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TITLE:

Design of an Open-Source, Low-Cost Bioink and Food Melt Extrusion 3D Printer

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KEYWORDS:

3D printing, additive manufacturing, melt extrusion, open source, food, bioprinting, bioinks

SUMMARY:

The aim of this work is to design and construct a reservoir-based melt extrusion three-dimensional printer made from open-source and low-cost components for applications in the biomedical and food printing industries.

ABSTRACT:

Three-dimensional (3D) printing is an increasingly popular manufacturing technique that allows highly complex objects to be fabricated with no retooling costs. This increasing popularity is partly driven by falling barriers to entry such as system set-up costs and ease of operation. The following protocol presents the design and construction of an Additive Manufacturing Melt Extrusion (ADDME) 3D printer for the fabrication of custom parts and components. ADDME has been designed with a combination of 3D-printed, laser-cut, and online-sourced components. The protocol is arranged into easy-to-follow sections, with detailed diagrams and parts lists under the headings of framing, y-axis and bed, x-axis, extrusion, electronics, and software. The performance of ADDME is evaluated through extrusion testing and 3D printing of complex objects using viscous cream, chocolate, and Pluronic F-127 (a model for bioinks). The results indicate that ADDME is a capable platform for the fabrication of materials and constructs for use in a wide range of industries. The combination of detailed diagrams and video content facilitates access to low-cost, easy-to-operate equipment for individuals interested in 3D printing of complex objects from a wide range of materials.

INTRODUCTION:

Additive manufacturing is a powerful manufacturing technology that has the potential to provide significant value to the industrial landscape[1,2]. The attractive features of additive manufacturing involve no tooling costs, high levels of customization, complex geometries, and reduced barriers to entry costs. No retooling costs allow for the rapid manufacturing of prototypes, which is desirable when trying to decrease “time to market”, which is a critical aim of industries in developed nations trying to remain competitive against low-wage competitors[1]. High levels of customizability allow for a wide variety of products to be fabricated with complex geometries. When these factors are combined with the low costs for set-up, materials, and operator specialization, there is a clear value of additive manufacturing technologies[3].

Additive manufacturing, also called 3D printing, involves layer-by-layer fabrication of an object in a computer numerical controlled (CNC) system[3]. Unlike traditional CNC processes such as milling, in which material is removed from a sheet or block of material, a 3D printing system adds material into the desired structure layer-by-layer.

3D printing can be facilitated through a range of methods including laser, flash, extrusion, or jetting technologies[4]. The specific technology employed determines the form of the raw material (i.e., powder or melt), as well as the rheological and thermal properties required for processing[5]. The extrusion-based 3D printing market is dominated by filament-based systems, which is due to filaments being easy to handle, process, and continuously supply large volumes of material to the extrusion head. However, this process is limited by the type of material able to be formed into filaments (mainly thermoplastics). Most materials do not exist in filament form, and the lack of modern low-cost platforms in the market represents a notable gap.

This protocol shows the construction of a reservoir-based extrusion system that allows materials to be stored in a syringe and extruded through a needle. This system is ideally suited to manufacture a wide range of materials including foods[6], polymers[7], and biomaterials[8,9]. Furthermore, reservoir-based extrusion techniques are typically less hazardous, lower in cost, and easier to operate than other 3D printing methods.

There is a growing number of university-led teams designing and releasing open-source 3D printing systems to the public. Beginning with the Fab@Home extrusion-based printer in 2007[10,11], researchers aimed to create a simple and cheap platform to drive rapid expansion in 3D printing technology and applications. Later in 2011, the RepRap project aimed to create a filament-based 3D printing platform designed with parts made by 3D printing, with the goal to create a self-replicating machine[12]. The cost of 3D printers has been dropping over the years, from $2300 USD for a Fab@Home (2006), $573 USD for a RepRap v1 (2005), and $400 USD for v2 (2011).

In previous work, we demonstrated how an off-the-self 3D printing system could be combined with a custom reservoir-based extrusion system to create complex 3D objects from chocolate[13]. Further design investigation has shown that considerable cost savings can be achieved compared to this prototype design.

The aim of this protocol is to provide instructions for the construction of a low-cost reservoir-based melt extrusion 3D printer. Presented here are detailed diagrams, drawings, files, and component lists to allow successfully construction and operation of a 3D printer. All components are hosted on the open-source (creative commons noncommercial) platform <https://www.thingiverse.com/Addme/collections>, which allows users to change or add additional features as desired. Viscous cream, chocolate, and Pluronic F-127 (a model for bioinks) are used to evaluate the performance of ADDME and demonstrate application of the ADDME 3D printer to the biomedical and food printing industries.

A laser cutter capable of cutting acrylic and a desktop 3D printer capable of printing PLA or ABS filaments are required for this protocol. A machined heating jacket and heater cartridge or silicone heater can be used to heat the material, depending on which equipment the operator has access to. All CAD files can be found at <https://www.thingiverse.com/Addme/designs>. For firmware and software to control the 3D printer, <http://marlinfw.org/meta/download/> and <https://www.repetier.com/> are provided resources, respectively. For detailed instructions about the control board, see <https://reprap.org/wiki/RAMPS_1.4>.

PROTOCOL:

CAUTION: There is a risk of burns caused by hot soldering irons and heating cartridges. The heating cartridge should never be powered when not secured inside of the heating jacket. There is also a risk of pinching or lacerations from the moving 3D printer axis.

1. Overview and preparation

NOTE: **Figure 1A** shows a computer-generated rendering of the printer and **Figure 1B** is a photo of the finished printer.

1.1. Procure all parts from the **Table of Materials**.

1.2. See <https://www.thingiverse.com/Addme/designs> for all acrylic parts to be laser cut. Insure that 6 mm acrylic is used or the frame will not fit together. Laser cutters use a high energy laser to cut material; a professional shop is preferred here.

1.3. See < https://www.thingiverse.com/Addme/designs> for all 3D-printed parts. It is important that the printing parameters specified with each part are used. Note that 3D printers have hot surfaces and moving parts, so use the help of a professional.

1.4. Manufacture the heating jacket part, which is found at <https://www.thingiverse.com/Addme/designs>. If there is no available access to manufacturing capabilities, a silicone heater (**Table of Materials**) can be purchased with the associated 3D printed holder found at <https://www.thingiverse.com/Addme/designs>.

[Place **Figure 1** here]

2. Frame assembly

NOTE: The parts shown in **Figure 2** are required to finish the frame assembly. The frame of the melt extrusion 3D printer is held together by a combination of 6 mm laser cut acrylic and M3 bolts and nuts (**Figure 3**). The bottom of the printer is further strengthened with a M10 threaded rod and nut combination.

2.1. Gather acrylic parts 1–9 and place them together into the configuration shown in **Figure 3A**. Check the figure labels to ensure that each piece is located correctly. Secure with M3 screws and nuts in the configuration shown in **Figure 3C** using the M3 Allen key.

2.2. Place the M10 threaded rod through the purpose made holes in acrylic members 6, 8, and 10. Secure them with M10 washers and nuts as shown in **Figure 3B,D**. Tighten with the variable spanner.

[Place **Figure 2** here]

[Place **Figure 3** here]

3. Y-axis and printing bed sub-assembly

NOTE: The parts outlined in **Figure 4** are required to finish the y-axis and printing bed sub-assembly. All screws are seen in **Figure 4,** and tools are listed in the **Table of Materials**.

3.1. Using the parts in **Figure 4**, assemble the printing bed sub-assembly head according to **Figure 5C**.

3.1.1. Slide two pillow blocks (19) onto each 8 mm shaft (21) according to **Figure 5C**. Slide the endstop (3DP 4) onto one of the 8 mm shafts (21) and secure the mechanical endstop (14) using M2 screws and an Allen key according to **Figure 5E**.

3.1.2. Secure all four pillow blocks (19) to the mounting bed (acrylic part 12) using the M4 screws and Allen key (**Figure 5C**). Secure the belt clamp (3DP 3) onto the mounting bed (acrylic part 12) using the M3 screws and Allen key (**Figure 5C**). Secure the printing bed (acrylic part 11) onto the mounting bed (12) (**Figure 5C**) using the M3 screw, nut, and spring arrangement according to **Figure 5F**.

3.2. Secure the remaining parts from **Figure 4** to the frame according to **Figure 5D,G**.

3.2.1. Secure two of the shaft holders (3DP 2) to both the back panel (acrylic part 6) and front panel (acrylic part 10) using the M2 screws and Allen key according to **Figure 5D,G**, respectively.

3.2.2. Secure the stepper motor holder (12) to the back panel (acrylic part 6) using the M3 screws and Allen key (**Figure 5D**). Secure the stepper motor (11) to the stepper motor holder (12) using the M3 screws and Allen key (**Figure 5D**). Secure the belt idler (3DP 1) to the front panel (acrylic part 10) using the M3 screws and Allen key (**Figure 5G**).

3.3. Place the printing bed sub-assembly into the frame by matching up each end of an 8 mm shaft (21) to a shaft holder (3DP 2) according to **Figure 5A,D,G**.

NOTE: It may be necessary to loosen the M12 washers on the front panel (acrylic part 10) to create space to place the printing bed sub-assembly into the frame.

3.4. Finally, to complete the y-axis and printing bed sub-assembly, screw the idler to the belt idler (3DP 1) by using an M3 screw, then secure the idler toothed to the stepper motor by tightening the M2 grub screw on the idler toothed with the M2 Allen key. Slide the belt (17) around the idler (17) and idler toothed (17) and into the belt clamp (3DP 3) to produce tension in the belt. Complete the section by tightening the belt clamp (3DP 3) with the M3 Allen key.

[Place **Figure 4** here]

[Place **Figure 5** here]

4. X-axis sub-assembly

NOTE: The parts outlined in **Figure 6** are required to finish the x-axis sub-assembly. All screws are seen in **Figure 6,** and tools are listed in the **Table of Materials**.

4.1. Using the parts in **Figure 6**, assemble the left side of the x-axis sub-assembly according to **Figure 7C**.

4.1.1. Place the brass nut (18) inside of the nut holder (3DP 5) and secure to the x-axis pillow left (3DP 8) using the M3 screws and Allen key (**Figure 7C**).

4.1.2. Secure the pillow block (19) onto the x-axis pillow left (3DP 8) using the M4 screws and Allen key (**Figure 7C**). Secure the x-axis idler 1 (3DP 9) to the x-axis pillow left (3DP 8) using the M3 screws and Allen key (**Figure 7C**).

4.1.3. Align the center holes of the idler (17), x-axis idler 1 (3DP 9), and x-axis Idler 2 (3DP 10). Secure using the M3 screws and Allen key (**Figure 7C**). Using the parts shown in **Figure 6**, assemble the right side of the x-axis sub-assembly according to **Figure 7D**.

4.1.4. Place the brass nut (18) inside of the nut holder (3DP 5) and secure to the x-axis pillow right (3DP 6) using the M3 screws and Allen key (**Figure 7D**).

4.1.5. Secure the pillow block (19) onto the x-axis pillow right (3DP 6) using the M4 screws and

Allen key (**Figure 7D**). Secure the x-axis right (3DP 7) to the x-axis pillow right (3DP 6) using the M3 screws and Allen key (**Figure 7D**). Secure the stepper motor (11) to the x-axis right (3DP 7) using the M3 screws and Allen key (**Figure 7D**).

4.2. Thread each of the threaded rods (18) into each of the brass nuts (18) according to **Figure 7B**. Slide two of the 8 mm shafts (20) into each of the pillow blocks (19) vertically, and two of the 8 mm shafts (20) horizontally according to **Figure 7B,C,D.**

4.3. Secure the remaining parts from **Figure 6** to the frame according to **Figure 7E,F**.

4.3.1. Secure two of the shaft holders (3DP 2) to both the top panel (acrylic part 2) and electronics enclosure top (acrylic part 5) using the M2 screws and Allen key (**Figure 7E,F**). Secure the pillow block bearings (15) onto the top panel (acrylic part 2) using the M3 screws and Allen key (**Figure 7E**). Secure the stepper motors (11) onto the electronics enclosure top (acrylic part 5) using the M3 screws and Allen key (**Figure 7F**).

