Development of a low-cost bioprinter based on a Kossel delta 3D printer platform

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Abstract

The core of bioprinting related research aims to reduce the gap between ex vivo cell cultures and in vivo cellular tissue models to further its application within the biomedical field. While additive manufacturing is touted as disruptive technology, bioprinter equipment costs exceed limited resource budgets of many research laboratories restricting the scope for further development for biomedical research and potential medical application. In line with this, a relatively low-cost bioprinter (SidneV1) was successfully designed and manufactured using a low-cost, commercially available FDM Delta 3D printer as a prototype base with a successfully custom designed and manufactured micro-extrusion printhead. Printing accuracies assessed were 65% (for width measurements) and 64% (for height measurements). This study aimed to demonstrate a way to achieve low-cost bioprinting and hopefully pave the way for future system modifications and refinements such that this technology becomes more accessible to under-funded research groups around the world. Although these findings are preliminary, further optimization of printing parameters, bioink formulations and sterilization techniques will allow for the engineering of viable, physiologically relevant tissue models using low-cost bioprinting technology.

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Keywords: 3D Printing, additive manufacturing, tissue engineering, bioprinting.

Specifications table

Hardware name	SidneV1 (SV1)
Subject area	Engineering and Material Science Biological Sciences (e.g., Microbiology and Biochemistry) Educational Tools and Open-Source Alternatives to Existing Infrastructure

Hardware name	SidneV1 (SV1)
Hardware type	Three-dimensional printer modifications Syringe extruder
Open-Source License	GPL
Cost of Hardware	**$ 1 007,52**
Source File Repository	https://doi.org/10.17605/OSF.IO/7TQKB

Introduction

Context

Three-dimensional *in vitro* human tissue models have the potential to act as models for disease which will not only reduce the use of animals as models for disease but may potentially increase turnover time associated with new drugs undergoing pharmaceutical trials [1]. The purchase and maintenance costs of commercially available bioprinters have meant this technology is inaccessible to most research labs whether in developed economies or developing countries [2]. There are, however, many facets of this cutting-edge technique (bioprinting) that are continuously being improved for its application, including the reduction in associated cost, design of bioinks, design of extruder systems and the ability to sustain post-printed viability of cells within the 3D structures [3,4]. General access to desktop & open-source additive manufacturing technologies has seen advancement in this field in recent years. Arguably, this has spurred the bioprinting revolution.

Extrusion based bioprinting has been largely focused on the manufacture of 3D tissue constructs or scaffold due to its many advantages over other methods, including, but not limited to; high cell viability [5], flexible geometric shapes [6], ability to incorporate multiple biomaterials and cell types [7], both homogenous and heterogeneous structures can be created [6,8]. In extrusion based bioprinting, the bioink is extruded out of a nozzle tip to form a continuous line structure driven by either pneumatic pressure or mechanical pistons. The extruded product is referred to as filaments instead of droplets [5,6,8]. A three-axis/cartesian automatic extrusion system is typically used in this type of bioprinting, equipped with a fluid dispensing nozzle [9]. This method has been extensively reported in many studies [10–16]. Most systems make use of standard plastic syringes which provide many benefits including; wide availability, low cost, aseptic, pyrogen free and compatibility with a range of needle sizes used as print nozzle heads [17]. Although the most widely employed method, it is not without its limitations. Most syringe-based extruders are designed to incorporate the fluid reservoir into the extruder carriage which is solely responsible for the high mass typically associated with this approach. A heavy extruder carriage can cause various issues during the printing process by affecting speed and resolution which may cause compromises in geometries of the printed constructs [12]. Reducing the volume of the fluid reservoir could provide a solution to the increased mass, however this will negatively impact the ability to print complex and larger constructs. Other larger volume systems have utilized the Bowden approach to minimize the weight of the extruder carriage. This approach makes use of Bowden tubing to connect the fluid reservoir (which is completely removed from the extruder carriage) to the nozzle. These systems are typically pneumatically driven which then brings in another separate set of limitations such as; poor retraction, the need for vacuum during printing and unstable extrusion pressures [12]. The ability of the printer to retract is important during the printing process as this prevents material from dripping during non-extruding moves which in turn reduces printing fidelity of the construct [12]. Pusch *et al.*(2018) have addressed the two major concerns of extruder carriage weight and inability to retract using the Large Volume Extruder (LVE) design. This design utilizes the Bowden tube approach with stepper motors and a lead screw driven extruder. The use of stepper motors is suggested to achieve retraction through straightforward reversal of direction and to apply constant pressure using a standard syringe [18].

The RepRapPro (RRP) Paste extruder utilized in this study also uses a Bowden tube approach along with a lead screw driven extruder system which makes use of stepper motors. As with the LVE approach, attaching the RRP paste extruder to the printer frame places all the weight on the printer frame and not the extruder

carriage. This adds minimal payload to printer movements and should in turn maximize the print speeds and reduce any vibration of the nozzle during printing which may lead to nozzle dripping.

Design Criteria

Overall, the bioprinting process has multiple facets to consider which have been described under one of the three pillars required for successful tissue engineering – hardware, wetware and software considerations [19]. The outcome should include low operational cost, ability to use a wide variety of materials, allow for fine deposition of materials with high viscosities, maintain high cell viabilities, reduced maturation time of printed constructs and finally, minimal handling of the constructs. Here, we discuss the criteria required for the hardware development process applied in this study. The overall design and development process outlined in this study is specifically for the printing of cell free scaffolds which then provides the necessary foundation for future cell-laden bioink bioprinting.

1.2.1 Choice of Printer & RepRapPro (RRP) Paste extruder

A commercially sourced, RepRap based Delta 3D printer kit was selected for modification to include a hydrogel paste extruder in the place of the original thermoplastic extruder. The high printing precision and accuracy expected of delta printers led to the choice for eventual cell-free scaffold bioprinting [20]. The ANYCUBIC Kossel Linear Plus Delta printer used in this project was based off the popular Kossel RepRap delta printer designed by Johann C. Rocholl. All documents and development kits for the Kossel Delta are available online [L1] with many components of the model made readily available from Thingiverse [L2] . This allows for successful modification to be made to the printer at relatively low-cost. The firmware supplied with the Delta printer is the open-source Marlin Firmware (originally developed by Scott Laheine, [L3]). This firmware allows for modifications allowing for paste extrusion with the Delta hardware which allows for a relatively quick transition from thermoplastic extrusion to paste extrusion.

Delta printers contain an effector plate where the extruder nozzle sits and moves around the build volume, but the extruder stepper motor is situated outside the printing space, attached to the frame. The extruder nozzle must therefore be lightweight and secured properly onto the effector plate to prevent unwanted movement. Suspending the extruder nozzle above the bed arguably eliminates any contamination from particles caused by friction during print moves. The dimensions of the extruder carriage and the mass allowed on the axis that holds the extruder therefore dictate the maximum dimensions of the hydrogel paste extruder.

Furthermore, the kit used in this study was selected because of the linear rail-based motion control allowing for increased XYZ precision required for bioprinting. The ball bearing design of the linear rail system allows for a more precise motion control compared to the linear rod systems, typically found in desktop 3D printers. There is a considerable reduction in binding occurrences (ball bearings getting caught on the rail during a movement) which contributes significantly to a much smoother printing movement and creates a jerk-free print. The stepper motors (NEMA17 with 1.8 step angle) used in conjunction with the linear rails also contribute significantly to the increased precision found in this system, where micro-stepping allows for more controlled moves.

With such a highly application specific technology such as bioprinting, the commercially available bioprinters tend to be available at a very high cost. This has forced many research groups to develop their own bioprinters based on their specific application requirements whilst also maintaining a low overall build cost. This also allows for the functionality of the printer to be expanded over time, as required. As in this case, where a bioprinter initially designed for cell-free bioprinting of scaffolds may be easily modified for future cell-laden bioink printing. In this study a low cost commercially available 3D printer was modified to accommodate for bioprinting with cell-free hydrogels. This provided for majority of the printer parts at a relatively low cost. All additional parts were either 3D printed using thermoplastics or purchased at a low cost. Although the linear rails are considerably more expensive than smooth rod systems, the increase in printing precision is imperative to successful bioprinting and so outweighs the potential overall increase in build cost.

Methods & Materials

Design description.

An Anycubic Kossel Linear Plus Delta 3D printer kit was selected for modification. Based on the criteria for bioprinting, both the hardware and software of the Delta printer was successfully modified to accommodate for tissue engineering purposes. The manufacture and assembly of the final delta bioprinter can be separated into three main parts; (1) the hardware modification of the 3D printer frame, (2) the replacement of the thermoplastic extruder for a cell-free hydrogel paste extruder, and (3) the software modifications for hydrogel paste extrusion. Majority of the added components were 3D printed – making it inexpensive and easily modified as necessary. A leadscrew driven hydrogel deposition concept is employed within the system and due to the modular nature of the extruder parts, the RRP system easily allows for adjustments regarding the use of different syringe diameters and volumes.

All 3D printed parts mentioned were printed using an ANYCUBIC Kossel Delta Linear Plus 3D printer following the same general printing parameters as listed: Layer height: 0.1 mm, Fill density: 100 %, Print speed: 80 mm/s, Printing temperature: 210 C, 100 % Flow, No support structures unless necessary and No platform adhesion. Poly-L-lactic acid (PLA) was used as the printing material (1.75 mm diameter eSUN PLA+ 3D filament). No additional post-print processing was required. The 3D modelling software SketchupMake2017 (v.17.2.2555) was used to design all printed parts (unless otherwise specified) and the slicing software Cura (v.15.04.2) used to prepare the design files for further 3D printing.

