Open-source hybrid 3D-bioprinter for simultaneous printing of thermoplastics and hydrogels

Fritz Koch a,⇑, Ole Thaden a, Kevin Tröndle a, Roland Zengerle a,b, Stefan Zimmermann a, Peter Koltay a,b,c

a Laboratory for MEMS Applications, IMTEK – Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, D-79110 Freiburg, Germany
b Hahn-Schickard, Georges-Koehler-Allee 103, D-79110 Freiburg, Germany
c Freiburg Center for Interactive Materials and Bioinspired Technologies (FIT), Georges-Koehler-Allee 105, D-79110 Freiburg, Germany

ARTICLE INFO

Article history:
Received 2 April 2021
Received in revised form 3 September 2021
Accepted 7 September 2021

Keywords:
Hybrid printing
3D-Bioprinting
Open-source
Rapid prototyping
Hydrogel extruder
3D-printer

ABSTRACT

3D-bioprinting is a promising technology applicable in areas such as regenerative medicine or in vitro organ model development. Various 3D-bioprinting technologies and systems have been developed and are partly commercially available. Here, we present the construction and characterization of an open-source low-cost 3D-bioprinter that allows the alternated microextrusion of hydrogel and fused deposition modeling (FDM) of thermoplastic filaments.

The presented 3D-bioprinter is based on a conventional Prusa i3 MK3 printer and features two independent printheads: the original FDM-head and a syringe-based microextrusion printhead for soft materials. Modifications were designed modularly to fit various syringe formats or heating elements to the device. The bioprinter is the first hybrid DIY 3D-bioprinter that allows switching between materials as often as required during a print run to produce complex multi-material constructs with arbitrary patterns in each layer.

For validation of the printer, two designs suitable for relevant bioprinting applications were realized. First, a porous plastic construct filled with hydrogel was printed, serving as a mechanically stable bone replacement tissue model. Second, a plastic chamber, which might be used in organ-on-a-chip applications, was printed with an extruded silicone sealing that enables the liquid-tight attachment of glass slides to the top and bottom of the chamber.

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Specifications table

| Hardware name | Prusa i3 MK3 with microextrusion module upgrade for hybrid 3D-bioprinting |
|---------------|--------------------------------------------------------------------------|
| Subject area  | Medical (e.g. Pharmaceutical Science) Engineering and Material Science |
| Hardware type | Bioprinter                                                                |
| Open Source License | GPLv3                                                                 |
| Cost of Hardware | 996.73 €                                                                |
| Source File Repository | https://doi.org/10.17632/ywb5zdjk5x.1                                   |

⇑ Corresponding author.
E-mail address: fritz.koch@imtek.uni-freiburg (F. Koch).

https://doi.org/10.1016/j.ohx.2021.e00230
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Hardware in context

Additive manufacturing has received much attention from researchers worldwide in recent decades. Benefits include significantly shorter processing times for the rapid production of prototypes and the minimal waste of material. Today, many low-cost 3D-printers based on Fused Deposition Modeling (FDM) technology are available for less than 1,000 € or as kits with unassembled parts for only a few hundred euros [1]. This low-cost entry has made it easy for this fabrication technology to find its way into many hobby applications as well as into scientific laboratories.

Bioprinting is a specialized method of 3D-printing that uses living cells, biomaterials, and other additives to produce artificial tissues that mimic the shape and function of native tissues. The most commonly used technology to deposit cell-laden hydrogels is syringe-based microextrusion, used in 57% of all commercially available bioprinting devices [2]. Compared to other bioprinting technologies, microextrusion finds a compromise between cell survival and deposition rate. Although the required actuation hardware for this printing technology is well established and technically straightforward to control, the high price of commercially available bioprinters, starting at about 10,000 € [3], makes it difficult for research groups to enter the field of bioprinting. In addition to the high instrument costs, high material costs and, subsequently, cost-intensive process development must be considered. This is particularly relevant in extrusion-based bioprinting, where a compromise needs to be found between cell viability and printability of the bioink by fine-tuning material and process parameters [4,5]. In this context, a very stiff and elastic material usually results in printed constructs with a high level of shape fidelity, but due to increased shear stress during printing also in reduced cell viability. On the other hand, a soft and viscous material often has a higher cell survival rate than a highly viscous elastic material due to the lower shear stress during the printing process, but a poor shape fidelity as the material flows away [6]. Therefore, it can be of great advantage to separate the mechanical and biological requirements of artificial tissue by using different types of materials in combination to form hybrid 3D-printed constructs [7,8]. Combining the benefits of two technologies can significantly speed up the printing process and enable printing designs that are not realizable with a single printing technology.

