rotation^[18].

The stepper motor used in the syringe pump prototype is a bipolar NEMA 17 motor. It provides 200 steps per revolution. Its motor driver has a sixteenth-step mode, which allows the motor to have 16 microsteps in one step. The lead screw used has a displacement of 1.22mm.

The total number of steps per revolution was found to be 3200 steps/rev. The lead screw displacement is taken into consideration to determine the overall resolution of the pump. The resolution, or the displacement per step, was found to be 380nm which is very suitable for the applications of the system. For a 1mL BD plastic syringe, the estimated volume extruded is 0.7 $\mu\text{L}/\text{step}$. This volume is sufficient for pumping bioink to the 3D bioprinter.

2.4 Syringe Pump Flow Rate Calculations

The flow rate of the syringe pumps was calculated to ensure that the liquid solutions emerge at a reasonable speed for the bioprinter. The process of calculating the flow rate consisted of collecting the extruded volume from the syringe over the period of one minute and weighing it with an analytical balance. The experiments were repeated thrice for each pump to confirm results. The next section discusses the results from these experiments. The mass of the extruded volume was measured in milligrams and used to calculate the flow rate by taking the density of water to be 1g/cm³. Because the

volume of water extruded is measured for a period of one minute, the value found is determined to be the flow rate of the pump at a particular speed in milliliters per minute.

2.5 Communication

The application requires a set of three syringe pumps operating simultaneously at different flow rates. The pumping system is synchronized with Arduino Mega 2560. The Accelstepper library enabled the speed and position control of the three pumps. The code was scripted to initialize three stepper motors each with separate variables: displacement, direction, and speed. The Accelstepper command “moveTo (Positions)” runs all three steppers at their denoted positions that is specified by the displacement variable in the positions array. The command “runSpeedToPosition()” runs the stepper motors at their designated speed.

The syringe pump circuit for all three pumps was connected in parallel to a 12V 20A DC power supply. The final circuit was printed on a Printed Circuit Board (PCB) as an Arduino Mega Shield (Fig. 4a). The layout was created using Altium Designer software. Pins for the components were soldered to the PCB after printing (Fig. 4b).

Fig. 4. (a) Printed Circuit Board (PCB) layout of triple syringe pump circuit. (b) Printed Circuit Board after soldering components

The final step to complete the system was the synchronization with the 3D bioprinting robotic arm. The system as a whole works as follows (Fig. 5). Gcode and STL files of 3D structures are sliced through Repetier slicer software. Print commands are sent to the robotic arm to begin movement. The Arduino IDE and MATLAB Graphical User Interface (GUI) send commands simultaneously to the syringe pumps to begin bioink extrusion. The pumps continue to pump bioink solutions into the extruder until the print is complete.

Fig. 5. The overall system, integrating syringe pumps with the customized 3D bioprinting robotic arm

The designed GUI offers a user-friendly interface to control the three syringe pumps in the integrated bioprinting system. The integration was achieved using MATLAB implementation. As MATLAB does not contain the libraries available in Arduino, nor does it have the option of speed control which is elemental in the program, both Arduino IDE and

MATLAB were used together to control the pumps in the most optimal way. This was done via serial communication, which allowed the system to benefit from both software platforms and improve its overall performance.

The MATLAB GUI features position and speed control for bioprinting applications. The GUI depends on two codes. The Arduino code is the main script responsible for the control of the pumps, while MATLAB runs the GUI code and sends commands serially to Arduino hardware. Fig. 6 shows a sample of the GUI.

Fig. 6. MATLAB Graphical User Interface

2.6 The 3D Bioprinting Process

Once the different components of the system were designed, tested, and optimized, the system was arranged as a whole for the 3D bioprinting process. The process starts with 3D modeling of the desired cell structure using Solidworks, and exporting the model as an STL file. Then, the file is imported to Repetier, a slicer software, which sends the printing command to the bioprinter. At the same time, the syringe pump begins to operate, once a command is sent via the MATLAB GUI. This will push the bioink to the 3D bioprinter nozzle and with the movements of the extruder, the cell structure begins to form. The syringe pump system is loaded with three biological solutions of different viscosities – peptide solution, phosphate buffer, and skin cells in media. As the pumps begin to move, the three solutions mix at the nozzle and form a hydrogel at the tip of the extruder.

3. THE RESULTS

After completion of the design process, the system was ready to be tested. The three syringe pumps were operated at different speeds to measure their flow rates. The experiment was repeated three times for each pump to ensure accuracy. The slope was calculated and served to be the conversion coefficient in the code. The MATLAB GUI was used to run test commands at different motor speeds. The flow rate was observed, calculated, and tabulated. From the results obtained, the final prototype was able to reach 4 $\mu\text{l}/\text{min}$, which was sufficient for bioprinting experiments. This was a good advancement as the bioprinting application required a flow rate of 10-25 $\mu\text{l}/\text{min}$. Thus, the syringe pump final prototype served our application. Fig. 7 shows the increase in flow rate as the speed of the motor increases.