NOTE: The coupler (16) is a component that is designed to connect two different shaft sizes.

4.3.2. Secure the coupler (16) over the shafts of the stepper motors (11) by tightening the lower grub screw with the M2 Allen key (**Figure 7F**).

4.4. Place the x-axis sub-assembly into the frame by aligning the vertical 8 mm shafts with the shaft holder (3DP 2) and tighten using the M2 screws and Allen key (**Figure 7E,F**). Secure the threaded rod (18) into the other end of the coupler (16) by tightening the upper grub screw with the M2 Allen key (**Figure 7E,F**).

NOTE: The top panel (acrylic part 2) may need to be temporarily removed so that the x-axis sub assembly can fit into the frame.

[Place **Figure 6** here]

[Place **Figure 7** here]

5. Extrusion sub-assembly

NOTE: The extrusion sub-assembly utilizes a dual stepper motor design to ensure that a high level of accuracy is achieved through the balancing of forces on each side of the plunger. The parts outlined in **Figure 8** are required to finish the extrusion sub-assembly.

5.1. Gather all parts shown in **Figure 8** and assemble the extrusion head according to **Figure 9**.

NOTE: **Figure 9B** is an exploded view of the extruder sub-assembly that shows how each component fits together. The following steps explain how this is done. All screws are seen in **Figure 8,** and tools are listed in the **Table of Materials**.

5.1.1. Secure the two pillow blocks (19) onto the extruder backplate (3DP 14) using the M4 screws and Allen key (**Figure 9B**). Secure the extruder belt clamp (3DP 13) onto the extruder backplate (3DP 14) between the pillows blocks (19) using the M3 screws and Allen key (**Figure 9B**).

5.1.2. Secure the extruder backplate (3DP 14) to the extruder motor holder (3DP 15) using the M3 hex screws and Allen key (**Figure 9B**). Secure the two stepper motors (11) onto the extruder motor holder (3DP 15) using the M3 hex screws and Allen key (**Figure 9B).**

NOTE: The coupler (16) is a component that is designed to connect two different shaft sizes.

5.1.3. Secure the couplers (16) over the shafts of the stepper motors (11) by tightening the lower grub screw with an M2 Allen key (**Figure 9B**). Secure the threaded screw (18) within the couplers (16) by tightening the upper grub screw (**Figure 9B**).

5.1.4. Slide the heating jacket or silicone heater into the extruder motor holder (3DP 15) according to **Figure 9B**. Secure the brass nuts (18) inside plunger lock 1 (3DP 11) using the M3 screws and Allen key.

5.2. Mount the extrusion head onto the x-axis according to **Figure 9A**.

5.2.1. Slide the 8 mm shafts found on the x-axis into the pillow blocks (19) on the extruder head according to **Figure 9A**.

5.2.2. Wrap the drive belt (17) through the idler (17) and idler toothed (17) located on the left and right x-axis assemblies and secure the drive belt (17) in the extruder belt clamp (3DP 13) using the M3 hex screws and Allen key (**Figure 9C**).

[Place **Figure 8** here]

[Place **Figure 9** here]

6. Electronics and wiring

6.1. Mount the Arduino into acrylic part 7 (electronics shroud, shown in **Figure 10A**) with M3 hex screws using a M3 Allen key. Insert a ramps board on top of the Arduino board oriented as shown in **Figure 10A,B** with the USB plug facing acrylic part 6 (back panel).

6.2. Mount the DC power supply jack in acrylic part 6 (back panel, as shown in **Figure 10A**) and connector to the power supply in **Figure 10B**. Connect the motor controllers, stepper motors, end stops, heater, and thermocouple to the respective pins (**Figure 10B**).

[Place **Figure 10** here]

7. Software, control, and calibration

NOTE: For more detailed instructions and troubleshooting information, see <https://reprap.org/wiki/RAMPS_1.4>.

7.1. Download firmware from <http://marlinfw.org/meta/download/>.

7.2. Install repetier <https://www.repetier.com/>.

7.3. Replace the file .configuration in the firmware found in <https://www.thingiverse.com/Addme/designs>.

7.4. Set buad rate in repetier to 112500 by navigating (in repetier) to **Configure | Printer Settings | Connection | Baud Rate: 115200.**

7.5. Click the **Connect** icon in repetier.

7.6. Once connected, full control over the printer is achieved. Navigate to **Manual Control** to move the printing bed and try setting the temperature.

CAUTION: Make sure that the maximum temperature of the syringe or housing components is not exceeded (see the discussion for more information). While the stepper motors have limited power, the movement of the axis presents a mechanical hazard.

NOTE: At this stage there is a fully operating printer. In the following section (section 8), the procedure for getting the printer ready for 3D printing is described.

8. Preparation for 3D printing

8.1. Load a 2 mL syringe with the desired material, such as viscous cream, chocolate, or pluronic (**Figure 11A**).

8.2. To place the syringe into the extrusion head, start by inserting the syringe into plunger lock 1 (3DP 11, **Figure 11B**). Next, insert the syringe into the heating jacket while carefully turning the threaded screws (**Figure 11C**).

8.3. Optional: if the bed has not been leveled, it is necessary to level it. Move the printing head left and right then up and down, and check if the distance between the bed and syringe nozzle is consistent. Slide a piece of paper between the syringe and bed and feel the friction (**Figure 11E**), then use the M3 Allen key (**Figure 11D**) to adjust the bed level if required.

8.4. Optional: if the chosen material needs to be heated, do this now. Navigate to the **Manual Control** tab in repetier and set the temperature to the desired level.

[Place **Figure 11** here]

REPRESENTATIVE RESULTS:

The performance of ADDME during 3D printing was evaluated using a viscous cream (150 mL, Nivea hand cream), chocolate (Cadbury, plain milk), and Pluronic F-127 (Sigma Aldrich). The viscous cream and chocolate were used as is, and the Pluronic was dissolved into a 20% wt solution with ultrapure water and stored refrigerated at 5 °C until needed[14,15].

Line testing involved printing a filament back and forth on the build plate in a basic pattern to evaluate individual filament properties such as thickness or consistency. Line tests were made with a series of movement commands called gcode as shown in Equation 1 below. The amount of material to extrude can be found using Equation 2. The printing parameters used can be found in Table 1, and results are shown in **Figure 12A,B,C**.

$$G01\ X10\ Y50\ Z0\ E0.014\ F500$$

Equation 1: Representative line of gcode to control 3D printer movement, where: G01 tells the printer to conduct a linear move between the current position and the position specified by X, Y, and Z mm; E is the amount of material to extrude (mm) during this linear move; and F is the speed (mm/min).

$$E = D\,\frac{\left(\frac{syringe\ inner\ diameter}{2}\right)^2}{barrel\ inner\ diameter^2}$$

Equation 2: Extrusion, where: E is the gcode value telling the extruder stepper motor how far down to push the syringe; and D is the distance that the printing head moves during the line of gcode.

To create complex 3D objects, we cannot manually input each line of code, which was done for line testing. To create complex 3D objects, the object to be printed must be inputted into a standard tessellation language (.stl) file into repetier and “sliced” into 3D printable gcode. It is critical that in the slicer configuration manager, the filament diameter is set to the size of the inner barrel diameter and the nozzle is set to the size of the syringe inner diameter. The full list of printing parameters is shown in Table 1, and results are shown in **Figure 12D,E,F**.

[Place **Table 1** here]

[Place **Figure 12** here]

To determine the dimensional accuracy of the ADDME printer in the X, Y, and Z directions when printing a semi-solid material, a 1 cm x 1 cm x 1 cm cube was printed, 3D-scanned, and dimensionally compared against the original cube CAD data. A viscous cream was used to print a 1 cm x 1 cm x 1 cm cube using a nozzle diameter of 0.33 mm (Birmingham Gauge needle 23), layer height of 0.33 mm, and infill of 15%. This cube was then scanned using a metrology rated 3D scanner (Artec Spider) capable of an accuracy up to 0.05 mm. The resulting data was compared using Cloud Compare (Open Source Project), 3D point cloud editing, and processing software.

[Place **Figure 13** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Additive manufacturing melt extrusion (ADDME) 3D printer. (**A**) Computer-generated rendering of the printer. (**B**) Photograph of a finished printer.

Figure 2: Components needed to assemble the frame.

Figure 3: Frame assembly. (**A**) Assembled frame. (**B**) An exploded view with labeled acrylic parts and supporting M10 threaded rods. (**C**) An exploded view showing how each acrylic part is connected to one another, using M3 screws and nuts to hold the frame together. (**D**) An exploded view showing how the threaded rod holds acrylic parts 6, 8, and 9 together with M10 nuts and washers.

Figure 4: Components needed to put together the y-axis and printing bed sub-assembly.

Figure 5: Additive manufacturing melt extrusion (ADDME) 3D printer. (**A**) Graphical rendering of the frame, y-axis, and bed. (**B**) Graphical rendering of the y-axis and bed. (**C**) Exploded view of the bed sub-assembly. (**D**) Labeled view showing how the y-axis connects to the back panel. (**E**) Zoomed-in view of the mechanical endstop. (**F**) Exploded view of the printing plate spring leveling system. (**G**) Labeled view showing how the y-axis connects to the front panel. (**H**) Side view graphical render of the y-axis and bed.

Figure 6: Components needed to put together the x-axis sub-assembly.

Figure 7: X-axis sub assembly. (**a**) Graphical rendering of the frame and x-axis. (**b**) Graphical render of the x-axis. (**c**) Exploded view of the left side of the sub assembly. (**d**) Exploded view of the right side of the sub-assembly. (**e**) Labeled view showing how the x-axis connects to the top panel. (**f**) Labeled view showing how the x-axis connects to the electronics enclosure.

Figure 8: Components needed to assemble the extruder.

Figure 9: Extruder sub-assembly. (**A**) Graphical rendering of the extruder sub-assembly. (**B**) Exploded view showing extruder components.

Figure 10: Electronics. (**A**) Graphical rendering of the electronics control board mounting

location. (**B**) Connection diagram of electrical components and motors to 3D printing board [Jos Hummelink (grabcab.com) provided the Arduino and Ramps CAD files]. (**c**) Image of the finished wiring. Wires can be seen leading from the Ramps board, then to the extrusion head and x/y axis motors.

Figure 11: 3D printing preparation. (**A**) A 2 mL syringe loaded with (from left to right) viscous cream (150 mL, Nivea hand cream), chocolate (Cadbury, plain milk), and Pluronic F-127 (Sigma Aldrich). (**B**) Plunger being inserted into the plunger lock 1 (3DP 11). (**C**) Shown is a syringe being inserted into the heating jacket, while the threaded screws are catching on the brass nuts. (**D**) Shown is an Allen key about to be inserted into the retaining M3 hex screw, allowing the level to be adjusted. (**E**) A business card is then slid under the syringe to check the distance between the bed and syringe.

Figure 12: ADDME 3D printing results. (**A)** Line testing with viscous cream. (**B**) Line testing with chocolate. (**C**) Line testing with Pluronic F-127. (**D**) Custom-made object 3D-printed with viscous cream. (**E**) Custom-made object 3D-printed with chocolate. (**F**) Custom-made object 3D-printed with Pluronic F-127.

Figure 13: 3D Scanning Comparison. (**A**) The 1 cm x 1 cm x 1 cm cube made into a CAD model. (**B**) The 3D scan of the printed cube (inset). (**C**) The original model and 3D scan were then compared using cloud compare. A histogram of distances from nodes in the 3D model and scanned cube are presented. The C2M distances represent the physical differences between points in both models. Both models are within a tolerance of -0.15 mm and +0.15 mm.

Table 1: Printing parameters used throughout all tests.