Bioprinter Frame (Hardware) modifications

Enclosure: The printer was enclosed using both printed and non-printed parts (Fig 1a). The side frames and mid-sections (later referred to as bioprinter frames, windows, and doors) were designed and files sent for manufacture to a Perspex laser cutting service (M&D Creations, Makhanda, South Africa) using 3 mm thickness clear Perspex sheets. The 3D printed parts consist of three separate corners.

Board box: The Anycubic Tri-gorilla controller board was moved from under the build plate (as per printer design) to outside the system; shown in Fig 1b. Wiring was adjusted to accommodate for the new board position. A board box was designed, and 3D printed to accommodate for the new location of the board. Furthermore, a few changes were made to the electronic board such that the hotbed power input cables and fans were removed.

Bed platform : A new bed platform was designed, and 3D printed, to hold two 220 mm diameter glass plates of 2 mm thickness (Fig 1b). The bed platform with the glass in place was designed to cover most of the base of the printer with a few openings to allow for air flow to reduce any pressure build-up.

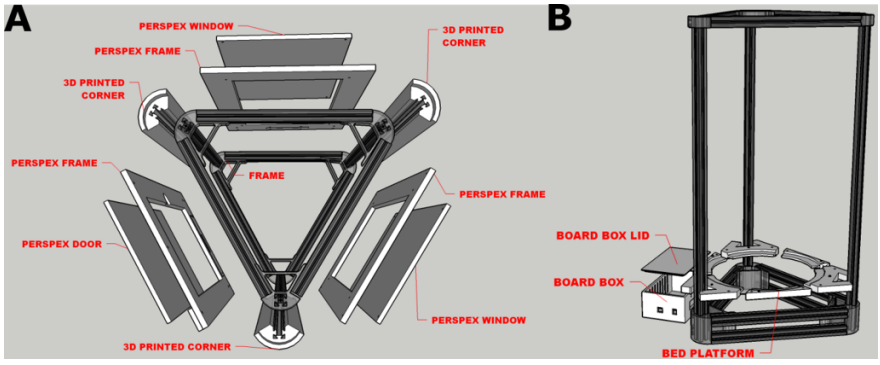

Fig. 1. CAD files used for encasing of the printer, repositioning of the electronic board and new printing platform.

Extruder Modifications

The syringe extruder was based off the RepRapPro Paste Extruder design (Licensed under the GPL; all files available from the RepRapPro Ltd Github repository at https://github.com/reprappro/Paste-extruder). The syringe-based extruder designed in this project is made up of printed parts, non-printed parts, and electronic components. Figure 2 below shows the full syringe-based extruder, including 6 parts printed directly from the RepRapPro Extruder design (available online). The part labelled "MOUNTING BRACKET" was custom designed and 3D printed. The extruder was assembled as per instructions given on the RepRapPro website[L1].

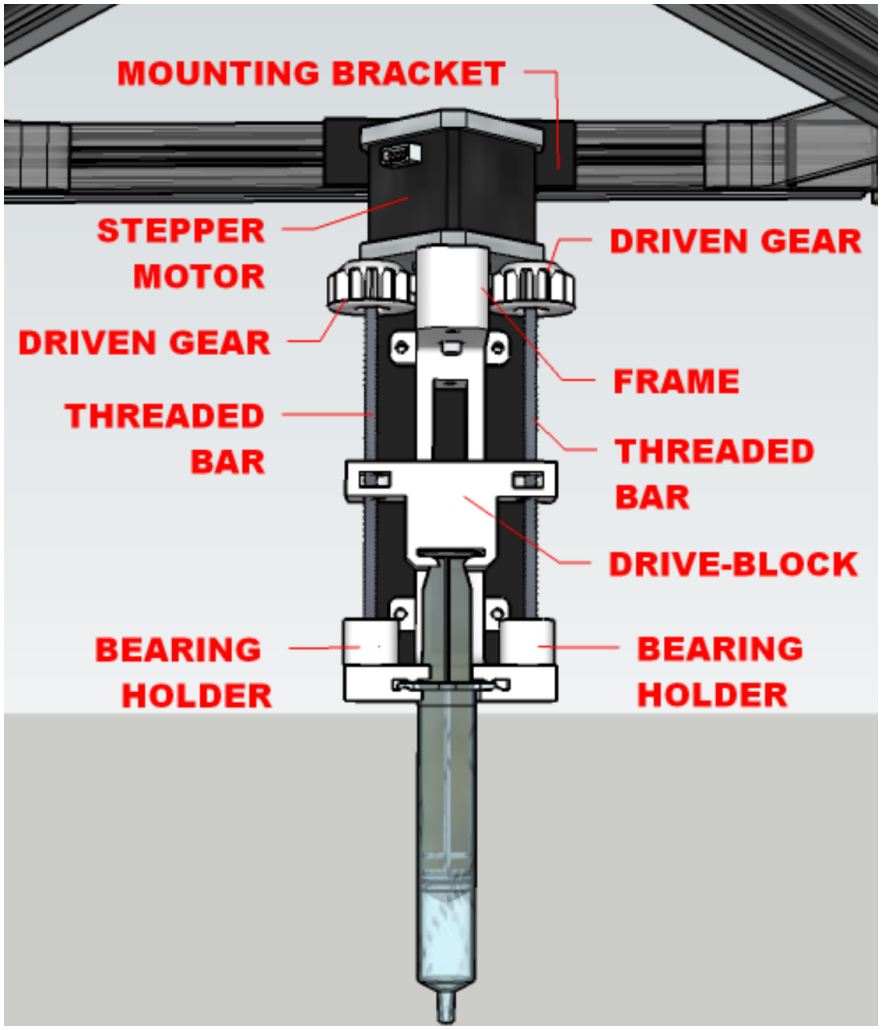

Fig. 2. Labelled CAD file of the syringe-based extruder model.

A Luer-lock effector plate adaptor was designed to attach to the original effector plate provided with the Delta printer and incorporates a male Luer-lock connection fitting and a Bowden tube connection fitting (Fig. 3).

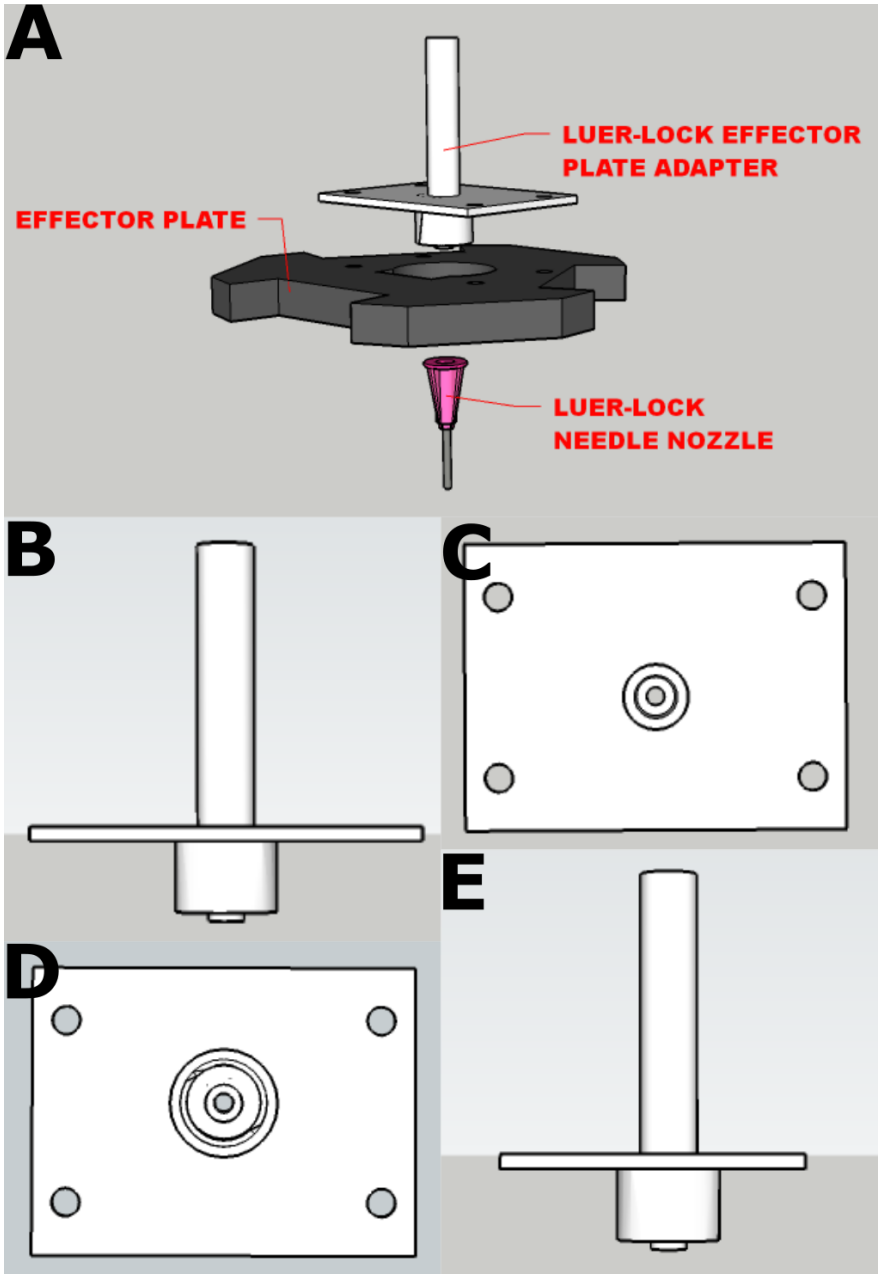

Fig. 3. Labelled CAD file of the Luer-lock effector plate adapter. A: Full assembly with effector plate and needle nozzle, B: Front view, C: Top view, D: Bottom view, E: Side view.

