Toward Economical 3D Bioprinters for High School Science Laboratories

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Abstract

An economical benchtop 3D bioprinter with < $300 price point is being designed, built, and tested for ubiquitous deployment and use in high school science laboratories. This device enables students to 3D print structures from soft materials – including living cells – in Liquid-Like Solid (LLS) media to create artifacts of educational interest, particularly for biological sciences, that cannot be easily fabricated through conventional 3D printing or other means. To benchmark and validate the prototype bioprinter's functions, a calibration object is needed that illustrates key capabilities. The proposed 3D bioprinter calibration object, 3DJelly, takes < 30 minutes to print in LLS and includes the following critical bioprinting features: 1) joining of two parts, 2) capsular structure, 3) controlled feature spacing, 4) modulated layer thickness, 5) one structure encapsulated in another, 6) curved tubes, and 7) mid-print material change. These attributes were demonstrated by creating the 3DJelly bioprinting calibration object on an established bioprinter at the University of Florida.

Keywords

3D BioPrinter, Liquid-Like Solid (LLS), Soft Materials, K-12 STEM Pipeline, 3DJelly, Ship Of Theseus Design

Introduction & Background

3D bioprinting is a cutting-edge technology for fabricating from soft and organic materials structures not easily created through conventional 3D printing or other means.[1-3] 3D bioprinters have been used to create patient-specific neurosurgical models,[4] tumor models, and miniature functioning organs such as kidneys.[5] Much like how access to conventional filament deposition 3D printing revolutionized middle school[6] and high school STEM education,[6] it is anticipated that 3D bioprinting will similarly positively impact K-12 biology instruction. Implementing 3D bioprinters in high schools will benefit teachers by enabling benchtop fabrication of new hands-on teaching resources. STEM students will benefit through exposure to what today is a revolutionary new technique but, in the future, may be an essential skill for STEM field employment. Moreover, this tool will provide students real-world context for abstract engineering ideas, attracting and endearing them to STEM fields. Nationally, 57.3% of biological sciences bachelor's degrees were earned by women, but women only accounted for 38.6% of bachelor's degrees awarded in all STEM fields.[7] The range of new fabrication abilities, especially in the biological and health sciences, afforded by 3D bioprinters could attract more female high school students to STEM fields creating a pipeline for their selection of and participation in college STEM majors.

National Science Foundation funded S3ed corporation attempted to enter the secondary education market[8] with a $5,000 3D bioprinter,[9] but the firm has since gone dormant in this marketplace. Key factors for developing successful K-12 STEM curricula by transitioning technologies normally found at tech companies and universities to middle and high school labs are 1) relevance, 2) accessibility, 3) affordability of the teaching tool or method.[10-14] While universities and biomedical companies require advanced and expensive bioprinters, the capabilities needed for high school applications are much less complex; an opportunity to dramatically reduce cost. While a $5,000 tool is out of reach of most K-12 schools, a much more realistic 3D bioprinter entry point is $300 as evidenced by the wide adoption of the ~$200 Ender-3 3D printer in American classrooms. We show here that a viable 3D bioprinter can be built for less than $300.

This work emphasizes the importance of prioritizing hands-on learning tools for high school STEM education and introducing students to forward-looking fabrication tools that will 1) further their interest in STEM careers, 2) inspire STEM passion projects carried out both in class and on their own, and 3) provide opportunities to emulate open-ended and creative problem solving expected of STEM students in college.

Methods & Materials

Our approach to developing a 3D bioprinter that is 1) relevant, 2) accessible, and 3) affordable for high schools is tasking a high school student to lead its design, construction, and testing. We capitalize on the College Board's AP Capstone Diploma™ program where high school students complete college-level academic research in a field of their choosing and are assessed on their ability to apply critical thinking, research, and collaboration skills.[15] We use as an design inspiration custom 3D bioprinters developed for research at University of Florida (UF), which print soft structures into a Liquid-Like Solid (LLS) matrix. Optically clear LLS provides structural support for suspended features while enabling near-real-time imaging via confocal microscopy to observe and record printing in progress.[16]

Our design methodology is the Ship Of Theseus technique[17] used previously in architecture education,[18] mathematics research education,[19] and creating engineering education laboratory kits.[20,21] Ship Of Theseus proceeds by identifying essential product subsystem functions then purchasing Off-The-Shelf (OTS) components to satisfy each identified function. These OTS components are stitched together into a discordant "Frankenstein" product that functions but lacks subsystem synergy. As the overall product is tested, it becomes better understood, and each OTS component is then systematically replaced with a well-designed custom element until none of the discordant original OTS parts remain and the product's final form is revealed.