DISCUSSION:

This protocol provides detailed instructions for constructing a low-cost melt extrusion-based 3D printer. Construction of the 3D printer can be broken down into subsections including frame, y-axis/bed, x-axis, extruder, electronics, and software. These subsections are presented with detailed diagrams, drawings, files and parts lists. The total price of an ADDME 3D printer comes to $343 AUD ($245 USD as of 01/17/2019), making this the cheapest, reservoir-based melt extrusion 3D printer currently known. It was aimed to make this device simple to manufacture through the use of laser-cut, 3D-printed, and off-the-shelf components. The functioning of this device has been demonstrated by line testing and 3D printing of organically shaped objects. The applicability of ADDME to diverse applications such as the biomedical and food industries has been demonstrated using viscous cream, chocolate, and Pluronic F-127 (as a model for bioinks).

3D printing parts for use in the construction of ADDME can be complicated due to difficulties arising from the differences in quality between each 3D printed object. Warping, shrinking, or expansion of 3D printing parts is known to be influenced by printing parameters and environmental factors. The use of polylactic acid (PLA) should significantly reduce errors that arise from shrinkage, expansion, or warping; however, environmental factors such as humidity can still cause problems. To minimize any potential issues, it should be ensured that 1) the printing parameters match those specified on <https://www.thingiverse.com/Addme/designs>, 2) the PLA filament is new (not affected by humidity), and 3) there is no airflow over the 3D printer (increased airflow can cause warping). All 3D-printed parts used in construction of ADDME have been specifically designed to be easy-to-print and do not require additional support material for overhanging geometry.

Also included are two methods to heat the syringe holding the printing material. The first option is a machined heating jacket with a heating cartridge, and the second is a silicone heating mat. The machined heating jacket provides uniform heating to the whole syringe and is recommended to be made from aluminum for high thermal conductivity. It may be difficult for individuals without proper expertise or access to facilities to procure a heating jacket. In this case, a silicone heater can be wrapped around the syringe to provide sufficient heating to the material. In both cases, the heating component is connected to the same pins on the electronics board and is controlled the same way.

The maximum temperature that can be applied to the syringe is limited by the syringe material and 3D printed materials surrounding the syringe. If a generic PLA is used, then the maximum temperature that can be applied to the syringe is ~60 °C; however, specialty high temperature PLA can be used to achieve a maximum temperature of ~110 °C. The syringe itself is made from a polypropylene (PP) barrel and high density polyethylene (HDPE) plunger. The syringe specified in this protocol does not specify a maximum operating temperature, but it is safe up to approximately 110 °C due to the jacket materials. It should be noted that syringes not listed in the **Table of Materials** may be made from materials with a lower melting point.

The results in **Figure 12** demonstrate the operation of this 3D printing system through line testing and object printing. When line testing, different printing parameters are used with viscous cream, chocolate, and Pluronic F-127 (**Table 1**) to achieve different results. The small nozzle size used with hand cream (**Figure 12A)** results in a thinner line, while the lower syringe to plate distance results in sharper corners. For chocolate, it was difficult to get a consistent flow of chocolate (**Figure 12B**), even with the flow set to 200%. In **Figure 12D,E,F**, it is clear that the chocolate and Pluronic F-127 show worse shape-retaining properties than the viscous cream as the height of the cone is reduced. Each of the printing parameters listed in **Table 1** have a significant impact on the final geometry of the filament produced, including syringe diameter, syringe-to-plate distance, temperature, speed, and extrusion.

The 3D cloud comparison of the CAD model and 3D scanned 1 cm x 1 cm x 1 cm cube in **Figure 13** show that the ADDME printer is capable of printing with a tolerance between -0.15 mm and +0.15 mm. There is a larger variance into the positive section when compared to the negative distances. This tends to occur at the base layers of the 3D printed parts, where the layers are programmed to print more thickly; as such, over-extruding occurs, and the needle tip drags additional print material over the part, as shown in **Figure 13B**. Additional geometrical accuracy may be achieved through finer tuning of printer parameters such as initial layer height and speed, extrusion flow rate, and ensuring that the build plate is level. These results indicate that the

ADDME printer is capable of achieving a level of print accuracy required for printing semi-solid materials such as viscous cream, chocolate, or Pluronic F-127.

The successful design and construction of the ADDME 3D printer has been verified by printing lines and objects made from different materials and printing parameters. It is demonstrated that there is an application of this printer in the biofabrication and food industries. The ADDME printer has improved upon previous generations of entry-level, reservoir-based, melt extrusion printers by reducing costs, minimizing the number of components, and using the latest electronic and software components/practices. The open-source nature of this project shows that in the future, other users can make changes or alterations for specific applications.

ACKNOWLEDGMENTS:

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DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Brettel, M., Friederichsen, N., Keller, M., Rosenberg, M. How Virtualization, Decentralization and Network Building Change the Manufacturing Landscape: An Industry 4.0 Perspective. *World Academy of Science, Engineering and Technology International Journal of Information and Communication Engineering.* **8** (1), (2014).
2. Gilchrist, A. Introducing Industry 4.0. *Industry 4.0.* 195–215, doi:10.1007/978-1-4842-2047-4_13 (2016).
3. Petrick, I. J., Simpson, T. W. 3D Printing Disrupts Manufacturing: How Economies of One Create New Rules of Competition. *Research-Technology Management.* **56** (6) 12–16 (2013).
4. Wong, K., Hernandez, A. A Review of Additive Manufacturing. *ISRN Mechanical Engineerin*g. **10** (2012).
5. Lanaro, M., Desselle, M. R., Woodruff, M. A. 3D Printing Chocolate: Properties of Formulations for Extrusion, Sintering, Binding and Ink Jetting. *Fundamentals of 3D Food printing and Applications.* (2018).
6. Godoi, F. C., Prakash, S., Bhandari, B. R. 3d printing technologies applied for food design: Status and prospects. *Journal of Food Engineering.* **179**, 44–54 (2016).
7. Stansbury, J. W., Idacavage, M. J. 3D printing with polymers: Challenges among expanding options and opportunities. *Dental Materials.* **32** (1), 54–64 (2016).
8. Zhu, W., Ma, X., Gou, M., Mei, D., Zhang, K., Chen, S. 3D printing of functional biomaterials for tissue engineering. *Current Opinion in Biotechnology.* **40**, 103–112 (2016).
9. Lanaro, M., Booth, L., Powell, S. K., Woodruff, M. A. Electrofluidodynamic technologies for biomaterials and medical devices: melt electrospinning. *Electrofluidodynamic Technologies (EFDTs) for Biomaterials and Medical Devices.* 37–69, doi:10.1016/B978-0-08-101745-6.00003-7 (2018).

10. Malone, E., Lipson, H. Fab@Home: the personal desktop fabricator kit Article information. *Rapid Prototyping Journal.* **13** (4), 245–255 (2007).
11. Vilbrandt, T., Malone, E., Lipson, H., Pasko, A. Universal Desktop Fabrication. *Heterogeneous Objects Modelling and Applications.* 259–284, doi:10.1007/978-3-540-68443-5_11 (2008).
12. Jones, R. *et al.* RepRap-the replicating rapid prototyper. *Robotica.* **29**, 177–191 (2011).
13. Lanaro, M. *et al.* 3D printing complex chocolate objects: Platform design, optimization and evaluation. *Journal of Food Engineering.* doi:10.1016/j.jfoodeng.2017.06.029 (2017).
14. Wu, W., DeConinck, A., Lewis, J. A. Omnidirectional Printing of 3D Microvascular Networks. *Advanced Materials.* **23** (24), H178–H183 (2011).
15. Paxton, N., Smolan, W., Böck, T., Melchels, F., Groll, J., Jungst, T. Proposal to assess printability of bioinks for extrusion-based bioprinting and evaluation of rheological properties governing bioprintability. *Biofabrication.* **9** (4), 044107 (2017).

Figure 1

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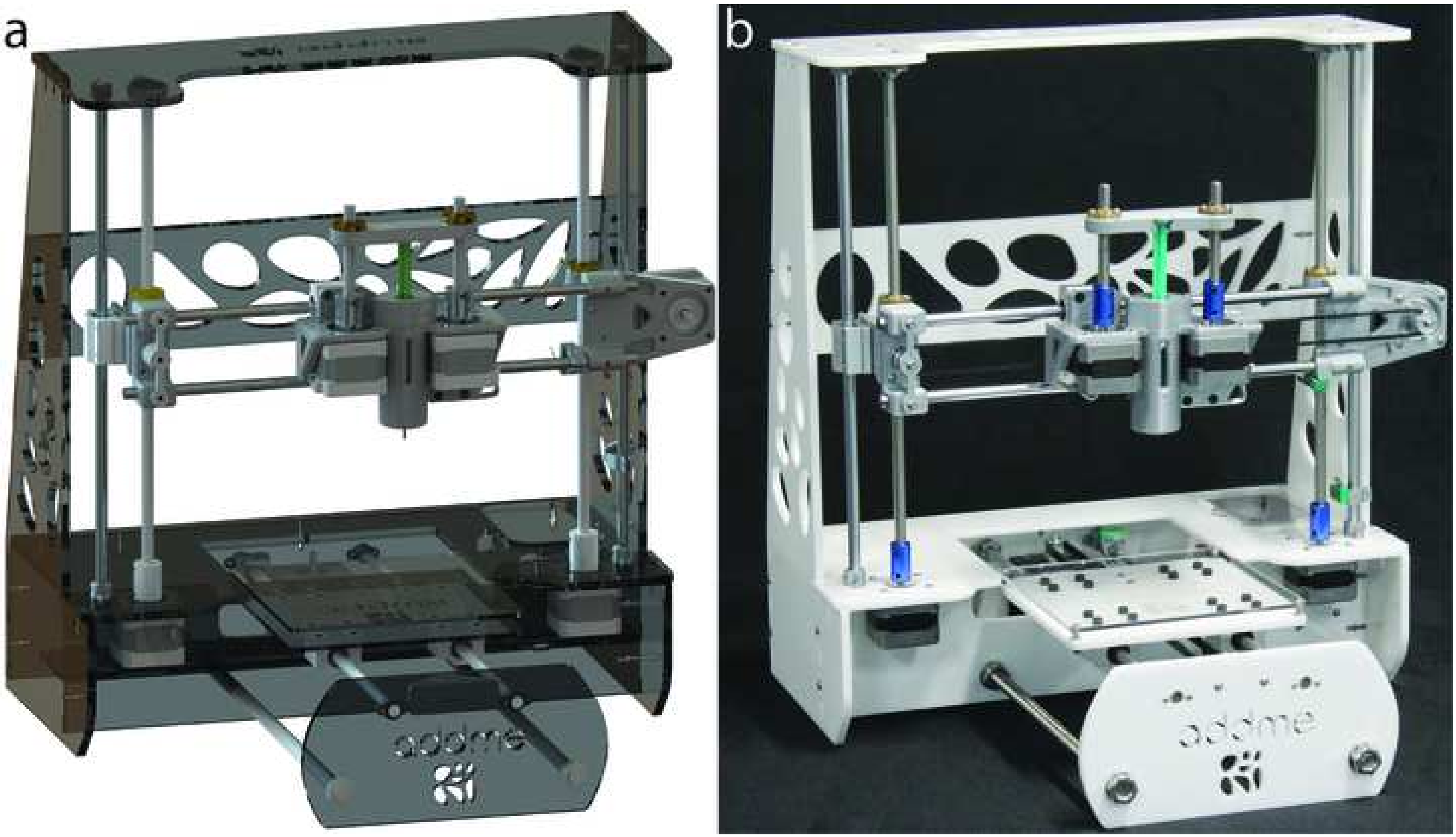