Marlin Firmware (Software) Modifications

Pronterface (printrun-2015031011 https://github.com/kliment/Printrun) host interface was used for controlling the printer X-, Y- and Z- movements as well as the extrusion motor. Default Pronterface settings were used at 250 000 baud rate. The firmware was modified to accommodate for hydrogel extrusion as described throughout the following sections.

Key strengths to the overall design approach

- The open-source nature of the project, including the use of the Anycubic Kossel Delta 3D printer and the GPL licensed RepRapPro Paste extruder allows for personal modifications at a low cost.
- The delta style 3D printer allows for a user-friendly modification process and requires few bigger modifications made to convert from thermo plastic to hydrogel printing.
- Making a few simple changes to the extruder and nozzle design will potentially allow for a multitude of different bioinks and cross-linkers to be used.
- This system is easily manufactured and more economically feasible compared to other commercially available bioprinters.

Design files

Bioprinter hardware design files

Design file name	Design file name	File type	Open source license	Location of the file
Enclosing components	Printed Corners	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7
	Frames	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7
	Window	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7
	Door	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7
	Handle	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7
	Glass Lid	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7
Electronic board	Box	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7
	Lid	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7
Bed platform	Platform supports	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7

Frames: The frames were manufactured in Perspex and secured onto the printer frame using M3 x 5 mm screws and T-nuts (Fig. 4).

Window: These are the mid-sections of the fully enclosed printer that attach to the Perspex frames using M3 x 8 mm screws and nuts (Fig. 4). These pieces were manufactured in Perspex.

Door and Handle: The door was manufactured in Perspex and attached to the one side of the printer frame using two hinges (Fig. 4). A handle was designed and added to the door for ease of use.

Printed corners: The corners were designed in such a way to allow sliding on to the aluminum extrusions without the need for screws (Fig. 5). The corners allowed for complete enclosure of the system (Fig. 5).

Glass lid: A glass lid was designed and manufactured in order to complete the full enclosure of the printer (Fig.6). Six M4 x 5 mm screws and six M4 T-nuts were used to secure the glass into place (two screws on each side). Three additional aluminum extrusions were added to the top of the printer (Fig. 6b) with 10 x 10 x 300 mm dimensions to create additional height and secured onto the existing extrusions using six 3D printed parts and twelve M4 x 5 mm screws and twelve M4 T-nuts (Fig.6). Nine 3D printed pieces were designed to fit into the aluminum extrusions and complete the finished look (Fig.6).

Electronic board box and lid: The Tri-gorilla electronic board was moved to outside the printer and the board box designed to house it. The board box consists of two parts: the box and the lid (Fig. 7). The electronic board was secured in place using two M3 x 10 mm screws and nuts (Fig. 7) and the box then secured onto the side of the printer using two M4 x 5 mm screws and T-nuts (Fig. 7).

Bed platform supports: The platform consists of six separate pieces; designed to hold two 220 mm diameter glass plates, with the top plate designed to hold a standard size petri dish (Fig. 7). The printed pieces were secured onto the printer's aluminum extrusions using two M3 x 25 mm cap-head screws and two M3 T-nuts per piece (Fig. 7).

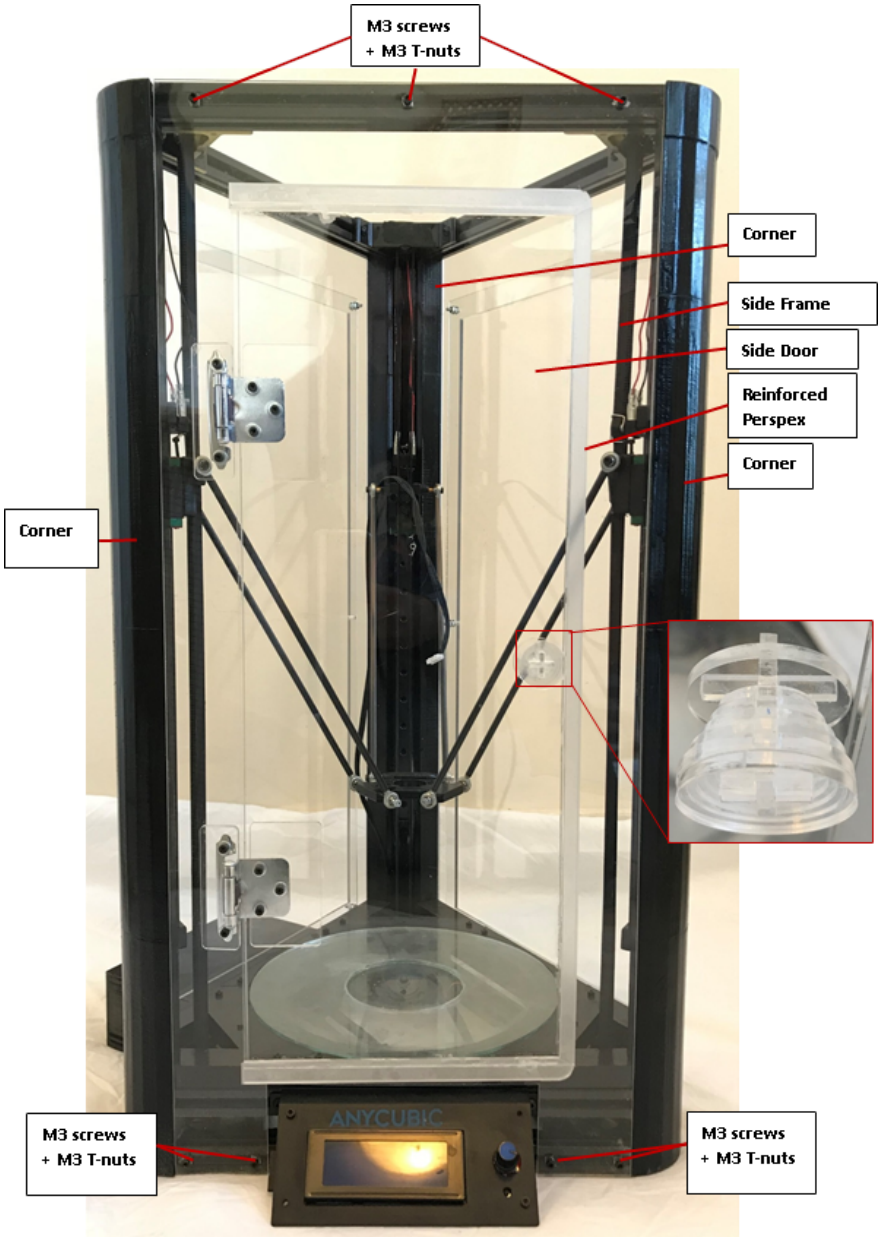

Fig. 4. Labelled image of fully assembled and enclosed Delta 3D printer. Door handle shown was assembled using Perspex.

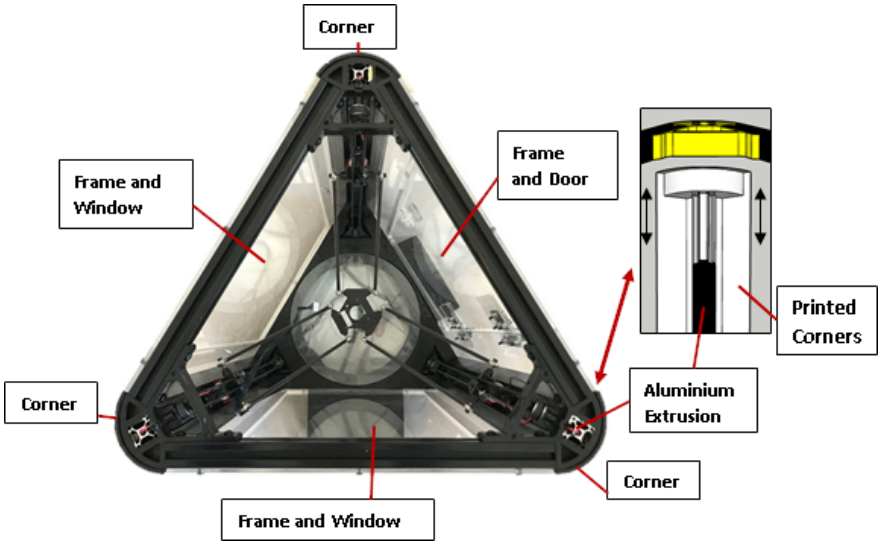

Fig. 5. Top view of fully assembled and enclosed Delta 3D printer. Insert highlighting how the corners were assembled onto the aluminum extrusions.

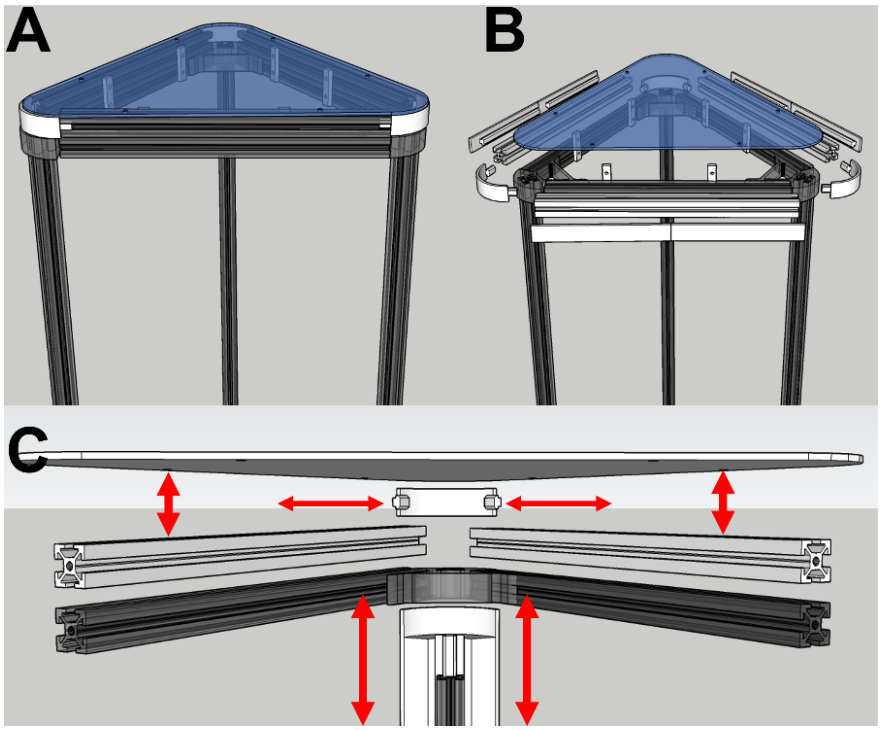

Fig. 6. CAD files of glass lid system designed to complete enclosure of the bioprinter. A: CAD file of all parts assembled (blue part indicating glass piece), B: Exploded image of all parts incorporated, including added aluminum extrusions and 3D printed parts, C: Method by which parts are assembled. All grey colored parts shown in the image indicate original parts.