Multi-nozzle bioprinters are commercially available, but rarely as DIY implementations for bioprinting. Krige already showed in his work [9], the potential in such a development to serve as an entry-level tool for many researchers, but did not yet use custom electronic hardware and firmware and did not show modular expandability. Still, most open access published DIY conversions of conventional 3D-printers to 3D-bioprinters replace the original FDM extruder and basically use the 3D-printer stage for moving a microextrusion printhead [10–14].

In all the previously presented approaches, the 3D-bioprinter cannot automatically alternate between plastic and gel extrusion during the printing process to create a hybrid multi-material construct. Either only a single material can be printed at a time, or it is possible to switch manually or semi-automatically between two different materials. So far, two different materials cannot be combined fully automatically within one layer to enable multi-material printing.

In this work, a custom-made syringe-based microextrusion printhead was developed as an add-on module for a commercially available Prusa i3 MK3 printer, as shown in Fig. 1. The microextrusion printhead was designed in a modular way to allow the dispensing of different materials by using different adapters. These adapters fit different syringe sizes and can feature the adjustment of the printing temperatures. The use of such a modular second printhead in addition to the FDM printhead at any point in the print run enables entirely new hybrid print designs. Examples of two potential applications that are feasible with the novel hybrid 3D-bioprinter are presented at the end of this article:

1. An application inspired by bone tissue engineering is printed, where FDM-printed polymer elements stabilize hydrogel structures to generate hybrid constructs with larger stiffness.
2. A microperfusion chamber for use with in vitro tissue models is printed, featuring a polymer body and soft silicone seals to integrate glass windows into the chip.

Hydrogels for bioprinting can have viscosities ranging from mPa s to several kPa s. This wide viscosity range leads to changing fluidic resistances and requires careful adjustment of the actuation pressure for pressure-driven extrusion systems. To provide a more robust and universal solution for hydrogel extrusion, the microextrusion system developed for this printer was designed as a piston-driven system to achieve constant volume extrusion regardless of the rheological properties of the material loaded into the syringe.

A significant advantage of the printer presented in this paper is the modular expandability of the bioprinter, which is usually not the case for commercially available products. The modularity of the printer shown here is demonstrated by using various adapters that allow the use of syringes with different volumes and could be expanded in the future to include additional features. Specifically, the hardware and firmware modifications are designed to enable further modification and improvement by implementing microextrusion principles other than those shown here, such as pressure-driven or rotating screw microextrusion. The use of a pneumatic- or piezoelectric-driven jetting-based dispenser, such as is frequently used in bioprinting, is also conceivable and might be a project for the future.
Hardware description

The basis for this hybrid bioprinter was a fully assembled 3D-printer Prusa i3 MK3. The Prusa printer features a fused deposition modeling (FDM) printhead that enables printing plastic filaments at elevated temperatures between 80 and 250 °C. This printhead has been complemented by a syringe-based microextrusion printhead that allows for the dispensing of low-viscosity hydrogels and liquids. The upgrade of the Prusa printer with both printing technologies can be helpful for many applications but is particularly suitable for hybrid 3D-bioprinting, where the cells are provided in a hydrogel-based bioink, and the FDM extruder is operated preferably with polycaprolactone (PCL) to integrated biocompatible structures into...
the construct and increase its mechanical stiffness. Therefore, alternating printing of two different materials within a single run is required.

The conversion of the original FDM 3D-printer involved three aspects:

1. The design and construction of the second printhead for syringe-based microextrusion and mounting as an easily exchangeable module on the FDM printhead, as shown in Fig. 2.
2. The modification of the electronic hardware to control the original hardware of the printer and the additional syringe extruder.
3. The adaptation of the firmware to control the new mainboard and the additional syringe extruder of the hybrid 3D-bioprinter.

The newly developed low-cost hybrid 3D-bioprinter can be helpful in many fields for bioprinting and tissue engineering and ease the entry into this topic for research groups. Highlights of the new development are the integrated control of both printheads for hybrid bioprinting, the modular expandability involving printing of essential components by the original printhead itself, and the broad possible use for both in vitro and in vivo applications.