Figure 2

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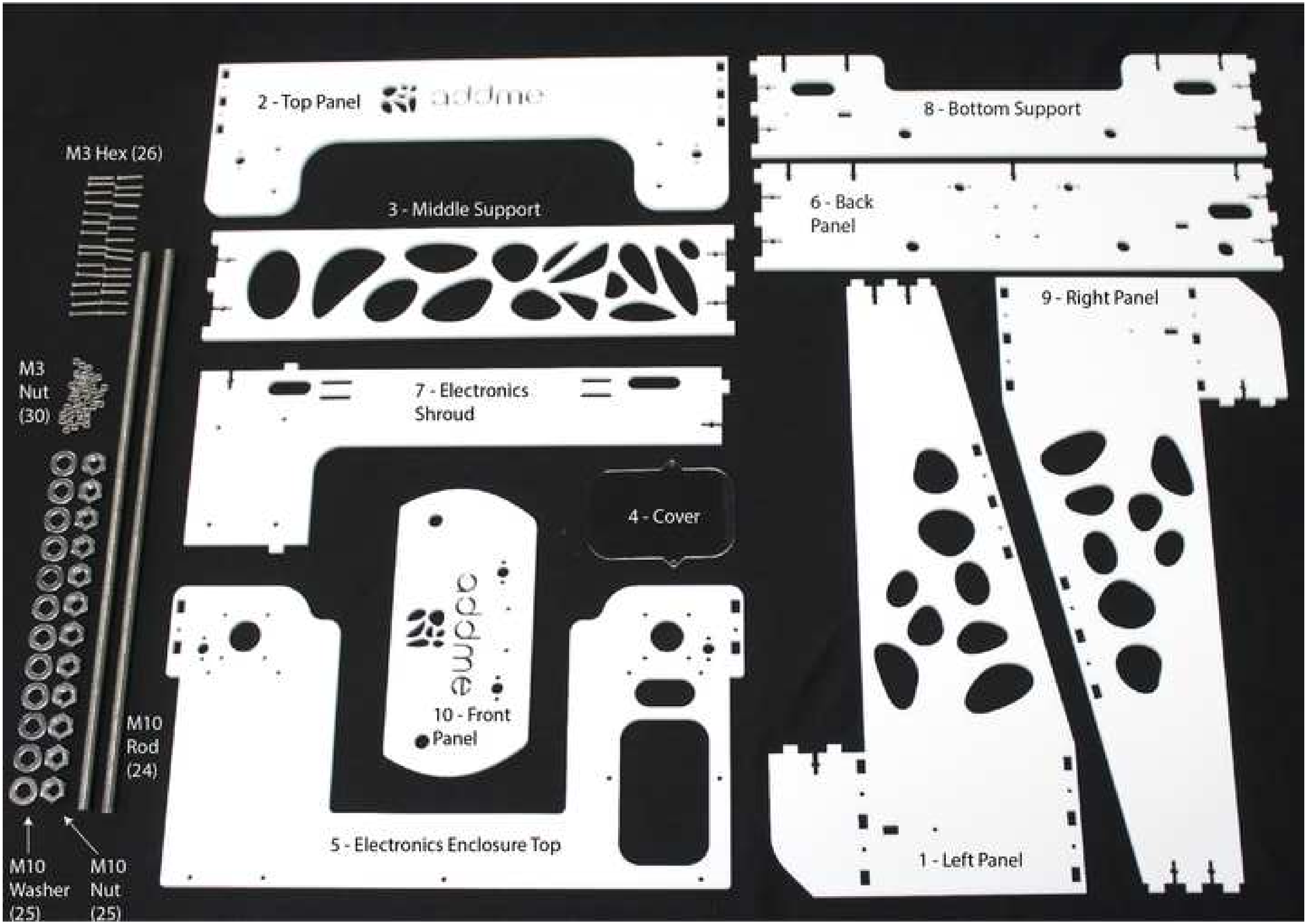

Figure 3

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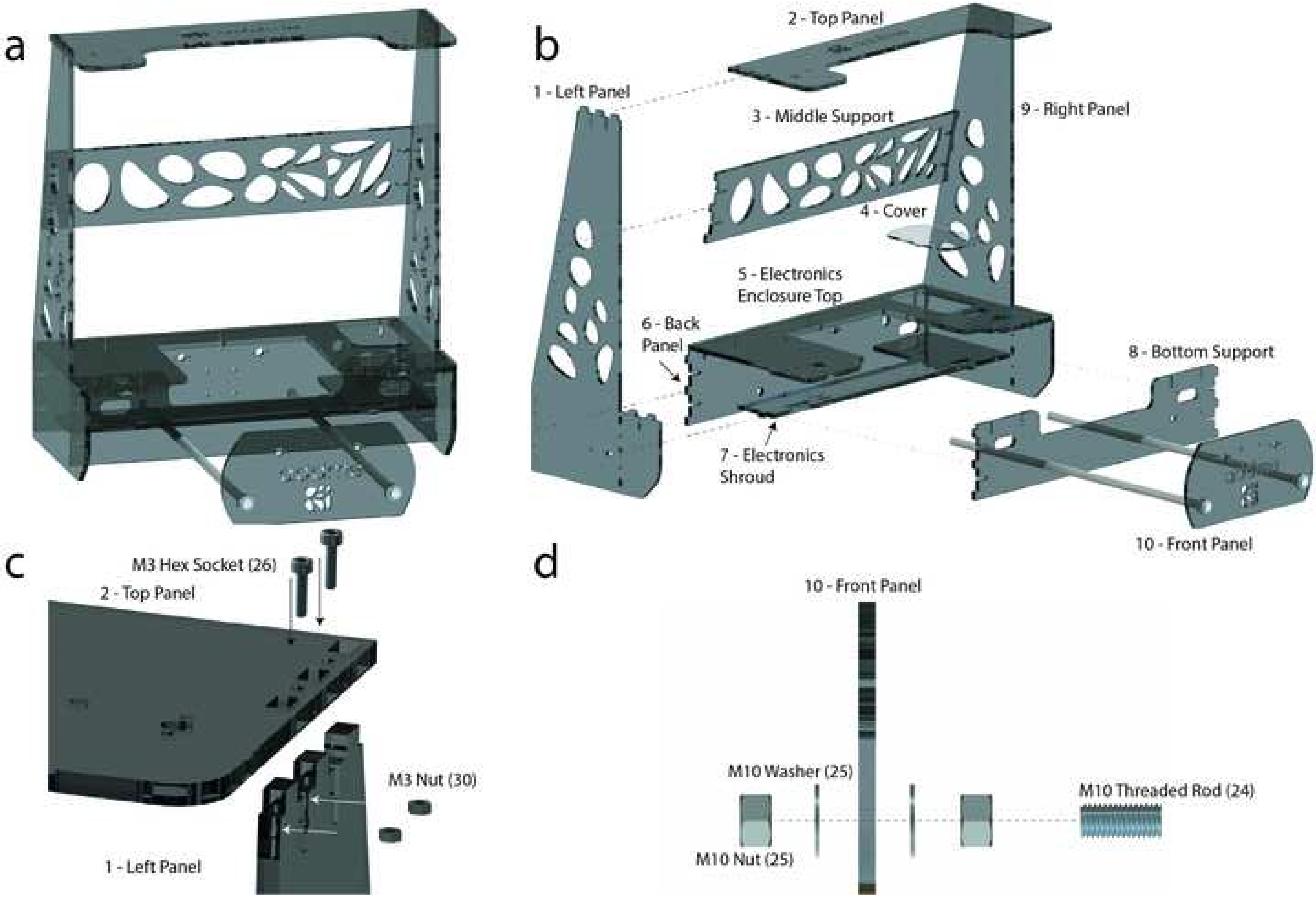

Figure 4

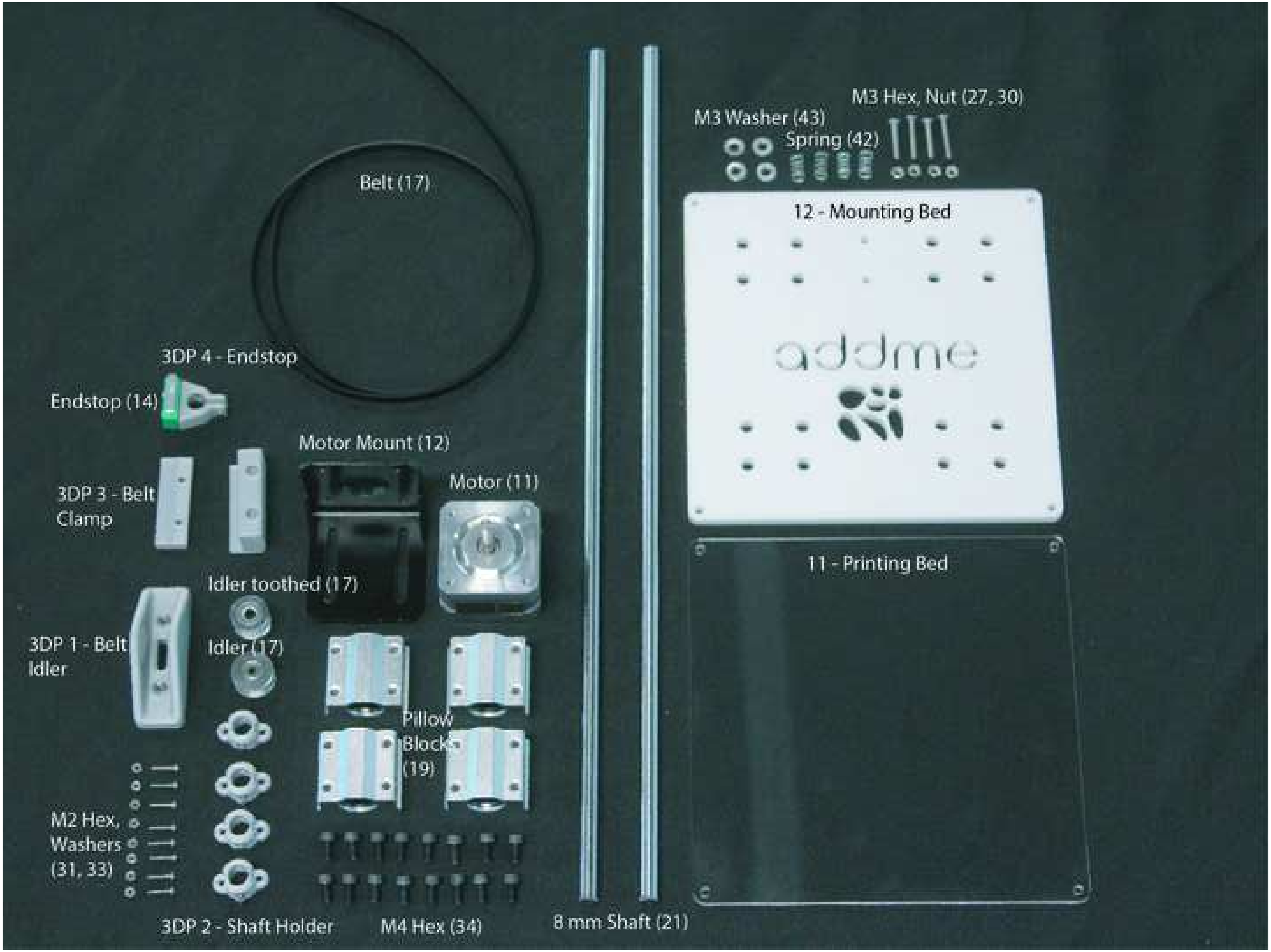
M3 Hex, Nut (27, 30)
M3 Washer (43)
Spring (42)
Belt (17)
12 - Mounting Bed
addme
3DP 4 - Endstop
Endstop (14)
Motor Mount (12)
Motor (11)
3DP 3 - Belt Clamp
11 - Printing Bed
Idler toothed (17)
3DP 1 - Belt Idler
Idler (17)
Pillow Blocks (19)
M2 Hex, Washers (31, 33)
3DP 2 - Shaft Holder
M4 Hex (34)
8 mm Shaft (21)