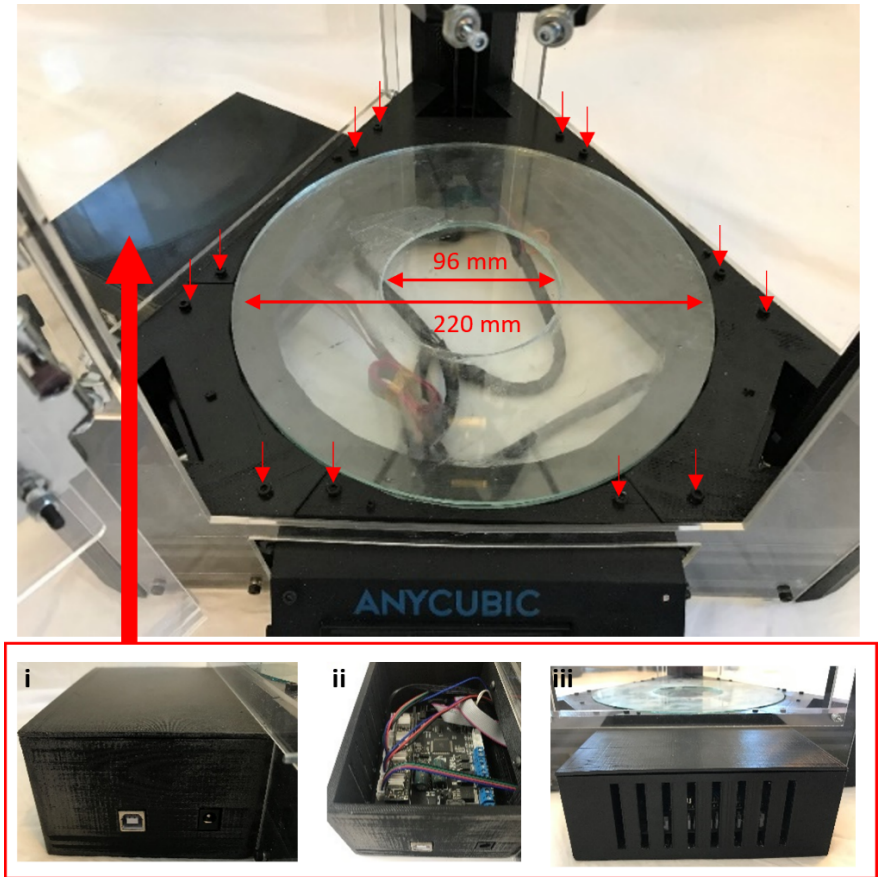

Fig. 7. Designed, manufactured, and assembled bed platform and electronic board box. Printed and assembled platform pieces holding two glass plates (vertical arrows indicating M3 x 25 mm cap-head screws used to secure pieces to aluminum extrusions. Larger red arrow showing newly positioned electronic board. The inset provided (i, ii, iii) shows the designed board box at various angles.

Extruder hardware design files

Design file name	File type	Open source license	Location of the file
Top strengthener	CAD & STL file	GPL	https://github.com/reprappro/Paste-extruder
Mounting bracket	CAD & STL file	GPL	https://github.com/reprappro/Paste-extruder
Driven gears	CAD & STL file	GPL	https://github.com/reprappro/Paste-extruder
Drive gear	CAD & STL file	GPL	https://github.com/reprappro/Paste-extruder
Frame	CAD & STL file	GPL	https://github.com/reprappro/Paste-extruder
Drive Block	CAD & STL file	GPL	https://github.com/reprappro/Paste-extruder
Bearing holder	CAD & STL file	GPL	https://github.com/reprappro/Paste-extruder
Luer lock effector plate adapter	CAD & STL file	LGPL	https://doi.org/10.17605/OSF.IO/7TQKB

Top strengthener: This component further secures the system in place, particularly the syringe. It is secured onto the frame using the same M3 x 35 mm screws used for attaching the bearing holders onto the frame. The top strengthener protects the syringe from moving, once secured into place for printing (Fig. 8b).

Mounting bracket: The mounting bracket is used to secure the fully assembled extruder onto the aluminum frames of the bioprinter (using M4 x 15 mm screws and T-nuts) (Fig. 8b).

Drive gear and driven gears: These components are used together with the NEMA17 stepper motor to rotate the threaded bars and therefore move the drive block (Fig. 8b). The drive gear mounts onto the NEMA17 motor shaft and is designed to be self-centering on the motor shaft. The driven gears are mounted onto adjacent corners of the motor face (meshing with the drive gear in the center) using two M2 nuts. The M5 x 95 mm threaded bars are secured in the center of the driven gears using two M5 nuts (Fig. 8b).

Frame: This is the main component of the extruder. The NEMA17 motor is secured onto the main printer frame along with the bearing holders and drive block. The design allows for a syringe to be secured in place whilst the plunger side is secured to the drive block (Fig. 8b).

Drive block: This component is secured to the end of the syringe plunger and is responsible for enabling actuation of the syringe plunger (extrusion and retraction). Two M5 x 95 mm threaded bars are used (one on either side of the drive block) which control the movement. These are secured into place using M5 nuts (Fig. 8b).

Bearing holders: The bearing holders are attached to the extruder frame and included to allow the rotation of the threaded bars which move the drive block (Fig. 8b). They are secured into place using 623 bearings, M3 nuts and M3 washers.

Luer lock effector plate adaptor: This component completes the extruder by connecting the mounted syringe extruder to the effector plate and nozzle (Fig. 8b,c). The design incorporates a male Luer-lock connection fitting and a Bowden tube connection fitting (Fig. 8c). It was secured onto the standard effector plate using four M3 x 8 mm screws and M3 nuts (Fig. 8c).

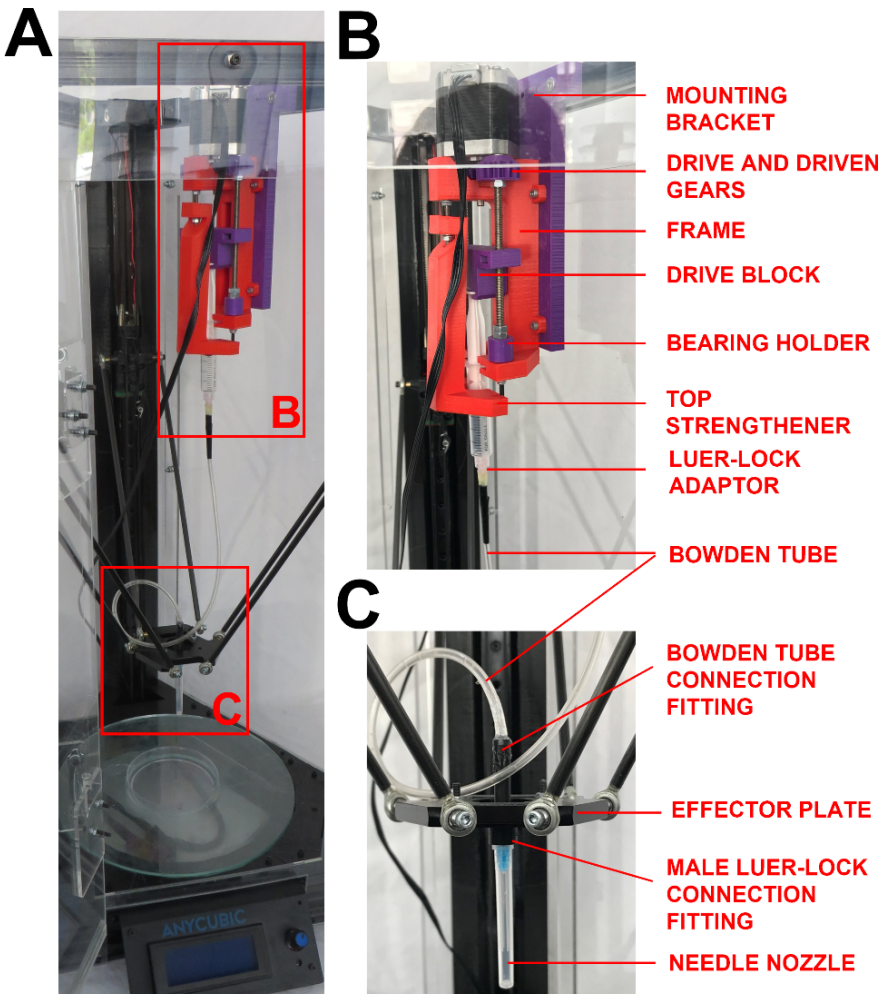

Fig. 8. Printed and assembled syringe-based extruder including the effector plate adaptor. A: Fully 3D printed and assembled syringe extruder highlighting the RepRapPro Syringe extruder (B) and the Luer-lock effector plate adaptor (C) including labelled parts.

