JMBE Journal of Microbiology & Biology Education

Inexpensive DIY Bioprinting in a Secondary School Setting

Leo Ming Pong Sun[a] and Andrew Ching-Yuet To[a]
[a]*Po Leung Kuk Laws Foundation College, Tseung Kwan O, Hong Kong*

KEYWORDS inexpensive, secondary school setting, 3D printing, bioprinting, bioprinter, bioink, alginate, algae, immobilized algae, structure-function relationship

INTRODUCTION

Recently, research in three-dimensional (3D) bioprinting has grown rapidly (1). Bioprinting allows custom printing of soft living tissue by harnessing the 3D printing technique (2). It has been shown clinically that bioprinting could help repair different types of human organs, including skin, bone, and ear (3). Bioprinting could also enhance drug discovery and allow development of new material (4, 5).

Given the great promise of bioprinting in future research and medicine, it is desirable to introduce bioprinting to secondary school students. Early exposure to bioprinting allows students to acknowledge the latest development of biotechnology, which might inspire them to become future professionals. The novelty and interdisciplinary nature of bioprinting technology makes it an attractive content for science, technology, engineering, and math (STEM) education. In addition, if bioprinting becomes available in secondary schools, cell-laden tissues could be produced on demand as teaching materials to facilitate the teaching of structure and function concepts in biology. However, unlike 3D printing of plastic, bioprinting is uncommon among secondary schools due to its high cost. A basic bioprinter might cost several thousand U.S. dollars, hindering the incorporation of bioprinting into secondary school STEM curricula (6). Researchers in the field have demonstrated ways to make do-it-yourself (DIY) bioprinters (7–9). However, those DIY bioprinters were mainly designed for research purposes, and the construction and operation of them requires uncommon reagents, professional equipment, and advanced engineering skills.

To enable exploration of bioprinting for concept clarification and experimentation in a secondary school setting, here we showcase how a Creality Ender 3 V2 3D printer can be converted into a bioprinter at low cost, using 3D-printed parts and readily available materials (Fig. 1). The DIY bioprinter prints by extruding bioink into a support medium layer-by-layer (10). The bioink contains living cells, culture medium, and sodium alginate, while the support medium contains calcium ions for solidifying the bioink and at the same time preventing the solidified bioink from collapsing during printing (10). We demonstrate how bioink and support medium could be prepared using reagents available in secondary schools. Teachers and students together used the bioprinter to print a coronary artery model and an algae-laden artificial leaf. The print quality of our bioprinter is sufficient for teachers to illustrate the rationale of bioprinting to students. Finally, the photosynthetic activity of the algae-laden artificial leaf was assessed using a hydrogen carbonate indicator, and this enabled students to observe the bioactivity of the bioprinted construct.

PROCEDURE

Modification of a commercial 3D printer into a bioprinter

The commercial 3D printer chosen to be modified in this work was a Creality Ender 3 V2 3D printer, which is currently one of the most popular low-cost 3D printers for hobbyists. As shown in Fig. 1, the 3D printer was converted into a bioprinter by using 3D-printed parts: a syringe, plastic tubes, bearing, screws, etc. The conversion was mainly done by replacing the original extruder and printhead with our bio-extruder and bio-printhead. The 3D files for our bio-extruder and bio-printhead are available online in STL format (see Appendix S1 in the supplemental material). The bio-printhead was of our own design, while the bio-extruder was designed by modifying an open-source paste extruder named Spritzstruder, designed by user kitingmare on Thingiverse (278905; licensed under the Creative Commons Attribution-Non Commercial 4.0 International open-source license). The detailed steps for assembling the bioprinter are described in Appendix S2.

Editor Dave J. Westenberg, Missouri University of Science and Technology
Address correspondence to Po Leung Kuk Laws Foundation College, Tseung Kwan O, Hong Kong. E-mail: tcy@plklfc.edu.hk.
The authors declare no conflict of interest.
Received: 2 August 2022, Accepted: 20 March 2023,
Published: 10 April 2023