Figure 5

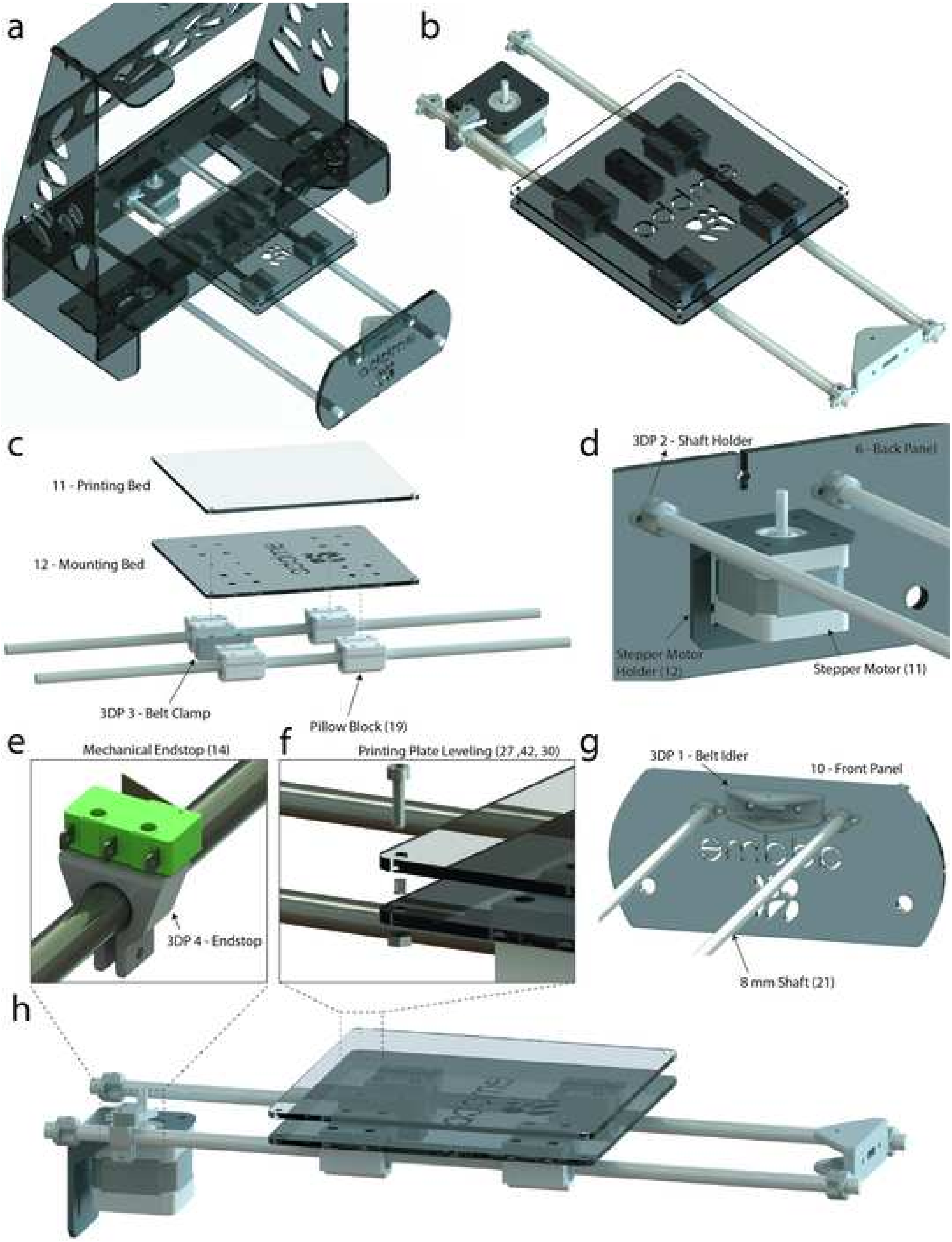
a
b
c
11 - Printing Bed
12 - Mounting Bed
3DP 3 - Belt Clamp
Pillow Block (19)
d
3DP 2 - Shaft Holder
6 - Back Panel
Stepper Motor Holder (12)
Stepper Motor (11)
e
Mechanical Endstop (14)
3DP 4 - Endstop
f
Printing Plate Leveling (27 ,42, 30)
g
3DP 1 - Belt Idler
10 - Front Panel
8 mm Shaft (21)
h

Figure 6

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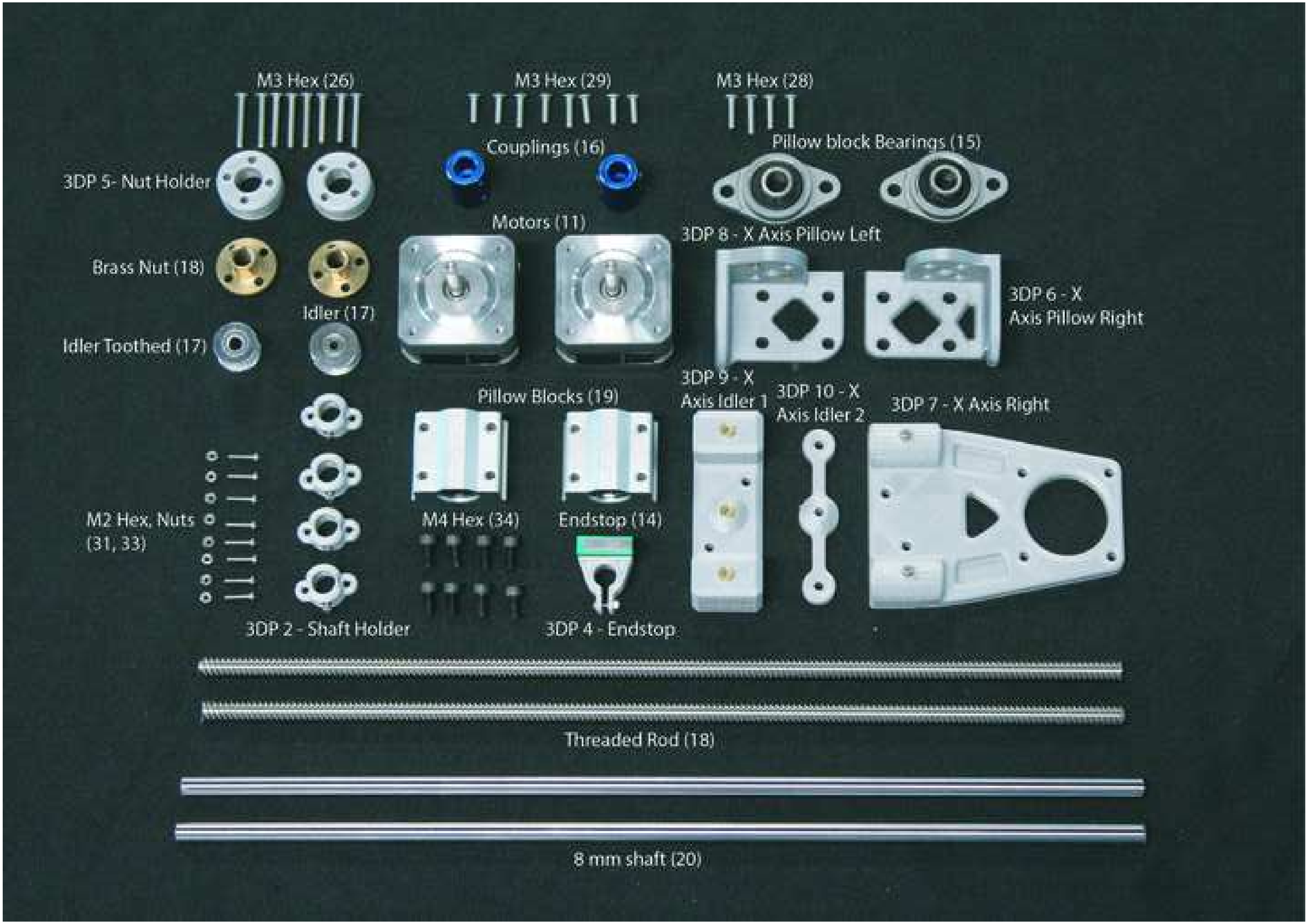

Figure 7

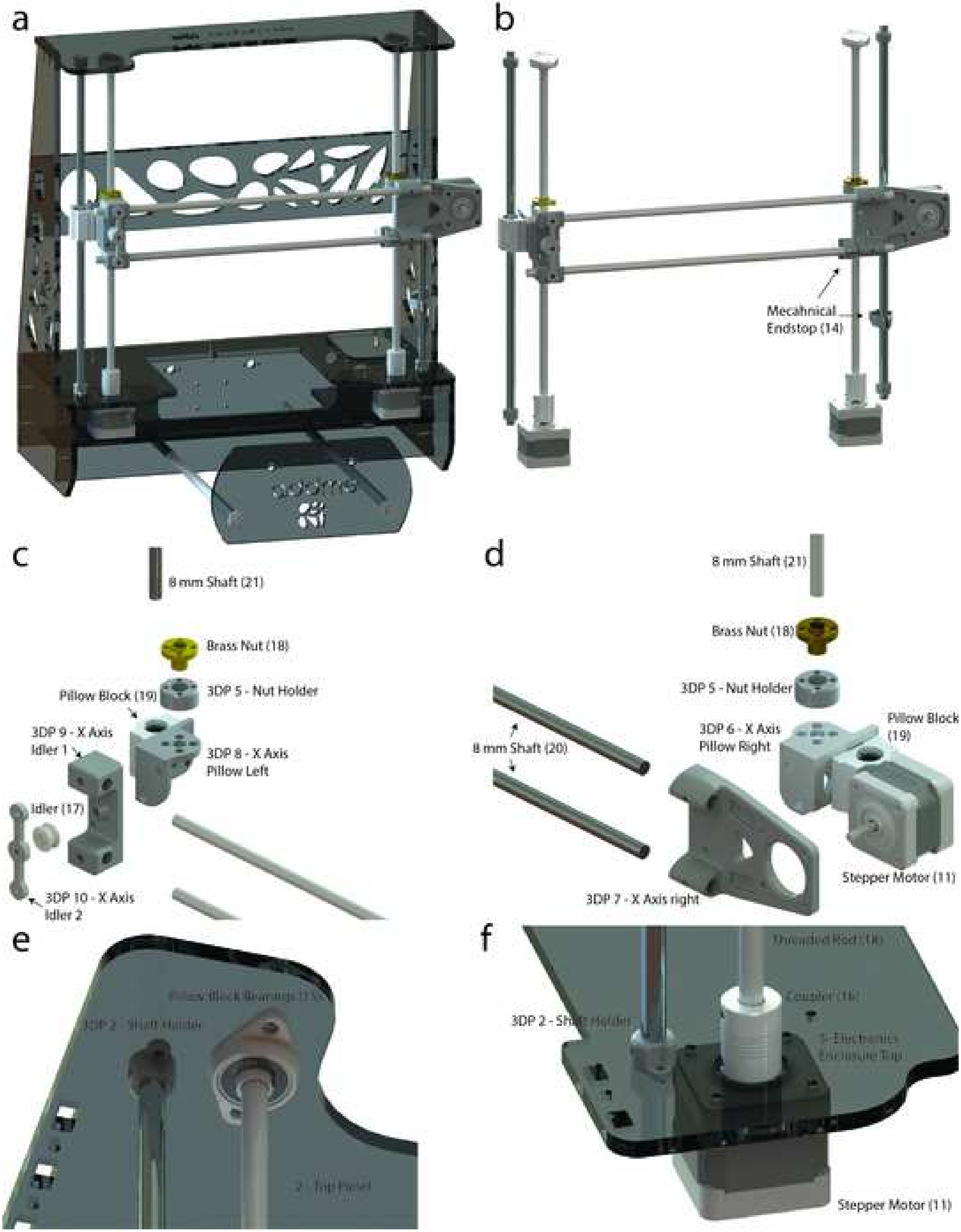
a
b
Mecahnical
Endstop (14)
c
8 mm Shaft (21)
Brass Nut (18)
Pillow Block (19)
3DP 5 - Nut Holder
3DP 9 - X Axis
Idler 1
3DP 8 - X Axis
Pillow Left
Idler (17)
3DP 10 - X Axis
Idler 2
d
8 mm Shaft (21)
Brass Nut (18)
3DP 5 - Nut Holder
3DP 6 - X Axis
Pillow Right
Pillow Block
(19)
8 mm Shaft (20)
Stepper Motor (11)
3DP 7 - X Axis right
e
f
Stepper Motor (11)