Marlin software design files

Design file name	File type	Open source license	Location of the file
Bioprinter marlin firmware 2019	.ino	GPL	https://doi.org/10.17605/OSF.IO/7T0 forked from https://github.com/MarlinFirmware/M

Basic settings:

Endstop inverting: the firmware was edited to invert the x-min and y-min endstop logic. This was done to accommodate for the Delta printer endstop set up.

#define X_MIN_ENDSTOP_INVERTING *false* set to #define X_MIN_ENDSTOP_INVERTING *true*#define Y_MIN_ENDSTOP_INVERTING *false* set to #define Y_MIN_ENDSTOP_INVERTING *true*

LCD character setup: the firmware script was edited from a Japanese character display setup to a western character display. #define DISPLAY_CHARSET_HD44780 *JAPANESE* set to #define DISPLAY_CHARSET_HD44780 *WESTERN*

Thermal Settings:

Bed minimum and maximum temperature: both initial lines in the script were changed to 0 degrees Celsius. This was done as the hotends were removed from the bed platform during modification of the printer into a bioprinter.

#define BED_MINTEMP *5* set to #define BED_MINTEMP *0*#define BED_MAXTEMP *120* set to #define BED_MAXTEMP *0*

1. **Cold extrusion:** The script was changed to allow cold extrusion. #define PREVENT_COLD_EXTRUSION set to // #define PREVENT_COLD_EXTRUSION
2. **Extruder minimum/maximum temperature:** As with the bed platform temperature settings, both were changed to 0 as the extruder hotend and thermistor were removed during modification. #define EXTRUDE_MINTEMP *170* set to #define EXTRUDE_MINTEMP*0*
3. **Preheating bed and extruder:** These temperatures were also set to 0 as there was no need for preheating, without hotends available. #define PREHEAT_1_TEMP_HOTEND *180* set to #define PREHEAT_1_TEMP_HOTEND *0*#define PREHEAT_1_TEMP_BED *70* set to #define PREHEAT_1_TEMP_BED *0*

Mechanical Settings:

1. **Printable radius:** The printable radius was reduced during the modification to accommodate for the smaller printing environment required. #define DELTA_PRINTABLE_RADIUS *116* set to #define DELTA_PRINTABLE_RADIUS *30*
2. **Y and Z probe offset from extruder:** The Y and Z probe offsets were changed to accommodate for the smaller, newly included extruder nozzle. #define Y_PROBE_OFFSET_FROM_EXTRUDER *-10* set to #define Y_PROBE_OFFSET_FROM_EXTRUDER *0*#define Z_PROBE_OFFSET_FROM_EXTRUDER *-3.5* set to #define Z_PROBE_OFFSET_FROM_EXTRUDER *-18.5*
3. **Invert Extruder motor direction:** The extruder stepper motor inversion was changed to false as it was extruding correctly. #define INVERT_E0_DIR *true* set to #define INVERT_E0_DIR*false*
4. **Z-Home position:** The distance between the nozzle and the printing platform was changed regularly to accommodate for the different extruder used and the different length needles. #define MANUAL_Z_HOME_POS *295.2* set to #define MANUAL_Z_HOME_POS *116.5*

Movement Settings:

Axis steps per unit: These values were changed based on the Stepper motor used. #define DEFAULT_AXIS_STEPS_PER_UNIT { 80, 80, 80, *96* } set to #define DEFAULT_AXIS_STEPS_PER_UNIT { 80, 80, 80,*200* }

Maximum/Default/Retraction/Travel Acceleration: All movement related script was changed to reduce the speed of the various movements during printing.

#define DEFAULT_MAX_ACCELERATION { *3000, 3000, 3000, 3000* } set to #define DEFAULT_MAX_ACCELERATION { *1000, 1000, 1000, 1000* } #define DEFAULT_ACCELERATION *2000* set to #define DEFAULT_ACCELERATION *1000*#define DEFAULT_RETRACT_ACCELERATION *2000* set to #define DEFAULT_RETRACT_ACCELERATION *1000*#define DEFAULT_TRAVEL_ACCELERATION *2000* set to #define DEFAULT_TRAVEL_ACCELERATION *1000*

Default E-Jerk: The E Jerk value is the minimum speed change that requires acceleration. This was reduced in order to create smoother print movements whilst printing smaller and finer constructs.

#define DEFAULT_EJERK *5.0* set to #define DEFAULT_EJERK*1.0*

Bill of Materials

The complete bill of materials for the final Bioprinter manufactured is listed in Table 1. The original 3D Delta printer used for modification was assembled according to manufacturer's instruction and all parts used in the final bioprinter model were from the original 3d printer, unless mentioned in the Bill of materials Table 1.

Build Instructions

Assembly of commercially available ANYCUBIC Kossel Delta 3D printer

For ease of access to build materials, an Anycubic Kossel Linear Plus Delta 3D printer was purchased from DIYelectronics (Durban, South Africa). All parts were provided within the build kit and assembly of the printer was done as per supplier's instructions. The printer technical specifications are outlined in Table 2.

Firmware Flashing

The Tri-gorilla control board (integrated with the function of the Mega2560+ramps 1.4; main control chip: ATMEGA256016AU), on the assembled delta printer, required installing (i.e. flashing) software (firmware) specifically written for controlling and monitoring the 3D printer. Open-source Marlin Firmware (v.1.1.0) was first used for flashing the board; available on the manufacturers' website [L4]. Firmware installation was performed through the Arduino Integrated Development Environment (IDE) desktop software (v.1.8.1.0, [L5]). The printer was first connected to a PC using a USB cable (USB 2.0-A Male to USB-B Male cable), the Arduino.ino file was then opened using the Arduino software environment. In the Arduino environment, Tools > Board > Arduino MEGA 2560 serial connection was selected. The Firmware was subjected to several changes as described in detail in section 1.1. Within the Arduino environment, the *Configuration.h*tab was used for the software modifications. The "upload" option was then selected to flash the Tri-gorilla board. The final modified firmware is available at https://doi.org/10.17605/OSF.IO/7TQKB.

Hardware modification

Based on the criteria for bioprinting, the delta printer was modified to accommodate for tissue engineering application purposes. The methods of modifications to the hardware are explained throughout the following sub-sections.

Enclosure of the printer

The printer was encased to maintain sterility during future bioprinting. All parts used were designed using CAD software and manufactured in PLA (corners), Perspex (sides) or Glass (lid). Assembly was as follows;

Corners:

1) Printed corners were pushed onto the aluminium frame.

Sides and door:

1) Each frame was secured to the aluminium extrusions of the printer using six M3 x 10mm cap-head screws, six M3 T-nuts and twelve M3 washers.

2) The windows, for the two sides of the printer, were secured onto the newly attached frames using four M3 x 8mm cap-head screws, four M3 nuts and eight M3 washers.

3) The door handle was fitted onto the door without the need for fasteners.

4) The door was assembled onto the frame of the front facing side of the printer using two hinges, ten M3 x 10mm cap-head screws, ten M3 nuts and twenty M3 washers.

Lid:

1. Additional aluminum extrusions were secured to each existing extrusion using 3D printed parts and twelve M4 x 5mm screws and twelve M4 T-nuts.

2. Glass was secured onto the top of the new extrusions using six M4 x 5 mm screws and six M4 T-nuts.
3. The finishing pieces were added simply by sliding into the new extrusion grooves.
4. Electronic board box

The Tri-gorilla electronic board (assembled according to manufacturer's instruction) was moved to outside the printer. A board box was designed and manufactured in PLA using a standard Delta 3D printer. The designed and printed board box consists of two separate pieces: the box and the lid. Assembly was as follows;

1) All wires were disconnected from the Tri-gorilla electronic board and the board removed from its original fixture (under the print bed).

2) The board was secured onto the newly designed and printed board box using two M3 x 10mm cap-head screws.

3) The board box was secured onto the aluminium extrusions of the correct side of the printer using two M4 x 5mm cap-head screws and two M4 T-nuts.

4) All wires were fed through the aluminium extrusions (from within the printer) into the board box and reconnected in their respective places.

Bed platform

A print bed platform was designed and printed using a standard Delta 3D printer using PLA as extrusion material. The platform consists of six separate pieces; designed to hold two 220 mm diameter glass plates, with the top plate designed to hold a standard size petri dish. The printed pieces were secured onto the printers aluminium extrusions using two M3 x 25mm cap-head screws and two M3 T-nuts per piece.

Extruder

The original thermoplastic extruder was removed from the effector plate and replaced with a newly designed syringe-based extruder. Assembly of the syringe-based extruder was carried out exactly as described online [L6]. Instructions up until attachment of the power-supply mounting bracket were followed exactly as described. The following changes were made from there;

Assembly onto mounting bracket and printer

1) The assembled drive block was attached to the mounting bracket using four M3 x 35mm cap-head screws and four M3 nuts.

2) The mounting bracket with attached drive block was attached to the printer frame aluminium extrusions using two M4 x 15mm screws with four M4 T-nuts.

3) The NEMA17 stepping motor was connected to the E0 port of the Tri-gorilla electronic board using a standard 4-pin extruder cable.

Assembly of Luer-lock effector plate adaptor

A "nozzle" was designed for the extrusion of biomaterials using the syringe-based extruder system. It was designed to attach to the original effector plate provided with the Delta printer. The nozzle was designed to incorporate a male Luer-lock connection fitting and a Bowden tube connection fitting. Assembly went as follows;

1) The printed adaptor piece was secured onto the effector plate using four M3 x 8mm cap-head screws and four M3 nuts.

2) The bowden tube was pushed into the bowden tube connection fitting and secured into place using insulating tape and silicone paste.

3) A Luer-lock syringe needle was then fastened into place using the male Luer-lock connection fitting part of the adaptor piece.