After achieving the desired flow rate for 3D bioprinting, the three syringe pumps were tested with the Robotic arm using bioink. This was done to determine the optimal flow rate for our bioprinter. The coaxial nozzle was loaded with the three biological solutions of different viscosities- peptide solution, buffer, and skin cells in media. The print command was sent through Repetier software and the syringe pumps were controlled through the MATLAB GUI. After multiple tests, the

optimum flow rates for the hydrogel to form were found to be 50 $\mu\text{l}/\text{min}$ for the peptide solution, 20 $\mu\text{l}/\text{min}$ for the phosphate buffer, and 10 $\mu\text{l}/\text{min}$ for the skin cells in media.

Fig. 7. Graphs showing flow rates of Pumps 1, 2, & 3 at different speeds

Once the optimal print settings and flow rates were set, a test ring structure with a height of 1 mm was bioprinted with human dermal fibroblast cells (Fig. 8a) and monitored for cell growth (Fig. 8b). A taller 3D construct of 10*10 mm was also bioprinted using human dermal fibroblast cells (Fig. 8c, d) and compared with the shorter ring structure for cell growth. The 3D printed construct had smooth walls and was a homogeneous mixture with a relatively stable structure. After 14 days of cell culturing (Fig. 9), the 3D bioprinted structures showed increased stiffness, indicating that the cells were growing and multiplying. The fluorescence microscopy of the skin fibroblast cells shows the state of the cells inside the constructs after printing (Fig. 8e-f). The microscopy images confirm that the cells are alive and growing. These results proved that the syringe pumps were pumping the solutions at the correct flow rates to allow for proper peptide hydrogel formation. The hydrogel was found to maintain its structure over time. Also, the coaxial nozzle allowed for smooth printing without any pressure buildup or blockage. Hence, the experiment was successful and the results obtained proved that the system was working as expected.

Fig. 8. (a) 3D bioprinted ring containing human skin fibroblast cells printed using peptide bioink, height of 1 mm (top view) (b) Fluorescence confocal microscopy images of human skin fibroblast cells after Day 14 of culture. (c) 3D bioprinted cylindrical structure printed using peptide bioink, height of 10 mm (front view) (d) 3D bioprinted cylindrical structure (top view) (e) Whole-mount tile scanning of the 3D bioprinted HDFn ring construct. (f) Bright-field and fluorescent green-tracker merge. Zoom in of picture e, showing HDFn cells distributed throughout the entire cylinder just after printing. (g) Maximum intensity projection of the fluorescent F-actin/DAPI stained HDFn in the printed cylinder after 14 days of culture. HDFn cells are stretched, connected among them and growth in all directions. Scale bars, 100 μ m. (h) Bright-field of picture g.

Fig 9. Representative fluorescence confocal microscopy images of human skin fibroblast cells bioprinted using peptide-based bioink (IVZK) (left panel). Fluorescence confocal microscopy 3D images of human skin fibroblast cells bioprinted using peptide-based b bioink (IVZK) (right panel). Cells were stained for F-actin (green) and nuclei (blue) for fluorescence microscopy studies. The printed structures using IVZK were cultured for 1, 3, 5, 7 and 14 days. Both 2D and 3D confocal microscopy images were taken at different locations on the same sample.

4. CONCLUSION

The proposed design was implemented successfully. An extruder was effectively designed to be compatible with the peptide based hydrogel and installed in a 3D bioprinting robotic arm for more enhanced printing. The core of the project, the syringe pump system, was successfully completed. The pumps were synchronized to run simultaneously during the printing process. The hydrogel extruded from the bioprinter withstood tests for structure and biological capabilities. The printed structures were able to continue the growth of cells without any discrepancies. Additionally, the total cost of the system was less than USD\$3,500, which is well under the prices of the commercial pumps in the market. All in all, the project met its goals and has great potential to contribute to the field of nanomedicine. This research has a large scope for future modifications and improvements. For faster and more advanced 3D bioprinting, the system can incorporate multiple robotic arms simultaneously. This would allow for multi-material printing which is essential for certain types of organ and tissue fabrication. The syringe pumps can be fully synchronized with the 3D bioprinter using one Graphical User Interface instead of multiple programs. A 3D scanning feature can be introduced in the system for a complete scan-to-print process.