Figure 8

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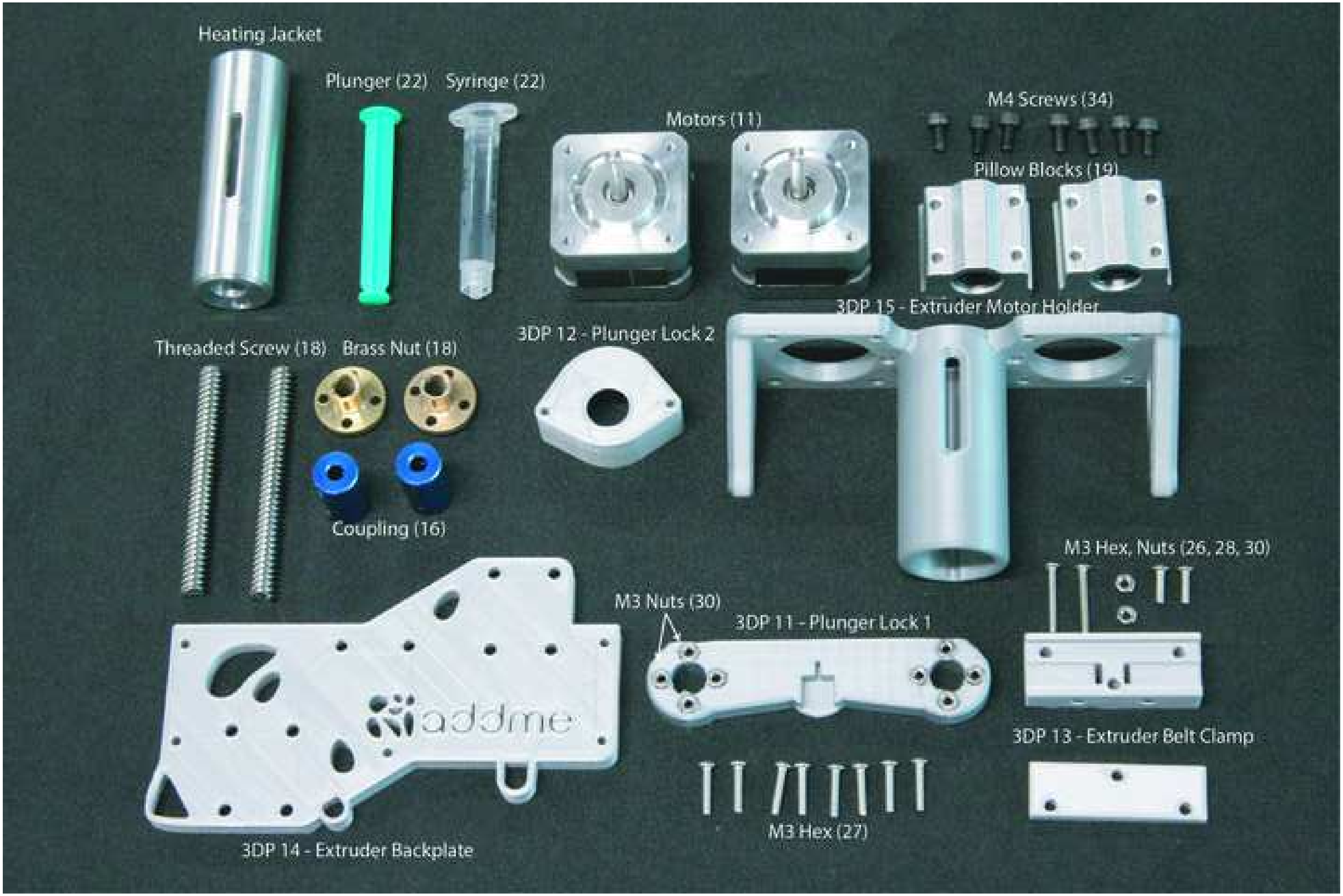

Figure 9

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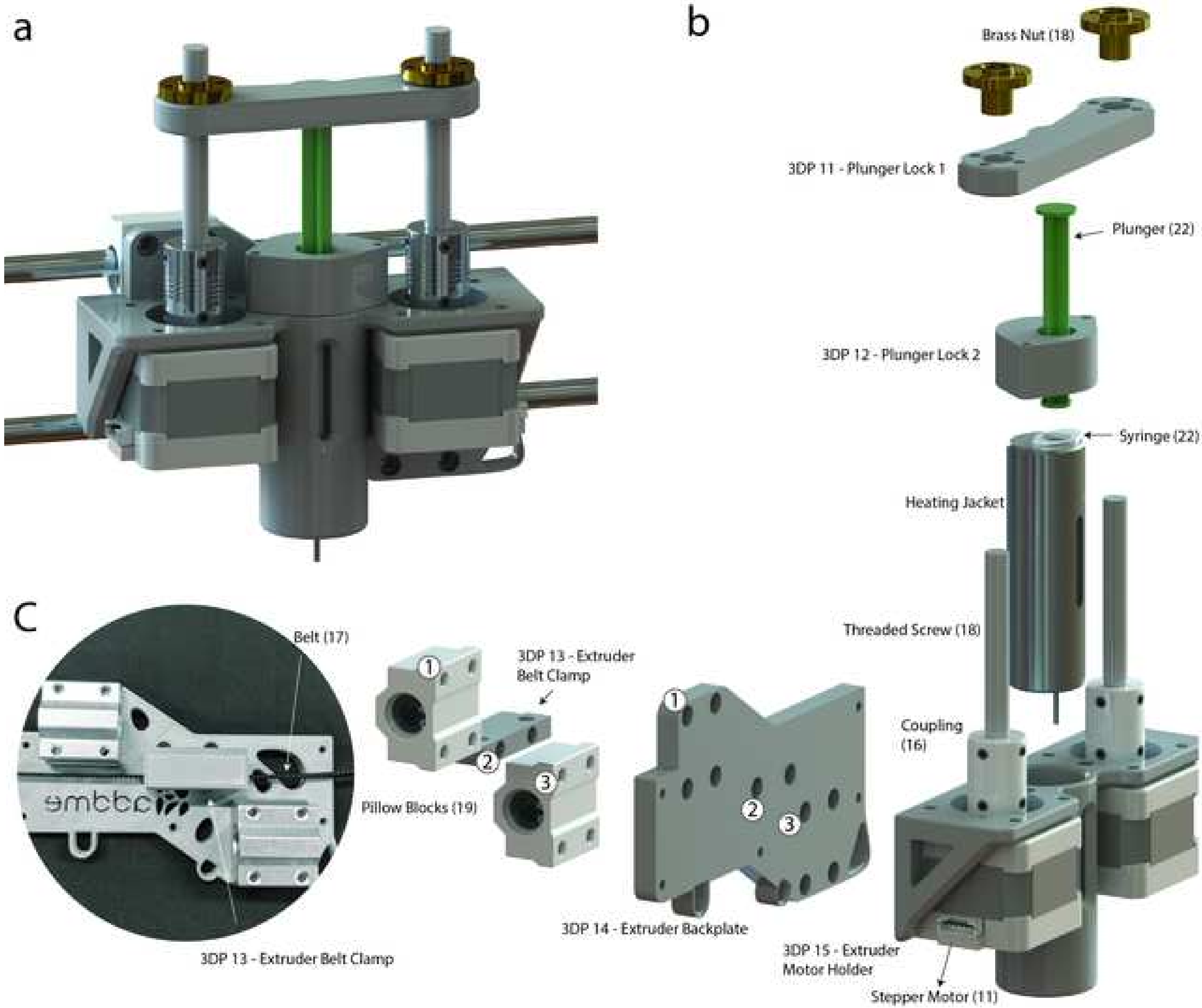

Figure 10

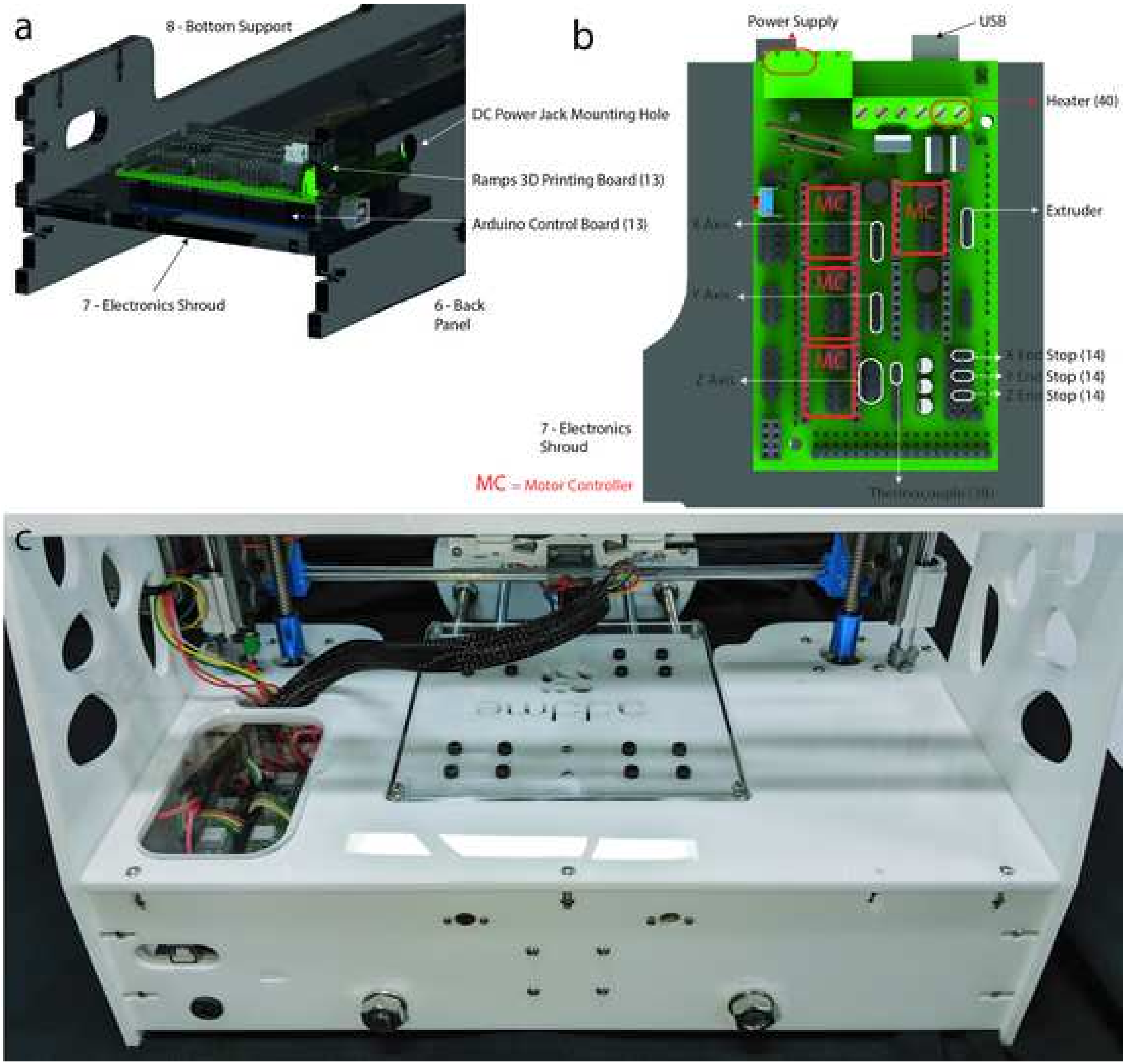
a
8 - Bottom Support
DC Power Jack Mounting Hole
Ramps 3D Printing Board (13)
Arduino Control Board (13)
7 - Electronics Shroud
6 - Back Panel
b
Power Supply
USB
Heater (40)
Extruder
MC
Stop (14)
Stop (14)
Stop (14)
7 - Electronics Shroud
MC = Motor Controller
c