Addition of syringe, top strengthener and tubing

1) A 5 ml Luer-lock syringe was added to the assembled and mounted syringe extruder.

2) The printed top strengthener was fitted onto the mounted syringe extruder using the threaded bar used to secure the motor to the frame and the two M3 x 35mm cap-head screws positioned on the other end of the frame.

3) The bowden tube was attached to the syringe using a Luer-lock adaptor, silicone sealant and insulating tape. The other end of the tubing was attached to the Luer-lock nozzle piece.

Operation Instructions

Software requirements

- The bioprinter was connected to the PC using a USB cable (USB 2.0-A Male to USB-B Male cable)
- An 18G needle was added to the nozzle of the bioprinter (Luer-lock fit)
- Pronterface (printrun-2015031011[,3] https://github.com/kliment/Printrun) software was initiated and used to manually count the Z home position. This depends on the 18G needle length used. Briefly, once the printer was connected to the software, the homing option was initiated. The distance between the needle nozzle and the printing platform was then calculated using the Z-axis 10 mm, 1 mm and 0.1 mm movement arrows within the software. This was repeated several times until the exact distance between bed platform and nozzle after homing was determined.
- The printer was disconnected from Pronterface and the correct Marlin firmware file was opened in the Arduino Environment.
- The firmware was changed, in the *Configuration.h* tab of the Arduino environment, to include the correct Z position calculated.
- The edited firmware was uploaded onto the printer by clicking "upload" button in the Arduino environment.
- The new Z home position was tested by, once again, connecting to Pronterface (printrun-2015031022).
- Setting up slicing software
- Cura (v.15.04.2) software was initiated.
- The printer was manually set up for desired bioprinter parameters starting with Machine settings. For this study, the following machine settings were chosen: E-steps per 1mm filament = 0, Maximum width = 60, Maximum depth = 60, Maximum height = 50, Extruder count 1, No heated bed, Machine centre 0,0 Yes, GCode Flavor Marlin/Sprinter, All printer head size options set to 0.0, Serial port Auto, Baudrate Auto.
- The desired bioprinting parameters were set up next, such as: Layer height = 0.1, Shell thickness = 0.4, Disable retraction, Fill density 100%, Print speed = 10, Printing temperature set to 0, No support or platform adhesion, Filament diameter = 1 and flow = 150. In the "Advanced" tab, all speeds were set to 5.
- The desired STL file was uploaded into Cura (v.15.04.2).
- The Gcode was saved and loaded onto an SD card which was then inserted into the printer awaiting print.
- Cell-free Bioprinting
- The tubes and printer environment were cleaned and sterilized thoroughly using 70 % (v/v) ethanol. The sterilizing solution was manually fed through the tube several times using a sterile syringe.
- The cell-freebioink solution was prepared (heating, cooling, mixing etc.)
- The bioink was then added to a 5 ml sterilized syringe and placed into the frame of the extruder with the top strengthener added to secure it into place.
- One end of the sterilized tube was attached to the bioink syringe, and the other end fastened to the top of the effector plate (via the luer-lock adaptor).
- A newly sterilized 18G Luer-lock needle was added to the bottom section of the effector plate adaptor.

- A sterilized petri dish was added to the printing platform where the glass plates permit.
- The bioprinter was once again connected to the PC using a USB cable (USB 2.0-A Male to USB-B Male cable)
- Using Pronterface (printrun-2015031033[3] https://github.com/kliment/Printrun) software, the M302 g-code command was entered to allow for cold extrusion and the extruder motor turned on to extrude until the bioink was fed through the sterilized tube to the tip of the nozzle needle.
- The bioprinter was then disconnected from the PC.
- The LCD display on the bioprinter was used to navigate to the saved GCode files on the inserted SD card.
- Printing was initiated.
- Once completed, the bioprinter door was opened using aseptic technique and cross-linker solution was added to the construct (if required). The construct was left to cross-link for 2 minutes followed by removal of the cross-linker solution.

Results

Validation and Characterization

The concept was assessed to test initial printability using a 6 % (w/v) sodium alginate (dissolved in sterile double distilled water) (see Appendix 1 for a detailed protocol). Cell-free bioprinting experiments were performed initially using an 18G syringe needle as the nozzle. Subsequent analysis on optimal parameters were then carried out. All materials used were purchased from Sigma-Aldrich Pty. Ltd. (South Africa), unless otherwise specified.

Proof of concept: does the extrusion mechanism work as intended and is it capable of printing geometrically complex constructs?

The computer aided design (CAD) software SketchupMake2017 (v.17.2.2555) was used to design a grid-like structure for initial printability tests. Dimensions include: Height = 19.80 mm

Width = 19.80 mm, Layer height = 2 layers (0.2 mm). Cura (v.15.04.2) was used as the slicing software as well as for the parametrization of the printing process. The printing parameters include Layer height (1 layer = 0.1 mm), Shell thickness (0.4 mm), No retraction, Bottom/Top thickness (0.4 mm), Fill density (100%), Print speed (10mm/s), Flow rate (150%), Nozzle diameter (18G needle).

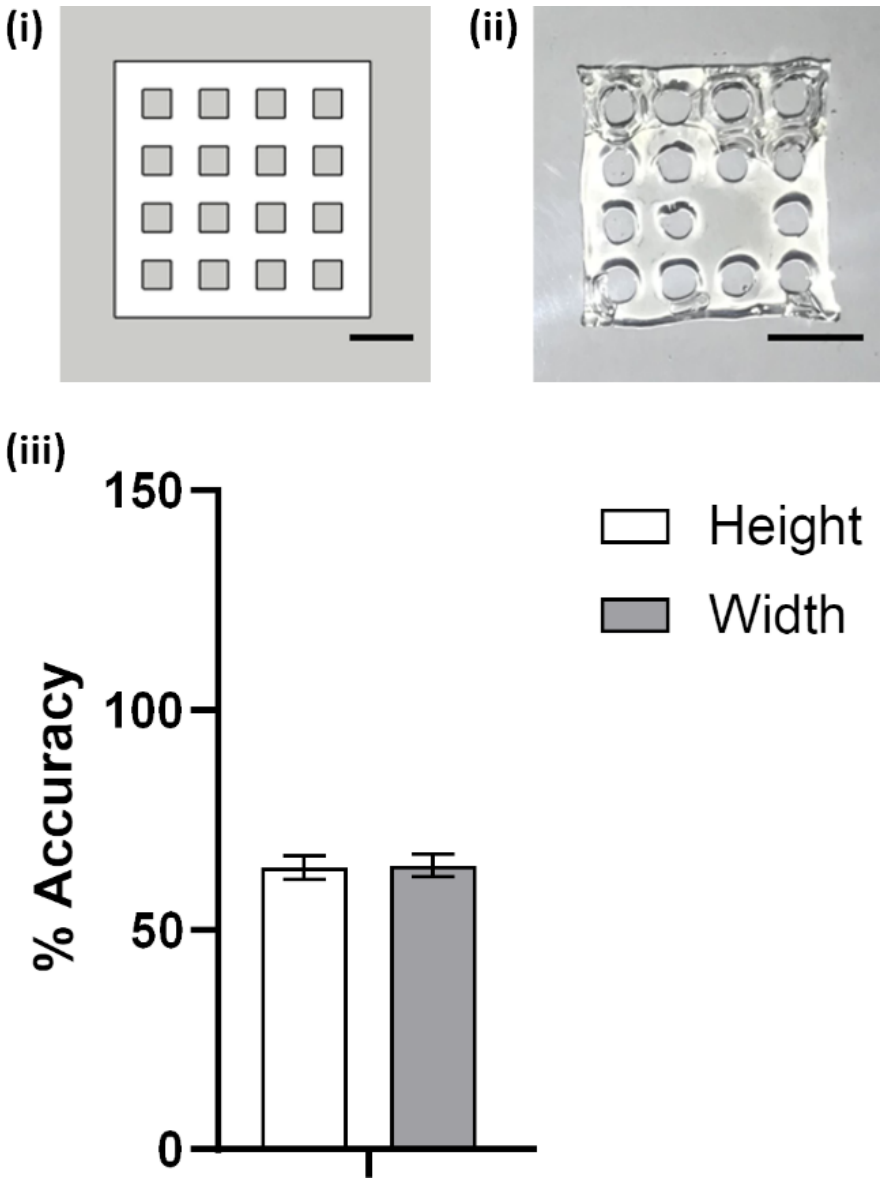

Fig. 9. Assessing printability.; (i) CAD file of construct, (ii) Post-printing photographs of the printed construct; printed using a syringe-based extruder, (iii) Post-printing accuracy (%) calculated for the printed alginate constructs (using height and width). Scale bars represent 5 mm. Data points are means; error bars represent SEM for n = 6.

Using the measurements of height and width (mm), of both Fig.9(i) and Fig.9(ii), the printing accuracies (%) were calculated for the assembled hydrogel extruder (Fig.9(iii)). Printing accuracies (%) for height and width were calculated at 64.13 % and 64.58 %, respectively. The settings chosen turned out to be suitable for experimental trials based on cell-free 6 % (w/v) concentrated hydrogel inks, hence these parameters were kept for the remaining experiments except for flow rate and needle gauge optimization studies.

Optimization studies: Does needle gauge and flow rate affect printability?

Needle gauge

The printing accuracy (%) was determined using the following equation;

$$\text{Printing Accuracy } (\%) \frac{\text{Experimental Value}}{\text{Theoretical value}} x\ 100$$

A 6% (w/v) alginate solution was used as extrusion material to print the designed constructs with subsequent cross-linking in $CaCl_2$ solution for 2 minutes. Images were taken of the cross-linked constructs and measurements determined with ImageJ software (v1.4.6.r). Prints were repeated three times for each needle gauge tested (15G, 18G, 23G and 25G). The flow percentage used in the experiment was 100%. This experiment aimed to determine the effect of nozzle diameter (using different needle gauges) on the printed construct shape quality, under certain constant parameters (flow rate = 100%, print speed = 10 mm/s).