In this paper, exemplary applications of hybrid multi-material bioprinting for in vitro models and for the field of regenerative medicine are shown. Due to the different materials that can be used in the FDM-head as well as in the syringe-based microextrusion head, many other applications are conceivable for bioprinting and beyond.

Fig. 3 shows three syringes adapters for using syringes with a volume of 5 ml and 2 ml with and without heat foil. The bioprinter’s modular, low-cost design leaves spaces for further improvements, such as using different adapters with integrated cooling systems or using other syringe modules such as coaxial nozzles.

The electronic hardware was implemented in a way that there is still space on the board to control further motor drivers and additional printheads. More syringe-based microextrusion printheads or a drop-on-demand printhead could be added, enabling high-precision dispensing of several cell types or growth factors into the printed construct.

**Design files**

Table 1 shows all design files with rendered images for the construction of the microextrusion printhead. Brief description of each part.

**Corpus**

The corpus is used to attach the syringe extruder to the FDM printhead. We tried to use as many fixation points on the FDM printhead as possible to distribute the mechanical load as evenly as possible, which is primarily generated by the weight of the Nema 17 motor attached to the top end. The corpus is a single CAD part, but it must be printed as two individual parts, joined together with screws because of its geometry.

**Modular extruder frame**

The modular extruder stabilizes the linear shafts and serves as a support for the motor. It is designed in such a way that adapters for different syringes can be mounted. Because syringes of different sizes and nozzles of different lengths can be used, the z-height of the nozzle can be adjusted over a wide range by two-cylinder head screws on the right and left sides of the modular extruder frame.

![Fig. 2. Computer-generated image of the modified syringe-based microextrusion printhead. A modular design was chosen to enable the use of different syringe sizes by using adapters (light gray). The design of the extruder is based on [15].](image-url)
Syringe adapter

The adapter is inserted in the modular extruder frame and can be easily exchanged via two screws. In addition to the syringe adapter for 2 ml BBraun syringes, an adapter for 2 ml BBraun syringes with heating block and an adapter for 5 ml BBraun syringes were also developed.

Heating block

The heating block should be made of aluminum or other material with high thermal conductivity. It is inserted into the syringe heating adapter and does not touch this adapter, as a distance to the plastic can be guaranteed via metal screws in the syringe heating adapter.

Modular ram

The modular ram is used to transfer the rotation of the Nema 17 motor into a movement of the syringe piston. It is mounted on the linear shafts via bearings.

Syringe ram

The syringe ram is fixed in the modular ram and can be easily exchanged via four screws. Besides the ram for 2 ml BBraun syringes, a ram for pistons of syringes with 5 ml was also designed. Each syringe ram should be used only together with the corresponding syringe adapter.

Rumba + shield

The Rumba + shield PCB provides a simple and straightforward connection to the SPI pins of the board using the pins of the display interface. The basis of the Rumba + shield was a version of a Rumba shield [16]. This shield was adapted to the Rumba + board by repositioning the exp3-connector and increasing the distance between the last two pins.

Bill of materials

Table 2 shows all commercially available parts needed to built the low-cost 3D bioprinter. Build instructions.

The modification of the printhead is divided into three parts. First, the syringe extruder is constructed and assembled (chapter 5.1). Second, the mainboard is exchanged and converted (chapter 5.2). Third, the firmware is adapted to the use of two printheads (chapter 5.3). During assembly and use of the modified printer, the same safety considerations apply as for a conventional Prusa printer. Due to the unshielded hardware, ESD protection must be observed. No particular danger results from the Rumba + mainboard with an electrical voltage of up to 24 V.

Assembly of the syringe-based microextrusion printhead

The syringe extruder’s fundamental element is the corpus, which provides a stable connection to the FDM printhead. A modular extruder, on which the motor and bearings are installed, is mounted on the corpus. Adapters with different syringes can be mounted in the modular extruder.
Corpus

Due to the complex and large design of the corpus, it must be divided into two parts for printing (lower and upper corpus). Therefore, a single CAD-file and two gcode-files are given in the chapter design files. The assembled corpus is shown in Fig. 4 A, and the boundary between the two parts of the mounted corpus is indicated with a red line.