Figure 1 illustrates the "Frankenstein" 3D bioprinter already constructed; the original unmodified Ship Of Theseus. It is assumed that any high school deploying a 3D bioprinter would supply its own computer capable of running MS Windows-compatible software. This 3D bioprinter system is built around, an IQCrew STEM Science Discovery 40X-500X Inverted Microscope ($60 retail at time of writing). The microscope's optical objectives observe samples from underneath allowing users to visualize structures and cells being printed without the deposition tip blocking the visual field. A 5.0 MP digital camara (included with the microscope) replaces the scope's eyepiece while included driver software allows users to capture still and video images of the microscope's view field for near-real-time visualization, just like UF's 3D bioprinter.

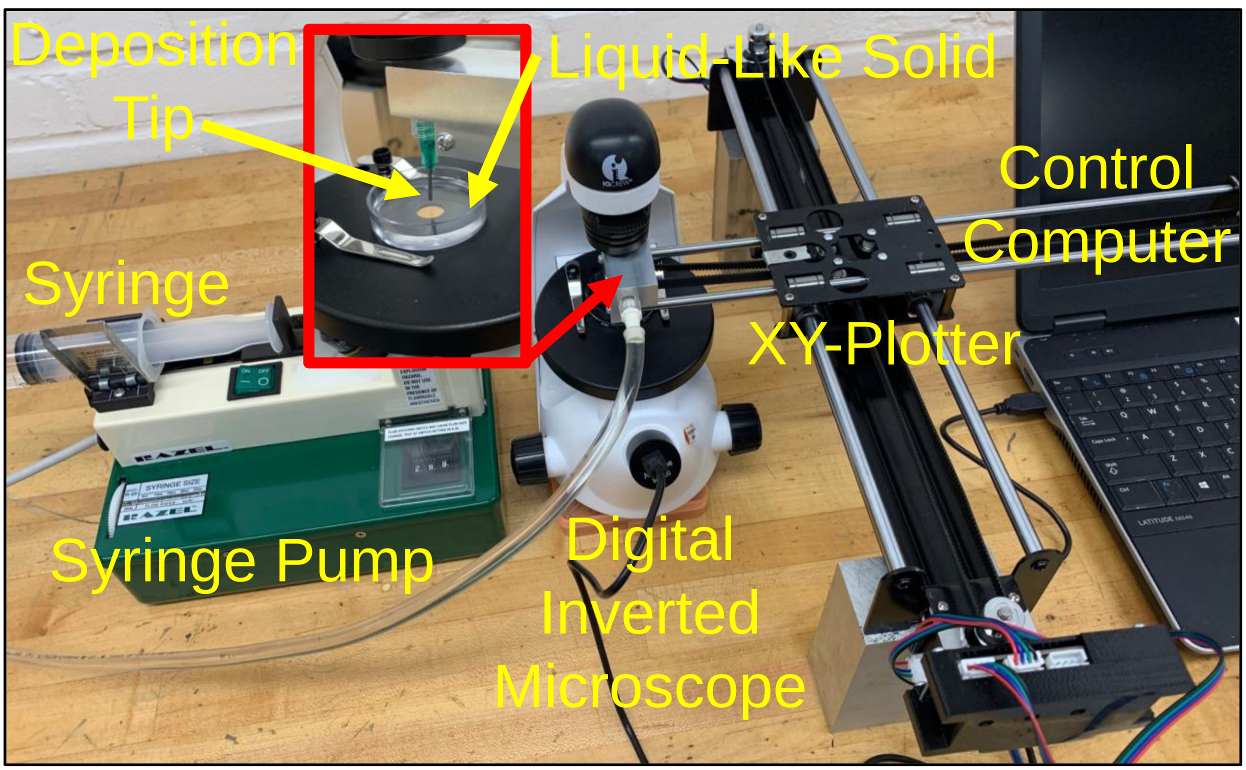

Figure 1. Version 1 of the < $300 3D bioprinter for high schools was designed following the Ship Of Theseus product development technique.