Figure 11

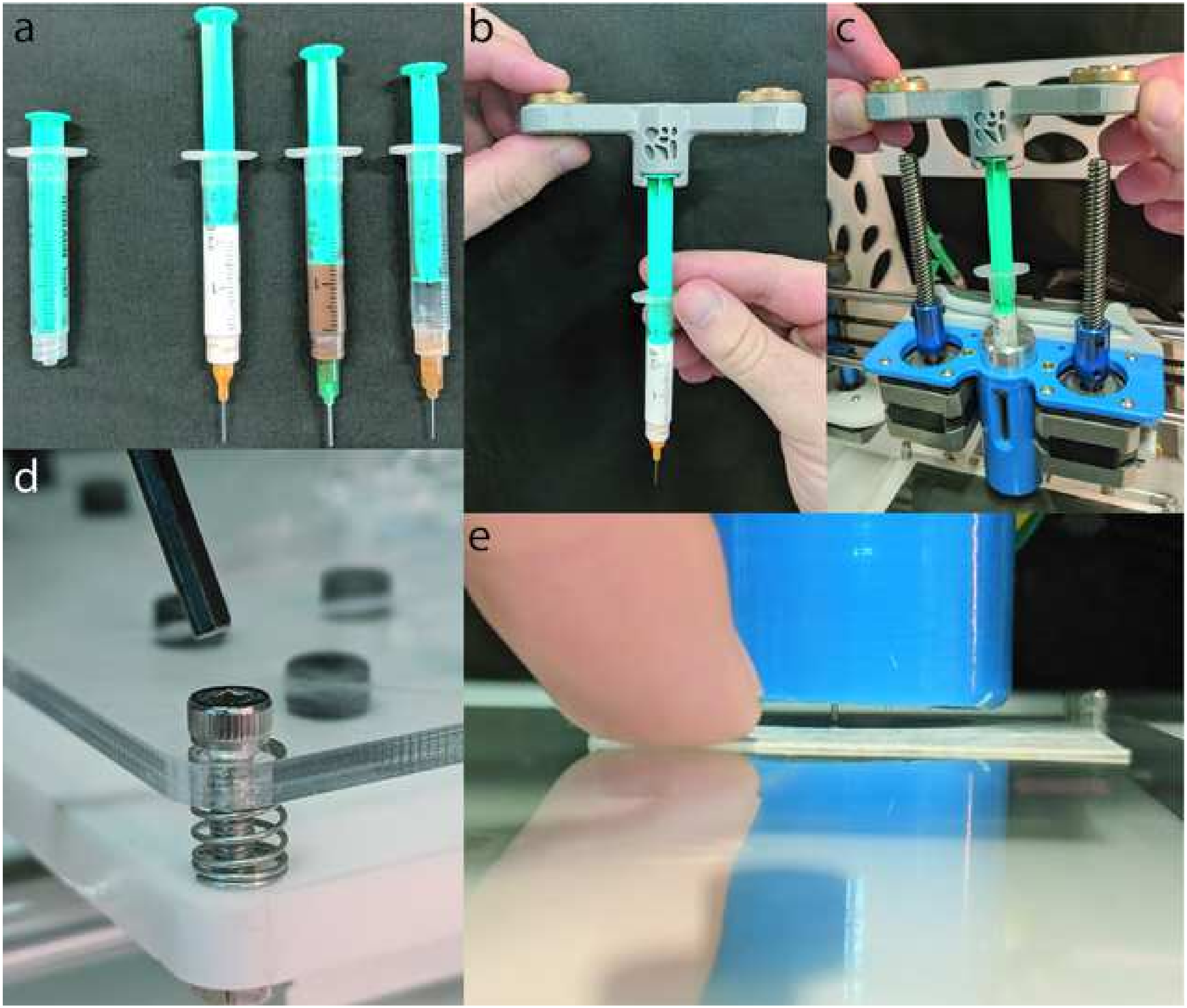

Figure 12

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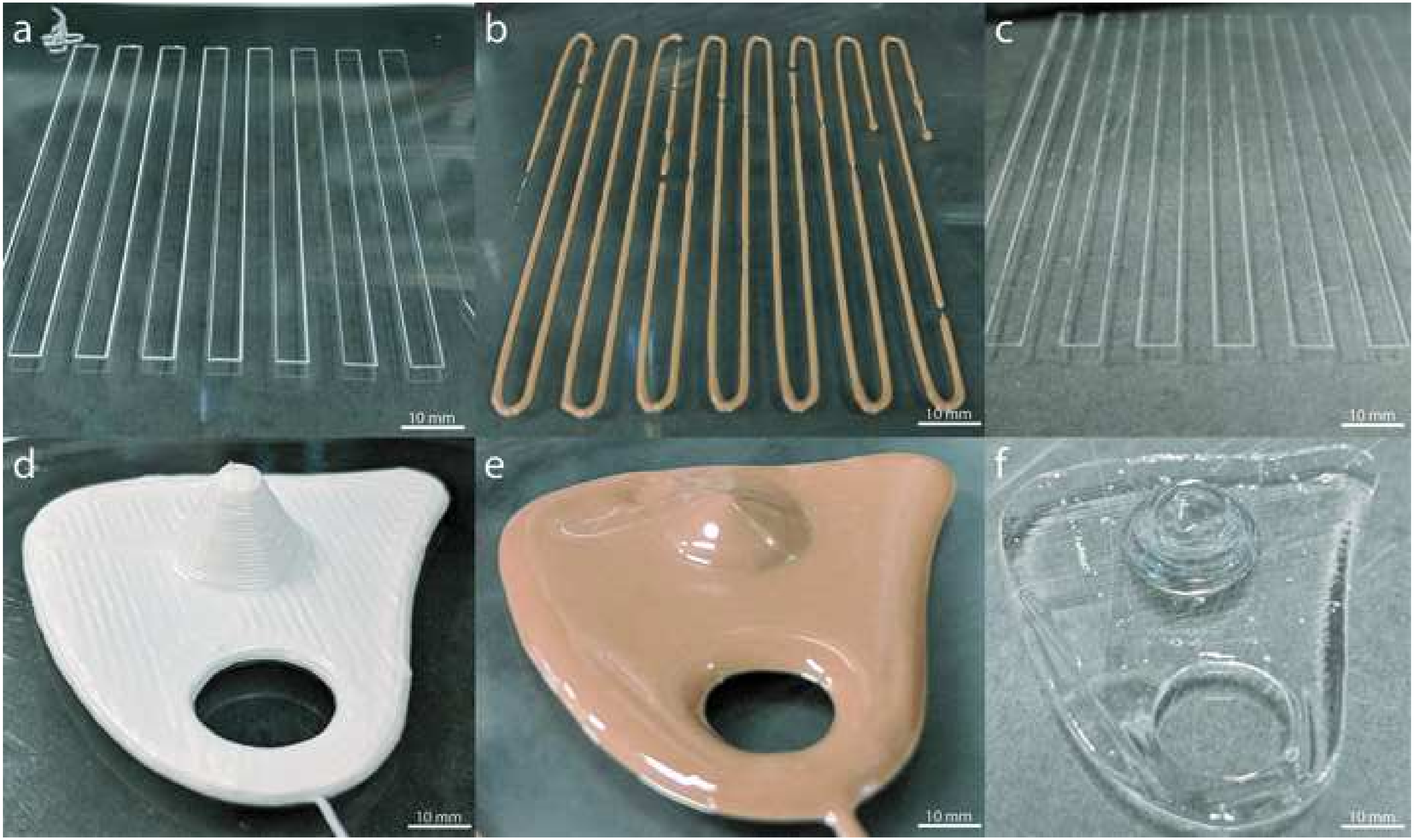

Figure 13

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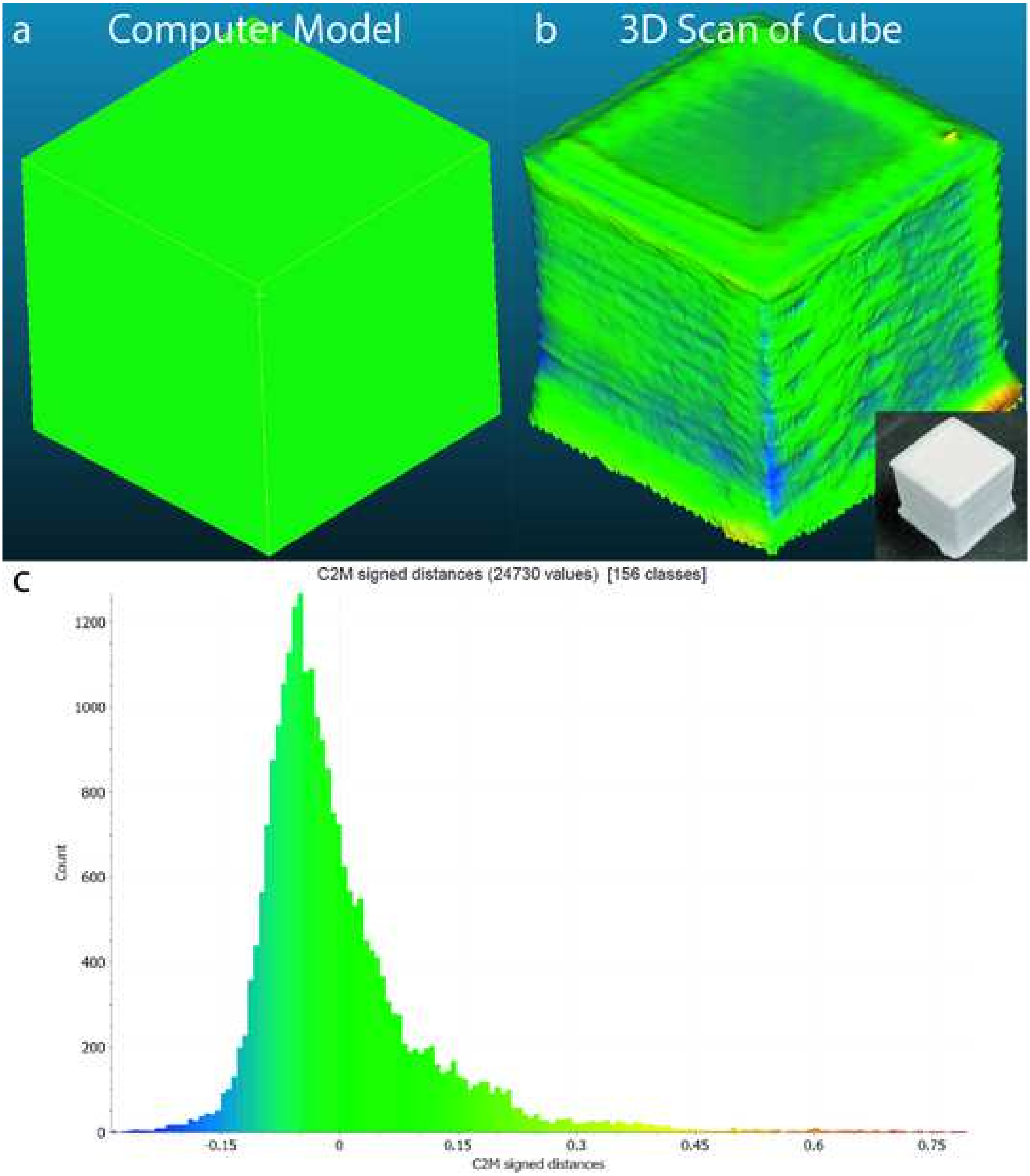

	Line Testing			3D Object	
Parameters	**Viscous Cream**	**Chocolate**	**Bioink**	**Viscous Cream**	**Chocolate**
Syringe Inner Diameter (mm)	0.33	0.84	0.33	0.33	0.84
Barrel Inner Diameter (mm)	9.35	9.35	9.35	9.35	9.35
Temperature (°C)	Room Temp	53	Room Temp	Room Temp	53
Speed (mm/min)	500	500	500	500	500
Extrusion (scalar)	100%	200%	150%	100%	200%
Syringe to Plate Distance (mm)	~0.3	~1	~0.5	~0.3	~1