Kahl *et al.* (2019) found that the needle diameters are essential in optimizing printing resolutions such that; the larger the needle diameter, the smaller the resolution and therefore the lower the printing accuracy. The choice in needle diameter, length and shape (i.e. conically shaped or cylindrical) has additional consequences in terms of cell viabilities. Findings suggest shorter, conically shaped needles allow for higher cell viabilities, post-printing, compared to cylindrically shaped needles [11,22,23]. These findings have major implications in cell-laden bioprinting approaches but little implications in cell-free approaches, as the cells are seeded onto the bioprinted constructs post-printing.

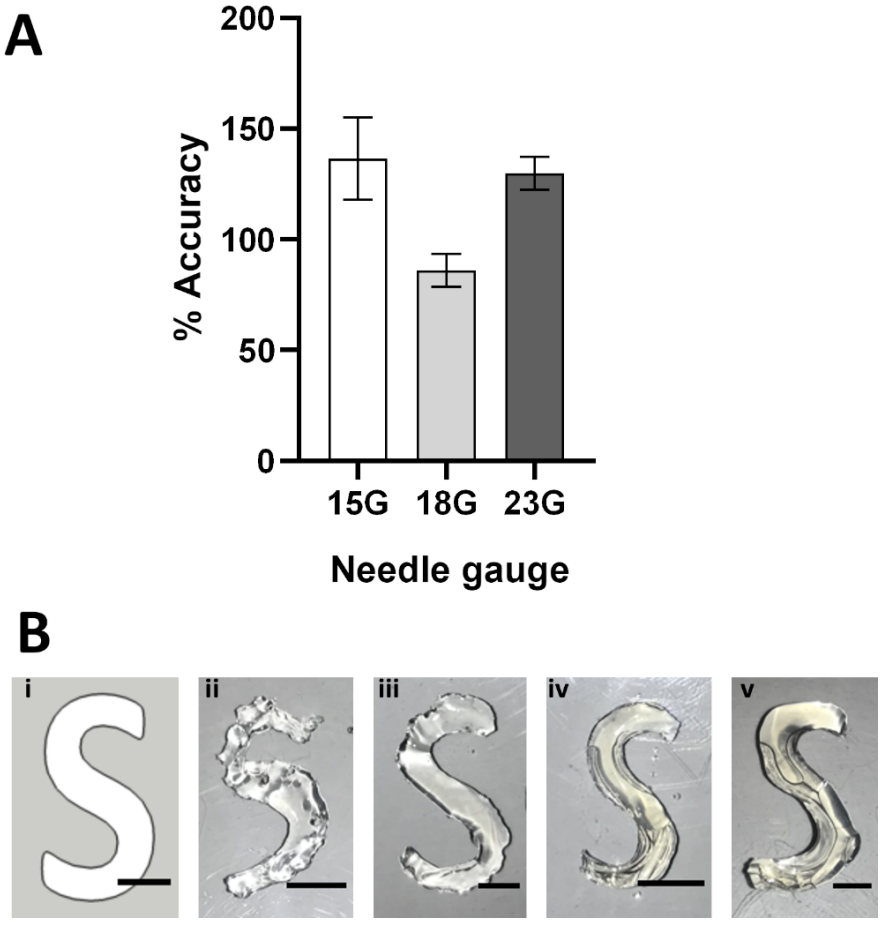

Fig. 10. Assessment of printability using different nozzle diameters. A: Post-printing accuracy (%) calculated for alginate constructs printed using various nozzle diameters (15G, 18G and

23G), 25G not included in graph. B: Post-printing photographs of alginate constructs printed using (ii) 25G, (iii) 23G, (iv) 18G and (v) 15G needle gauge. (i) CAD file for printed construct. Scale bars represent 5mm. Data points are means; error bars represent SEM for n=3. No statistical significance was found between groups (p = 0.0522; p > 0.05).

Figure 10 shows the images of the printed constructs (post-printing and cross-linking), printed using various needle gauges; 15G, 18G and 23G. The inner diameters for each needle gauge are as follows; 15G = 1.372 mm, 18G = 0.838 mm, 23G = 0.337 mm. A final needle gauge was included in the experiment; 25G (inner diameter = 0.260 mm), however, the printed constructs produced were not uniform and therefore printing accuracies based on filament width were unable to be calculated; Fig. 10b(ii)**.** The printing accuracies (%) produced for each needle gauge experiment include; 136.53 %, 86.01 % and 129.93 % for 15G, 18G and 23G, respectively. Although no statistical significance was found between groups, the 18G needle produced a printing accuracy closest to 100 % (Fig. 10a).

Flow rates

The spreading ratio was determined at different flow percentages (100%, 125%, 150%, 200%) using the following equation:

$$Spreading\ ratio = \frac{\text{Filament diameter}}{\text{Nozzle diameter}}$$

The flow percentages listed are extrusion multipliers. These values instruct the printers to extrude more material during the prints. Post-printing images were taken immediately after printing. Calcium chloride was not added to the printed constructs for cross-linking. A nozzle diameter of 0.838 mm (18G needle) was used for the syringe extruder based on the results obtained from the needle gauge printability experiment. Three constructs were printed for each flow percentage tested. An average of the width, measured at various positions along the filament, of the printed filaments were divided by the needle diameter which remained constant throughout the experiment; 0.838 mm. The constructs were printed using 6% (w/v) alginate hydrogel with no post-printing cross-linking methods employed.

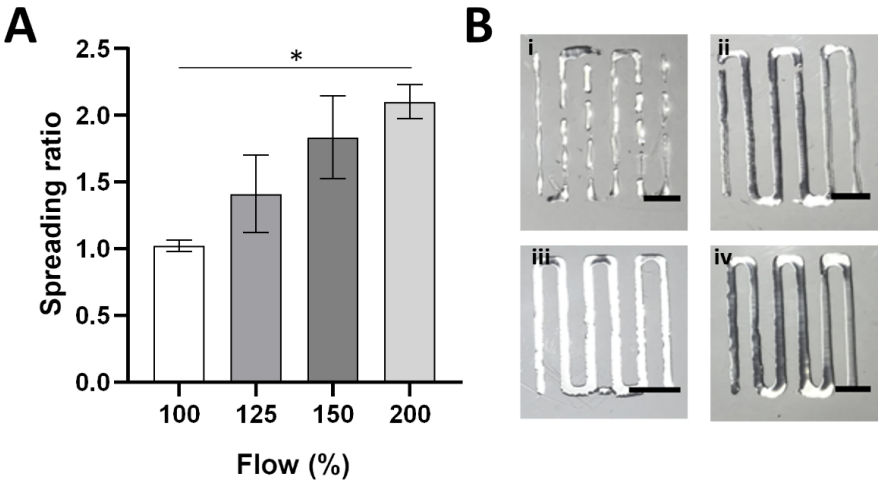

Fig. 11. A comparison of hydrogel printability. A: Post printing spreading ratio's (filament diameter/needle diameter) determined for various flow rates using 6% (w/v) alginate. B: Post printing photographs of printed constructs for each flow rate (i) 100%, (ii) 125%, (iii) 150% and (iv) 200%. Data points are means; error bars represent SEM for n = 3. Asterix represents statistical significance (p = 0.0363; p < 0.05), no Asterisk shows no statistical significance (p > 0.05). Scale bars represent 5 mm.

Figure 11 shows a positive correlation between flow percentage and spreading ratios obtained. The spreading ratios obtained for 100 % and 200 % flow percentage groups showed a statistically significant difference; $p = 0.0363$; $p < 0.05$, whereas no other statistical significance was found between groups. Similarly, Kahl *et al.*(2019) shows the higher the extrusion rate, at a constant nozzle diameter, results in lower shape fidelity of the printed construct. Images in Fig. 11 show the inconsistency in filament structure produced when printing at a 100 % flow percentage, even though a low spreading ratio was noted. Due to the complex nature of bioprinting and the necessity to print complex geometries at extremely high resolutions, the final printed construct must be highly precise and accurate. A high spreading ratio is, therefore, an undesirable characteristic and further optimizing must take place in order to accommodate for this [24].

Conclusions

The overall aim to create a low-cost bioprinter from a commercially available thermoplastic 3D delta printer was achieved. The final bioprinter designed and manufactured consists of an already established syringe-based extruder, incorporated with minor alterations to fit the delta style 3D printer, and an easily built commercially available Delta printer with added enclosure and easily modifiable firmware. Converting the thermoplastic Delta 3d printer into a bioprinter cost a total of $ 1,007.52, making it affordable to most research laboratories. This system allows for the printing of geometrically complex structures using cell-free hydrogel based bioinks and can be easily modified (i.e., firmware parameters) for other paste extrusion materials and future cell-laden hydrogel bioprinting. There is, however, much room for improvement where this study only aims to provide the much needed and promising foundation work for what could be a fully functional low-cost bioprinter with multiple applications. The modifications made to the hardware during the conversion from 3D printer to bioprinter were successful and allowed for an increased sterile environment for the sensitive nature of bioprinting, as set out as a priority criterion. To further increase sterility, one improvement would include the addition of HEPA-filtered laminar air flow to the system. Smaller sterility-related improvements may include the addition of UV lights to the system and re-designing of the internal hardware components (i.e., extruder carriage) into fully enclosed and easily cleaned singular components.

Securing the extruder carriage to the frame of the printer had the desired effect of removing the excessive weight from the effector plate which then allowed for smoother printing movements and higher printed construct quality. Another important design criterion stipulated was that of producing highly complex shapes with precise geometry. This criterion was partially met but remains an area that requires much improvement. Lowering the extruder carriage closer to the nozzle would aid in reducing the distance between the syringe feedstock and the nozzle which in turn allows for more control over the extrusion of the bioinks. More control over the extrusion means less possibility of under or overextrusion; gaps forming within the construct versus leakage of bioink during travel moves, respectively.