The next essential subsystem is a Vevor X-Y plotter ($120 retail at time of writing) which provides planer movement of the deposition tip. This plotter does not provide Z-axis motion, rending this first bioprinter iteration only capable of automatic plotting in two dimensions. The third spatial plotting dimension could be enabled by placing the plotter on a lab jack and changing deposition depth manually with each printed layer. Alternatively, an inexpensive conventional 3D printer like Ender-3 could have been adapted instead of an X-Y plotter to automate Z-axis motion. However, for a first Ship Of Theseus iteration the 2D plotter provides adequate framework to inspire later custom X-Y-Z motion design. Moreover, Vevor X-Y plotter runs on open source GRBL software platform, making it easy to convert drawings in MS PowerPoint into G-code toolpaths without need for 3D modeling or slicing software.

The plotter's pen-holding mechanism was replaced with a custom deposition tip holder part (see Figure 1 inset) that passes bio ink through a 1.3-cm-long hypodermic luer lock syringe needle to deposit print material. The deposition tip holder also contains two through holes connecting the part to the X-Y plotter and a threaded hole to mount the plotter's belt cog. While the part shown in Figure 1 was machined from aluminum, we also successfully tested an identical replica made by conventional 3D printing in PTEG (filament cost < $1). In future production-scale systems, needed biocompatibility for high school applications will determine ultimate material choice.

For deposition tips, a 120-piece pack of Brostown 1/2" (1.3 cm) dispenser needles with luer locks was purchased ($11 at time if writing) containing 14-, 15-, 18-, 20-, 21-, 22-, 23-, 25-, 27-, and 30-gage blunt tips. We will experiment in the future with the range of tip diameters, fluid volume flow rates, and deposition tip velocity through the LLS to identify the most promising combinations of these variables for high-quality bioprinting printing.

Bio ink forced through the deposition tip is deposited into an LLS-filled clear petri dish. As described elsewhere, LLS is like a collection of fluid-filled microbeads that behave like liquid water when sheared. Once bio ink is deposited, static LLS behaves like an amusement park ball pit, holding in suspension printed soft materials deposited therein.[22] A commonly available fluid with LLS microstructure is hand sanitizer. It can be found both in oil-based and water-based generic brands for about $2 per bottle.

The final essential hardware element is a syringe pump, which pushes bio ink out the deposition tip needle by compressing a conventional liquid-filled disposable plastic syringe. The syringe is connected via luer fittings and a flexible plastic tube to the deposition tip holder part described above. The syringe pump pictured in Figure 1 is a Razel Scientific R-99-EJ variable speed model borrowed from UF and outside the target price range. Once a viable syringe pump compression speed range was empirically determined, this item was replaced by a Razel Model A fixed speed syringe pump purchased from a surplus supplier ($25). This pump accommodates disposable plastic syringes ranging from 10 mL to 60 mL ($1 each). Bio ink volume flow rate from the deposition tip can be varied by swapping in different diameter syringes. Following the Ship Of Theseus iterative design approach, the syringe pump is the first subsystem that will be replaced with an inexpensive custom-built alternative possessing both variable speed and ability to be reversed. Reversibility allows bio ink and cells deposited in LLS to be later retrieved for additional assays and analyses. Multiple literature examples show researchers are building their own custom variable syringe pumps for less than $100.[23,24]

In addition to the major and minor component costs described above, we estimate an additional $15 spent on tubing, plastic fittings, and accompanying parts. The bio ink itself is a colloidal suspension which we use in such tiny volumes that its cost is trivial. Summing the component costs reveals the total price of materials for the described early prototype 3D bioprinter: $235. This cost is below the $300 threshold deemed viable for wide adoption of this 3D bioprinter as a successful commercial product in the middle and high school laboratory equipment marketplace. Moreover, through application of design customization, Design For Manufacturing, volume materials purchasing discounts, and other cost reducing innovations, the cost to create this product could be driven even lower! Lastly, this version can be modified to mate with any syringe pump, inverted microscope, or X-Y plotter a school may already have on hand. Thus, schools with common lab equipment on-hand would not need to buy all the 3D bioprinter pieces in order to build one. OTS material choices for this early 3D bioprinter iteration were driven cost, accessibility, and functionality. Following the Ship Of Theseus design approach, the final $300 3D bioprinter will contain an open-source X-Y-Z motion stage, deposition tip holder, and syringe pump that can be built by high school students using parts that are either 3D printed or standardized and easily accessible from an engineering supplier like McMaster-Carr.