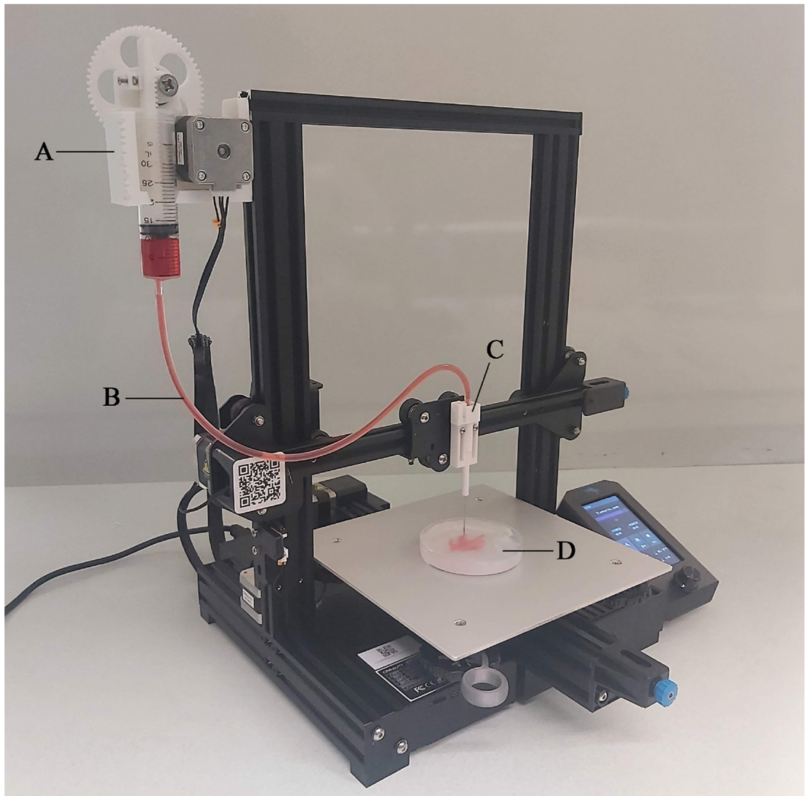

FIG 1. Components of the DIY bioprinter, converted from a Creality Ender 3 V2 3D printer. (A) A bio-extruder unit that is made up of 3D-printed parts, stepper motor, syringe, screws, and bearing. (B) A plastic tube connecting the bio-extruder and the bio-printhead. (C) The bio-printhead is made up of 3D-printed parts, 100-mm 22-gauge blunt-end needle and a luer lock adaptor. (D) Support medium on a petri dish for solidifying and supporting the bioprinted structure.

Preparation of bioink

To prepare for bioprinting, the syringe in the bio-extruder needs to be filled with bioink. To prepare 100 mL of bioink, dissolve 2 g of sodium alginate in 100 mL of cell culture to obtain bioink with 2% (wt/wt) sodium alginate. Sodium alginate can be polymerized into hydrogel when it encounters calcium ions in the support medium. In our work, we prepared an algae culture by referring to a previously described method (11). Cell culture could be replaced with colored water for testing or demonstration purposes.

Preparation of support medium

The biostructure is printed on a petri dish containing support medium. To prepare 600 mL of support medium, dissolve 4 g of agar in 400 mL of 11 mM $CaCl_2$ to give 1% (wt/wt) agar in $CaCl_2$. After solidification, add 100 mL 11 mM $CaCl_2$ solution to the agar and use a spatula to smash the agar into pieces. Then, transfer the agar solution mix to a blender and blend the mixture for 3 min to generate a Bingham plastic-like slurry. Put the slurry in a 4°C refrigerator and wait for a week to remove the air bubbles, as the presence of air bubbles could undermine print quality. If a centrifuge is available, one can quickly remove the bubbles by centrifuging the slurry at 8,000 rpm for 1 min. The prepared support medium can be stored at 4°C.

Hands-on bioprinting

To prepare 3D models for bioprinting, open-source models can be downloaded on websites like Thingiverse or NIH 3D print exchange. G-code can be generated using a slicing setting, as described in Appendix S3. The G-code could be stored in a micro-SD card and plugged into the bioprinter for printing. Before printing, the syringe in the bio-extruder is filled with bioink and a petri dish filled with support medium needs to be placed at the middle of the build plate. To ensure that the nozzle is completely filled with bioink before printing, one could press the plunger until seeing bioink coming out from the bio-printhead. After that, printing can be initiated. The printing time depends on the volume of the print. For example, printing the coronary artery, as shown in Fig. 2, requires 15 min. After printing, leave the petri dish overnight to ensure complete solidification. The 3D printed biostructure can then be isolated by washing away the support medium carefully in

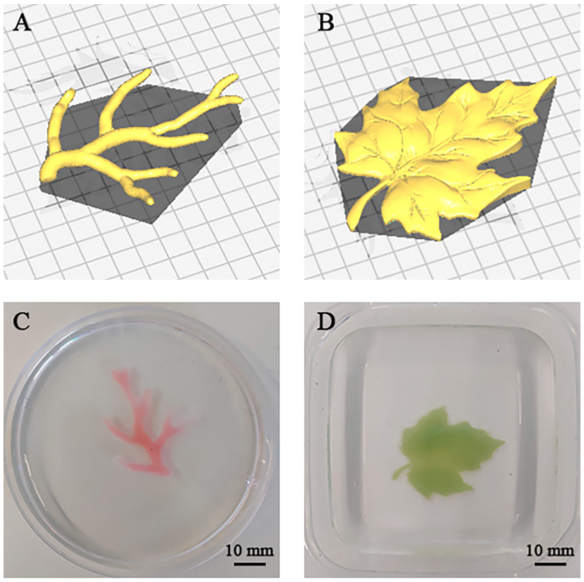

FIG 2. Two example constructs printed by the DIY bioprinter. 3D models (A and B) in STL format were used to bioprint a right coronary artery tree (C) and a maple leaf (D). Scale bar, 10 mm. The right coronary artery tree is a test print printed with 2% sodium alginate with red food coloring. The maple leaf was printed with algae culture with 2% sodium alginate.

running water. To obtain optimal print quality, see Appendix S4, which outlines questions and answers for troubleshooting.