Bioink
0.33
9.35
:oom Temp
500
150%
~0.5

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
15 W 12V DC 50x100mm Flexible Silicon Heater	Banggood	1280175	Optional; AU$4.46
3D Printer	Lulzbot		https://download.lulzbot.com/TAZ/6.02/documentation/manual/9780989378482_interior_r6.
3D Printer	Ultimaker	Ultimaker 2+	
AC 100-240V to DC 12V 5A 60W Power Supply	Banggood	994870	AU$12.7
Acrylic Sheet White Continuous Cast 1200x600mm	Mulford Plastics		AU$36.95
Allen Keys	Metric		
Arduino MEGA2560 R3 with RAMPS 1.4 Controller	Geekcreit	984594	AU$28.91
Carbon Steel Linear Shaft 8mm x 350mm	Banggood	1119330	AU$13.44
Carbon Steel linear Shaft 8mm x 500mm	Banggood	1276011	AU$19.42
Chocolate	Cadbury		
Computer with internet access	Dell		
Coupler 5-8mm	Banggood	1070710	AU$6.93
Hand Cream	Nivea	80102	
Heating Cartridge	Creality 3D	1192704	AU$4.75
K Type Temperature Sensor Thermocouple	Banggood	1212169	AU$2.37
Laser Cutter	trotec	Speedy 300	https://www.troteclaser.com/fileadmin/content/images/Contact_Support/Manuals/Speedy-4
M10 1mm Pitch Thread Metal Hex Nut + Washer	UXCELL		AU$8.84
M10 1mm Pitch Zinc Plated Pipe 400mm Length	UXCELL		AU$11.62
M2 - 0.4mm Internal Thread Brass Inserts	Ebay		AU$5.65
M2 Nuts	Suleve	1239291	AU$9.17
M2 x 10 mm Button Hex Screws	Suleve	1239291	AU$9.17
M2 x 5mm Button Hex Screws	Suleve	1239291	AU$9.17
M3 - 0.5mm Internal Thread Brass Inserts	Suleve	1262071	AU$7.5
M3 Nuts	Suleve	1109208	AU$7.85
M3 Washer	Banggood	1064061	AU$3.05
M3 x 10mm Button Hex Screws	Suleve	1109208	AU$7.85
M3 x 20mm Button Hex Screws	Suleve	1109208	AU$7.85
M3 x 6mm Button Hex Screws	Suleve	1109208	AU$7.85
M3 x 8mm Button Hex Screws	Suleve	1109208	AU$7.85
M4 x 8mm Button Hex Screws	Suleve	1273210	AU$4.32
Needle Luer Lock 18 - 27 Gauge	Terumo	TGA ARTG ID: 130227	AU$3.57
NEMA 17 Stepper Motor	Casun	42SHD0001-24B	AU$54
NEMA Stepper Motor Mounting Bracket	Banggood	ptNema17br90	AU$4.79
Pillow Block Flange Bearing 8mm	Banggood	KFL08	AU$5.04
PLA Filament	Creality 3D	1290153	AU$24.95
Pluronic F127	Sigma Aldrich	P2443-250G	
SC8UU 8mm Linear Motion Ball Bearing	Toolcool	935967	AU$21.6
SG-5GL Micro Limit Switch	Omron	1225333	AU$4.5
Soldering Station			Solder, Wires, Heat shrink e.c.t.
Spring	Banggood	995375	AU$2.53
Syringe 3ml Luer Lock Polypropylene	Brauhn	9202618N	AU$3.14
Timing Pulley GT2 20 Teeth and Belt Set	Banggood	10811303	AU$11.48
Trapezoidal Lead Screw and Nut 8mm x 400mm	Banggood	1095315	AU$29.02
Variable Spanner			

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General Response,

Dear Editor,

We would like to thank yourself and all of the reviewers for taking the time to provide much appreciated feedback of our work. We believe that with your help we have been able to produce a superior manuscript that will provide the Journal of Visualized Experiments and wider bioprinting and food printing communities with a valuable piece of work. We have taken the time to address each of the concerns.

We noted a few recurring themes in the feedback,

1. The title mentioned that we designed a "Bioink" printer but did not use a bioink to characterise the performance.
2. More information about how to go about printing an object is needed.
3. The accuracy of the printer needed to be validated through our results.
4. The layout of the protocol was hard to follow and did not proceed in a stepwise manner.

Firstly, to make sure that our printer has wide appeal, we have introduced another material into our results section, Pluronic F-127 (Sigma Aldrich) as a model for bioinks to demonstrate the application of our printer to bioprinting.

Secondly, we have provided more information on how to go about printing an object by introducing a new section into the protocol, '8. Preparation for 3D Printing'. This will help bridge the gap between constructing an ADDME 3D printer and generating gcode to print an object.

Thirdly, to better characterise materials in a way that displays the accuracy and fidelity of our 3D printer we have added a new section in the results. In this section we use a 3D point cloud comparison of a computer generated object and the same object that has been 3D printed and 3D scanned.

Lastly, we have gone through our protocol and made sure that it proceeds in a step wise manner that is better aligned to the journals standards.

Again thank you for your constructive feedback, we hope our paper is now suitable.

Kind regards,

Matthew Lanaro

Editors Comments	Comment	Response	Line Numbers
E1.1	Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues	We have made a number of changes to all the sections within the manuscript.	All
E1.2	For each step/substep, please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.	Thank you for your feedback, we have gone through and made significant changes to the wording of the protocol.	Protocol
E1.3	Do you mean the Table of Materials here?	Yes, the name has been changed to table of materials.	Line 128-129
E1.4	Should part 9 also be listed here?	Yes, part 9 has been added.	Line 163
E1.5	Can you expand a little more here about how to actually print something?	We have added an additional section into the protocol “8. Preparation for 3D Printing” in addition to figure 11.	Lines 377 - 400
E1.6	Please correct the inverted parentheses around volume numbers.	The inverted parentheses have been corrected.	References
E1.7	Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.	We have included the materials and equipment used throughout the protocol.	Table of Materials

Reviewer 1	Comment	Response	Line Numbers
R1.1	The work in question does not reach an appropriate level to be published.	We have made additional changes to further support our work. Firstly, we have included an ink (Pluronic F-127) as a model for bioinks to demonstrate the use of our 3D printer in the biofabrication field. Secondly, we have added a 3D scanning comparison of a 3D printed object with the CAD model to verify the accuracy and fidelity of the printer. We trust that these changes raise the quality of our work to an acceptable level.	General

Reviewer 2	Comment	Response	Line Numbers
R2.1	The title indicated the bioink (polylactic acid) was also suitable for this equipment. However, author only used cream and chocolate as the materials, no evidence can prove polylactic acid can be the materials if printing by this equipment.	We have included Pluronic F-127 (Sigma Aldrich) as an ink (a model for bioinks) to demonstrate our printers usefulness to bioprinting. Please note that polylactic acid is only used as a material to construct the printer, not test it. We acknowledge that this is confusing and we have changed our language in the protocol to make this clearer.	Line 467
R2.2	Author mentioned the cost of this equipment was "cheap", as far as I know, the cheapest extrusion 3D printer was less than 500 US dollars. Cost accounting is necessary for this manuscript.	Yes, we found and note in the introduction that the RepRap v2 is $400 USD. In the discussion we mentioned that the cost of our 3D printer was reduced to $245 USD. We have provided a detailed cost breakdown of each component in the table of materials.	Lines 84, 460 and the table of materials
R2.3	Except the accuracy, some other parameters such as printing speed, size of syringe and compactness is also important for 3D printing, author need more statement for this part.	We have added expanded the discussion to include information on the importance of various printing parameters. Additionally, we have added a section in the results comparing a 3D scan of an object to the CAD model. This will characterise the geometric accuracy of the printer.	Line 504 - 506
R2.4	More optional accessories are good for this equipment. For instance, nozzles of different calibre and material, or syringes of different size.	We aim to support the modularisation and customization of	

	It can extend the scope of application of this equipment. Author can added more modularity components for this equipment to make it easy to use.	our equipment by releasing it as open source. This gives researchers full access to all the CAD files, enabling further accessories to be made.	
R2.5	The introduction part needs to be reversed. Author should focus on the development of 3D printer, listing the deficiencies of 3D printer existed, at the same time emphasize the superiorities of designed 3D printer.	We thank the reviewer for their suggestions regarding the layout of the introduction. We have made several changes to the arrangement of the introduction to better assist the reader to introduce our subject matter.	Introduction

Reviewer 3	Comment	Response	Line Numbers
R3.1	Please take out "bioinks" from the title. Bioinks is essentially defined as softer biomaterials printed along with live cells. Any hand cream cannot be considered as a bioink. Unless the authors would like to demonstrate the bioprinting with a more popular bioink material like alginate, GelMA etc. (https://www.sciencedirect.com/science/article/pii/S0734975016301719)	We have included Pluronic F127 as an ink (a model for bioinks) to display the versatility of our 3D printer as a bioprinter.	Results
R3.2	The printed structures have not been measured (width/height of the lines in Fig. 11). There is no mention of the number of printed samples. It is crucial to demonstrate the print fidelity of this low-cost assembled printer. The print tolerances need to be mentioned.	We have added a results section comparing a CAD model with a 3D scan of the same printed model. We are able to report the geometric difference between the 3D model and 3D scanned model down to 50 µm.	Line 441 - 453
R3.3	The operation of the printer is not written clearly for a user to follow. Ideally, the operation should be in a step-wise protocol format.	We thank the reviewer for this comment, we note that the editor had a similar comment. We have gone through our protocol and made changes to insure that the protocol is clearly written and better follows the step-wise format.	Protocol
R3.4	For any extrusion-based printing, shear-thinning is a major property of the material/biomaterial. The manuscript current doesn't mention anything about the material properties.	Yes, shear thinning is an extremely important materials property, especially for chocolate. We have aimed to make the results section about validating the protocol (in this case the printer) and not the materials.	
R3.5	What does the parameter "flow" imply in the table.	We have changed the term “flow” for extrusion. Extrusion (calculated from equation 2) is a dimensionless value that tells the electronics control board how much material to extrude. We can apply a scalar	

		value to this extrusion, to extrude more or less than the value calculated from equation 2.	
R3.6	Is there any way to calculate the extrusion pressure?	Yes, for newtonian fluids the relationship between shear rate is easy to calculate, shear rate = 8* velocity/diameter. Other research papers measure the extrusion pressure directly with a sensor. We aimed to include results that validated the design of our printer and focused on geometrical accuracy.	
R3.7	What are the sensitivities for the machined heating jacket and silicon heating jacket?	The thermal limits of the heating supplies should not matter as the maximum temperature is limited by the 3D printed housing materials. The geometric sensitivities are forgiving as the housing, not the heater is the load bearing element. Put another way, the heaters are low tolerance parts.	
R3.8	Can the authors provide more detailed instructions for electronics and wiring? It will be nice to include an illustrated figure on this.	We have included an image of the wiring in figure 10 and added some explanation.	Line 344 – 347, figure 10
R3.9	For the software setup, it will be nice to provide screenshots of the installation process so the readers know where exactly to change the settings and configurations.	We decided to include additional navigation information so the readers can better follow along.	Line 325 - 328
R3.10	Please include important notes and safety instructions for the assembly protocol.	We have expanded the safety notes in a number of locations	

		throughout the document.	
R3.11	Can the authors provide a summary or list of basic tools needed to assemble the 3D printer? For example screwdriver, Hex keys, etc.	We have updated the table of materials to include the tools needed to assemble the 3D printer.	
R3.12	It will be helpful to add numbers, callouts for each of the parts used in the machine.	We have added a number near each component in the figures which links to each respective component in the table of materials.	
R3.13	Line 352- The labels should be in lower case.	We have changed the labels.	Line 352, now Line 393
R3.14	It would be helpful to put all the online links as an Annex at the end of the paper	We have included a link to all online links in a note at the start of the paper.	Line 111 - 121
R3.15	Line 408 - Please take out "biofabrication". The printer has not been demonstrated for any biomaterials printing.	We have included an ink (as a model for bioinks) (Pluronic F-127) to demonstrate the versatility of our printer for biofabrication.	
R3.16	Some of the figures have a dark background and hence when printed on paper do not come out clearly. Since it is a protocol paper and it is reasonably expected for potentially interested users to print it and follow for assembly.	We recognise this may be a problem, unfortunately we do not have any other colour of acrylic available (such as black) to remake those figures. Due to time constraints we are not able to wait until new acrylic arrives.	