Overall, the results achieved with the final bioprinter prototype model were satisfactory and provide the much-needed foundation work for future optimization studies. Incorporating the use of cell-laden bioinks and assessing the different modes of cell-based bioprinting will be employed in future work, along with the assessment of various bioink formulations and cross-linking methods. While Delta style bioprinters are commercially available (Pensees Vitarix and VitarixW) the systems are registered design closed-source premium systems. The leading goal of the SV1 was toward the development of an open-source system.. Relative to commercial systems the substantial cost reduction presented may prove to be impactful to research laboratories in lower resourced economies as well as in developed countries.

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Conflict of interest

Authors have no conflict of interest relevant to this article.

Human and animal rights

Not applicable.

References:

[1] S. Melissaridou,, E. Wiechec,, M. Magan,, M.V. Jain,, M.K. Chung,, L. Farnebo,, K. Roberg, Cancer Cell Int. 19(1) (2019) 1–10. 10.1186/s12935-019-0733-1.

[2] J. Mason,, S. Visintini,, T. Quay, CADTH Issues Emerg. Heal. Technol. (2016).

[3] R. Jamee,, Y. Araf,, I. Bin Naser,, S.K. Promon, Regen. Ther. 18 (2021) 133–45. 10.1016/j.reth.2021.05.006.

[4] B. Yenilmez,, M. Temirel,, S. Knowlton,, E. Lepowsky, Bioprinting (January) (2019) e00044. 10.1016/j.bprint.2019.e00044.

[5] E.S. Bishop,, S. Mostafa,, M. Pakvasa,, H.H. Luu,, M.J. Lee,, J.M. Wolf,, G.A. Ameer,, T.C. He,, R.R. Reid, Genes Dis. 4(4) (2017) 185–95. 10.1016/j.gendis.2017.10.002.

[6] F. You,, B.F. Eames,, X. Chen, Int. J. Mol. Sci. 18(7) (2017) 1597. 10.3390/ijms18071597.

[7] H. Ding,, R.C. Chang, Appl. Sci. 8(3) (2018). 10.3390/app8030403.

[8] Z. Xia,, S. Jin,, K. Ye, SLAS Technol. Transl. Life Sci. Innov. (2018) 247263031876051. 10.1177/2472630318760515.

[9] F. Liu,, C. Liu,, Q. Chen,, Q. Ao,, X. Tian,, J. Fan,, H. Tong,, X. Wang, Int. J. Bioprinting 4(1) (2018) 1–15. 10.18063/ijb.v4i1.128.

[10] Z. Zheng,, J. Wu,, M. Liu,, H. Wang,, C. Li,, M.J. Rodriguez,, G. Li,, X. Wang,, D.L. Kaplan, Adv. Healthc. Mater. 7(6) (2018) 1701026. 10.1002/adhm.201701026.

[11] J.A. Reid,, P.A. Mollica,, G.D. Johnson,, R.C. Ogle,, R.D. Bruno,, P.C. Sachs, Biofabrication 8(2) (2016) 025017. 10.1088/1758-5090/8/2/025017.

[12] K. Pusch,, T.J. Hinton,, A.W. Feinberg, HardwareX 3 (2018) 49–61. 10.1016/J.OHX.2018.02.001.

[13] W. Jia,, P.S. Gungor-Ozkerim,, Y.S. Zhang,, K. Yue,, K. Zhu,, W. Liu,, Q. Pi,, B. Byambaa,, M.R. Dokmeci,, S.R. Shin,, A. Khademhosseini, Biomaterials 106 (2016) 58–68. 10.1016/j.biomaterials.2016.07.038.

[14] T.J. Hinton,, Q. Jallerat,, R.N. Palchesko,, J.H. Park,, M.S. Grodzicki,, H.-J. Shue,, M.H. Ramadan,, A.R. Hudson,, A.W. Feinberg, Sci. Adv. 1(9) (2015) e1500758–e1500758. 10.1126/sciadv.1500758.

[15] C. Amza,, A. Zapciu,, D. Popescu, Technologies 5(3) (2017) 50. 10.3390/technologies5030050.

[16] B. Wijnen,, E.J. Hunt,, G.C. Anzalone,, J.M. Pearce, PLoS One 9(9) (2014) e107216. 10.1371/journal.pone.0107216.

[17] L. Banović,, B. Vihar, J. Open Hardw. 2(1) (2018) 1–10. 10.5334/joh.6.

[18] J.W. Tashman,, D.J. Shiwarski,, A.W. Feinberg, HardwareX 9 (2021) e00170. 10.1016/j.ohx.2020.e00170.

[19] S. Fanucci,, M.W. Barwick,, E. Prinsloo, South African Med. J. 109(8b) (2019) 34. 10.7196/samj.2019.v109i8b.13838.

[20] B.M. Schmitt,, C.F. Zirbes,, C. Bonin,, D. Lohmann,, D.C. Lencina,, A. Da Costa Sabino Netto, Mater. Res. 20 (2017) 883–6. 10.1590/1980-5373-mr-2016-1039.

[21] M. Kahl,, M. Gertig,, P. Hoyer,, O. Friedrich,, D.F. Gilbert, Front. Bioeng. Biotechnol. 7(July) (2019) 1–12. 10.3389/fbioe.2019.00184.

[22] T. Billiet,, E. Gevaert,, T. De Schryver,, M. Cornelissen,, P. Dubruel, Biomaterials 35(1) (2014) 49–62. 10.1016/j.biomaterials.2013.09.078.

[23] A. Faulkner-Jones,, S. Greenhough,, J. A King,, J. Gardner,, A. Courtney,, W. Shu, Biofabrication 5(1) (2013). 10.1088/1758-5082/5/1/015013.

[24] A.C. Daly,, S.E. Critchley,, E.M. Rencsok,, D.J. Kelly, Biofabrication 8(4) (2016) 045002. 10.1088/1758-5090/8/4/045002.

List of linked webpages:

[L1] https://reprap.org/wiki/Kossel

[L2] https://www.thingiverse.com/

[L3] https://github.com/MarlinFirmware/Marlin

[L4] http://www.anycubic3d.com/support/show/594034.html

[L5] https://www.arduino.cc/en/Main/Software

[L6] http://reprapltd.com/reprappro/documentation/building-the-paste-extruder/index.html

Tables and Figures:

Table 1: Bill of Materials for the final Bioprinter build (given in South African Rands and United States Dollar).

Designator	Designator	Component
3D printer modified for bioprinting	3D printer modified for bioprinting	Anycubic Kossel 3D Delta printer with all comp
3D printed parts	Bioprinter	Corners (A1, A2, A3)
	Electronic board box	Box
		Lid
	Bed platform	Support type 1
		Support type 2
	Extruder	Top strengthener
		Frame
		Drive gear
		Mounting bracket
		Driven gears
		Drive block
		Bearing holders
	Nozzle	Luer-lock effector plate adaptor
Perspex parts	Bioprinter Frame	Frame (B1, B2, B3)
	Bioprinter sides	Window (C1, C2)
	Bioprinter door	Door (C3)
		Handle (C4)
Mechanics	Bioprinter Frame	M3 cap-head screws
		M3 T-nuts
		M3 Washers
	Bioprinter door	M3 cap-head screws
		M3 nuts
		M3 Washers
		Hinges
	Bioprinter sides	M3 cap-head screws

Designator	Designator	Component
		M3 T-nuts
		M3 Washers
	Electronic board box	M3 cap-head screws
		M4 screws
		M4 T-nuts
	Bed platform	M3 cap-head screws
		M3 T-nuts
	Nozzle	M3 cap-head screws
		M3 nuts
		M3 Washers
	Extruder	M3x35mm cap-head screws
		M3 nuts
		M3 nyloc nut
		M3 plain washer
		623 bearings
		M5x95mm cap-head screw
		M5 nut
		M3x8mm cap-head screw
		M3x50mm threaded bar
		M4 cap-head screw
		M4 T-nut
Miscellaneous	Bed platform	Circular glass bed (? 220 mm)
		Circular glass bed (? 220 mm with ? 96 mm cen
	Nozzle	200mm Bowden tube
		18G Luer lock syringe needle
	Extruder	5 ml Luer lock syringe
Electronics	Extruder	NEMA 17 stepping motor
		4-pin extruder head cable

Table 2: Anycubic Kossel Delta 3D printer technical specifications.

Make	Anycubic
Model	Kossel Linear plus
Software input formats	.STL, .OBJ, .AMF
Software output formats	GCODE
Technology	Fused Deposition Modelling
Frame	Delta
Year	2017
Print area	Φ230 x 300 mm
Max. bed temperature	100@C
Nozzle size	0.4 mm
Max. nozzle temperature	260@C
Max. Z-axis resolution	0.1 – 0.4 mm
Z-axis accuracy	0.0025 mm
X/Y-axis accuracy	0.0125
Max. print speed	60 mm/s
Max. travel speed	60 mm/s
Printable materials	PLA, ABS

Interface	LCD display
Connectivity	SD card
Printer size	380 mm x 680 mm
Printer weight	7 kg
Power input	AC 110-220V AC, 50/60Hz
Firmware	Marlin
Board	Tri-Gorilla control board

Appendix

Detailed protocol for preparing a 6 % (w/v) Alginate bioink

A 6% (w/v) alginate solution was prepared by dissolving alginic acid sodium salt in double distilled water (ddH_2O) under continuous stirring on a hot plate. The alginate was prepared and used immediately before each experiment. A 0.3 M $CaCl_2$solution was prepared and used for cross-linking of the alginate post-printing.