Calibration & Testing

Yes, a viable prototype 3D bioprinter can be built for less than $300, but what are its capabilities, repeatability, and print quality? How are these performance metrics assessed? All the subsystem elements of the Version 1 prototype have been shown to work independently. The immediate next step is to print a pattern of horizontal bio ink lines in LLS on the stage of the IQCrew Inverted Microscope stage while imaging the process in real time to show all the elements function together as a viable bioprinter.

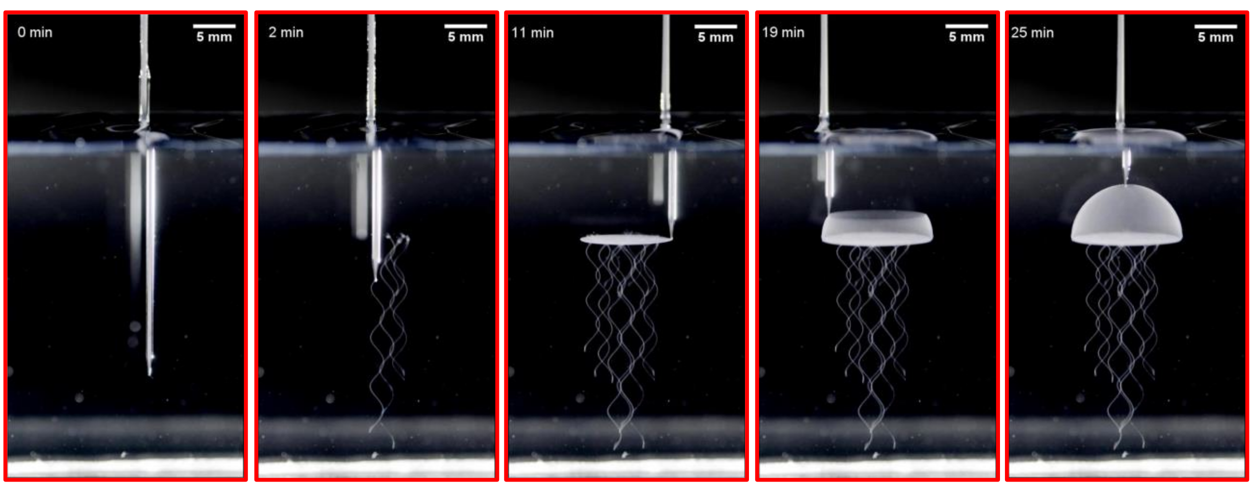

Figure 2. Time lapse printing of 3DJelly, a 3D bioprinting calibration object by UF.

To further test and evaluate the capabilities of any 3D bioprinter, a 3DBenchy equivalent for bioprinting is needed. 3DBenchy is a proprietary, copyrighted 3D computer model and calibration object designed to test the capabilities of 3D printers through difficult-to-print features.[25] For bioprinting, we propose use of 3DJelly, shown in Figure 2, which takes UF < 30 minutes to print in LLS and includes the following critical bioprinting capability features: 1) joining of two parts, 2) capsular structure, 3) controlled feature spacing, 4) modulated layer thickness, 5) one structure encapsulated in another, 6) curved tubes, and 7) mid-print material change. Using 3DJelly as a benchmark for the $300 bioprinter will demonstrate which essential functions it can perform and quantify how well these capabilities are executed. The $300 3D brioprinter's performance on the 3DJelly assessment will be presented in a future paper.

Conclusions & Next Steps

While our goal is to produce a 3D bioprinter for secondary educational institutions with as many capabilities as systems used in research universities or industry, at a minimum 3D bioprinters targeted for high school classrooms must be 1) relevant, 2) accessible, 3) affordable to be viable. The initial prototype $300 3D bioprinter described here will be evaluated at West Port High School by inviting teachers and students in STEM fields to propose projects for the 3D bioprinter that are beneficial in their classes. The first such project is already underway. A human vein replica made from clear soft PDMS material and capable of carrying artificial blood will be printed. This model will be used in the West Port High School phlebotomy course allowing students to practice needle skills with a vein easily visible below the clear artificial tissue surface before moving on to mannequins with hidden veins.

Analysis of the 3D bioprinter's utility will include both quantitative and qualitative measures consisting of a cost benefit analysis, a 10-point checklist of functionalities, and an end-user survey. To measure affordability, the engineering program's budget will benchmark and generate a cost-benefit analysis to determine its cost efficiency. The functionality of the $300 3D bioprinter will be measured by printing the 3DJelly benchmark object and observing its features relative to the same object created using a research-quality UF 3D bioprinter.

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