Bioprinting an algae-laden artificial leaf and assessing its bioactivity

In this bioprinting activity, students downloaded a maple leaf model on Thingiverse (number 25938 by user MakerBot) and printed it with an algae-laden bioink, as shown in Fig. 2 (see Appendix S1 for the STL file). To assess the bioactivity of the bioprinted leaf, it is submerged in 30 mL of hydrogen carbonate indicator (see Appendix S5). Photosynthetic and respiratory activities can be demonstrated by color change of the indicator. Photosynthesis under bright light results in a net consumption of carbon dioxide and turns the indicator purple, while respiration in the absence of light releases carbon dioxide and turns the indicator yellow.

Safety issues

This work does not involve hazardous chemicals or pathogenic microorganisms. To eliminate the risk of overheating during printing, the original heater must be disconnected from the mainboard when assembling the bioprinter (see Appendix S2).

CONCLUSION

This work demonstrates how DIY bioprinting can be done in a secondary school setting by adapting an available 3D printer at low cost. We have showcased how cell-embedded biostructures can be printed with satisfactory quality and how their bioactivity can be observed by students to enhance curiosity, interest, and motivation. We believe that our work could stimulate innovative and inspirational teaching and learning attempts in secondary schools. Not only might this work stimulate the learning of bioprinting in secondary schools, but also it might stimulate creative attempts in enhancing the teaching of structure-function concepts in biology. It is recommended that educators further explore ways to integrate DIY bioprinters into structured curricula.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, DOCX file, 1 MB.

ACKNOWLEDGMENTS

No external funding was used for this work.

We declare that the work was conducted in the absence of any relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Daly AC, Prendergast ME, Hughes AJ, Burdick JA. 2021. Bioprinting for the biologist. Cell 184:18–32. https://doi.org/10.1016/j.cell.2020.12.002.
2. Mancha Sánchez E, Gómez-Blanco JC, López Nieto E, Casado JG, Macías-García A, Díaz Díez MA, Carrasco-Amador JP, Torrejón Martín D, Sánchez-Margallo FM, Pagador JB. 2020. Hydrogels for bioprinting: a systematic review of hydrogels synthesis, bioprinting parameters, and bioprinted structures behavior. Front Bioeng Biotechnol 8:776. https://doi.org/10.3389/fbioe.2020.00776.
3. Agarwal S, Saha S, Balla V, Pal A, Barui A, Bodhak S. 2020. Current developments in 3D bioprinting for tissue and organ regeneration: a review. Front Mech Eng 6:90. https://doi.org/10.3389/fmech.2020.589171.
4. Hong N, Yang GH, Lee J, Kim G. 2018. 3D bioprinting and its in vivo applications. J Biomed Mater Res B Appl Biomater 106:444–459. https://doi.org/10.1002/jbm.b.33826.
5. Balasubramanian S, Aubin-Tam ME, Meyer AS. 2019. 3D printing for the fabrication of biofilm-based functional living materials. ACS Synth Biol 8:1564–1567. https://doi.org/10.1021/acssynbio.9b00192.
6. Tong A, Pham QL, Abatemarco P, Mathew A, Gupta D, Iyer S, Voronov R. 2021. Review of low-cost 3D bioprinters: state of the market and observed future trends. SLAS Technol 26:333–366. https://doi.org/10.1177/24726303211020297.
7. Koch F, Thaden O, Tröndle K, Zengerle R, Zimmermann S, Koltay P. 2021. Open-source hybrid 3D-bioprinter for simultaneous

printing of thermoplastics and hydrogels. HardwareX 10:e00230. https://doi.org/10.1016/j.ohx.2021.e00230.
8. Engberg A, Stelzl C, Eriksson O, O'Callaghan P, Kreuger J. 2021. An open source extrusion bioprinter based on the E3D motion system and tool changer to enable FRESH and multimaterial bioprinting. Sci Rep 11:21547. https://doi.org/10.1038/s41598-021-00931-1.
9. Honiball JR, Pepper MS, Prinsloo E. 2021. Step-by-step assembly and testing of a low-cost bioprinting solution for research and educational purposes. MethodsX 8:101186. https://doi.org/10.1016/j.mex.2020.101186.
10. McCormack A, Highley CB, Leslie NR, Melchels FPW. 2020. 3D printing in suspension baths: keeping the promises of bioprinting afloat. Trends Biotechnol 38:584–593. https://doi.org/10.1016/j.tibtech.2019.12.020.
11. Mitra M, Broom SM, Pinto K, Wellons SD, Roberts AD. 2020. Engaging inexpensive hands-on activities using Chlamydomonas reinhardtii (a green micro-alga) beads to teach the interplay of photosynthesis and cellular respiration to K4-K16 Biology students. PeerJ 8:e9817. https://doi.org/10.7717/peerj.9817.