ACKNOWLEDGEMENTS

The research reported in this publication was supported by funding from the King Abdullah University of Science and Technology (KAUST) and the KAUST Office of Sponsored Research under CRG 5. The authors also acknowledge the Seed Fund Grant awarded to the spin-off company PepPrint by the Innovation and Economic Development, KAUST. We would like to thank Dhakshinamoorthy Sundaramurthi for his support in confocal microscopy.

REFERENCES

- [1] V. Mironov, N. Reis, and B. Derby, "Bioprinting: A beginning," *Tissue Eng.*, 12(4), pp. 631–634, 2006.
- [2] Anand, Archit, Arun, Hemant, & Yuwei, "3D Printing - Biological Applications." Presentation, National University of Singapore, 2013.
- [3] Y. Ikada, "Challenges in Tissue Engineering," *Journal of the Royal Society Interface*, The Royal Society, 2006. Web. 4 Sept. 2016.
- [4] K. Hölzl, S. Lin, L. Tytgat, S. Van Vlierberghe, L. Gu, & A. Ovsianikov, "Bioink properties before, during and after 3D bioprinting," *Biofabrication*, 8(3), 1-5, 2016.
- [5] D. Sher, "The Top 15 Bioprinters. 3dprintingindustry.com," Retrieved 2 May 2018, from <https://3dprintingindustry.com/news/top-10-bioprinters-55699/>
- [6] F. Melchels, M. Domingos, T. Klein, J. Malda, P. Bartolo, and D. Hutmacher, "Additive Manufacturing of Tissues and Organs," *Prog. Polym. Sci.*, 37(8), pp. 1079–1104, 2012.
- [7] M. Nakamura, S. Iwanaga, C. Henmi, K. Arai, & Y. Nishiyama, "Biomatrices and biomaterials for future developments of bioprinting and biofabrication," *Bio-fabrication*, 2 (1), 2010.
- [8] X. Tao, J. I. Rodriguez-Devora, D. Reyna-Sarano, M. Bhuyan, L. Zhu, K. Wang, and Y. Yuan, "Principles of Bioprinting Technology," *Regenerative Medicine Applications in Organ Transplantation*, Elsevier, San Diego, CA, pp. 67–79, 2013.
- [9] S. Rauf, K. Kahin, S. Alshehri, S. Abdelrahman, H.H. Susapto, S.J. S. Asad, D. Sunderamoorthy, C.A.E. Hauser, In situ 3D bioprinting under physiological pH using ultrashort self-assembling peptides, (submitted), 2018.
- [10] W.Y. Seow, C.A. Hauser, "Short to ultrashort peptide nanogels for biomedical uses," *Materials Today*, 17, pp. 381-388, 2014.
- [11] E.C. Wu, S. Zhang, C.A. Hauser, "Self-assembling peptides as cell- interactive scaffolds," *Advanced Functional Materials*, 22, pp. 456- 468, 2012.
- [12] C.A.E. Hauser, R. Deng, A. Mishra, Y. Loo, U. Khoe, F. Zhuang, D.W. Cheong, A. Accardo, M.B. Sullivan, C. Riekel, J.Y. Ying, U.A. Hauser, "Natural tri- to hexapeptides self-assemble in water to amyloid β -type fiber aggregates by unexpected α -helical intermediate structures," *Proceedings of the National Academy of Sciences*, 108, pp. 1361-1366, 2011.
- [13] A. Mishra, Y. Loo, R. Deng, Y.J. Chuah, H.T. Hee, J.Y. Ying, C.A. Hauser, "Ultrasmall natural peptides self-assemble to strong temperature-resistant helical fibers in scaffolds suitable for tissue engineering," *Nano Today*, 6, pp. 232-239, 2011.
- [14] I. Ozbolat, and Y. Yu, "Bioprinting toward Organ Fabrication: Challenges and Future Trends," *IEEE Trans. Biomed. Eng.*, 60(3), pp. 691–699, 2013.
- [15] J. Chung, S. Naficy, Z. Yue, R. Kapsa, A. Quigley & S. Moulton, "Bio- ink Properties and Printability for Extrusion Printing Living Cells," *Biomaterials Science*, 1(7), 763-773, 2013.
- [16] S. Murphy, & A. Atala, "3D bioprinting of tissues and organs," *Nature Biotechnology*, 32(8), pp 773-785, 2014. <http://dx.doi.org/10.1038/nbt.2958>
- [17] 3D CAD Design Software. *Solidworks.com*. Retrieved 2 May 2018, from <https://www.solidworks.com/>
- [18] B. Wijnen, E. J. Hunt, G. C. Anzalone, & J. M. Pearce, "Open-Source Syringe Pump Library," *PLOS One*, 9(9), 1-5, 2014.