Table 1
The following parts were created as computer-aided design (SLDPRT) and manufactured (STL and GCODE) using 3D-printing to construct a syringe-based microextrusion printhead on a Prusa i3 MK3 printer. The design file for the PCB is given as a brd-file and pdf-file.

| Design file name              | Rendered image | File type            | Open source license | Location of the file                  |
|------------------------------|----------------|----------------------|---------------------|---------------------------------------|
| Corpus                       |                | CAD, stl, and gcode  | GPLv3              | https://doi.org/10.17632/cvjt57xspg.1 |
| Modular extruder frame       |                | CAD, stl, and gcode  | GPLv3              | https://doi.org/10.17632/cvjt57xspg.1 |
| Syringe adapter (2 ml)       |                | CAD, stl, and gcode  | GPLv3              | https://doi.org/10.17632/cvjt57xspg.1 |
| Syringe heating adapter      |                | CAD, stl, and gcode  | GPLv3              | https://doi.org/10.17632/cvjt57xspg.1 |
Screw the lower part of the corpus to the FDM printhead by replacing the screw that fixates the print fan to the FDM printhead with a 16 mm M3 screw, as shown with number 1 in Fig. 4 A.

Then mount the upper corpus to the FDM printhead with two 16 mm long M3 screws next to the filament insert and with two 30 mm long M3 screws and wing nuts onto the lower corpus, shown in Fig. 4 A as number 2 and number 3, respectively.

Modular extruder frame

- Place the linear bearings LM8UU on the linear shafts and screw both linear shafts into the modular extruder with four 16 mm M3 screws and nuts (matching holes for these nuts are designed in the CAD file). Screws are indicated with number 1 in Fig. 4B.
The following parts were purchased from the specified source and used to build the 3D-bioprinter, including the FDM 3D-printer, the syringe microextruder, and its electronic control hardware for a budget of less than 1,000 € in total.

| Designator | Component | Number | Cost per unit - currency | Total cost - currency | Source of materials | Order number | Material type |
|------------|-----------|--------|--------------------------|-----------------------|---------------------|--------------|---------------|
| FDM 3D-printer | Prusa i3 MK3 | 1 | 769.00 € | 769.00 € | Prusa Research | PRI-MK35-KIT-ORG-PEI | Non-specific |
| Syringe extruder | Nema 17 stepper motor | 1 | 27.16 € | 27.16 € | OMC CORPORATION LIMITED | 17HS13-04045-PG27 | Non-specific |
| Syringe extruder | Trapezoidal threaded shaft | 1 | 12.10 € | 12.10 € | Nanotec Electronic GmbH & Co. KG | SCREW-ABA-TJBA-200 | Metal |
| Syringe extruder | POM-Thread nut | 1 | 12.70 € | 12.70 € | Nanotec Electronic GmbH & Co. KG | LSNU-T-A3A-TJBA | Metal |
| Syringe extruder | Shaft coupler 6 mm/4 mm | 1 | 1.87 € | 1.87 € | Christians Technikshop | RS20-6-4 | Metal |
| Syringe extruder | Precision linear shaft h6, 150 mm | 2 | 5.20 € | 10.40 € | Dold Mechatronik | 30162-0TLS-150 | Metal |
| Syringe extruder | igus GFM-0608-06 Bearing Ø 6 mm | 1 | 0.65 € | 0.65 € | Conrad Electronic SE | 1416573-62 | Metal |
| Syringe extruder | Linear bearing LM8UU | 2 | 5.98 € | 11.96 € | Conrad Electronic SE | 1013,428 | Metal |
| Syringe extruder | Hotend heater cartridge E3D 24 V 40 W | 1 | 7.40 € | 7.40 € | Prusa Research | PRUSA-HEATER-24 V-40 W | Metal |
| Syringe extruder | Hotend thermistor E3D | 1 | 9.74 € | 9.74 € | Prusa Research | E-SEMITEC-50-MOLEX-INC-CABLE | Metal |
| Syringe extruder | TOOLCRAFT hexagon nut M3 | 12 | 0.10 € | 1.20 € | Conrad Electronic SE | 888,718 | Metal |
| Syringe extruder | TOOLCRAFT 100 × washers ID: 3.2 mm M3 | 1 | 1.79 € | 1.79 € | Conrad Electronic SE | 814,628 | Metal |
| Syringe extruder | TOOLCRAFT 100 × cylinder head screws M3 16 mm | 1 | 5.49 € | 5.49 € | Conrad Electronic SE | 839,672 | Metal |
| Syringe extruder | TOOLCRAFT 100 × cylinder head screws M3 20 mm | 1 | 7.49 € | 7.49 € | Conrad Electronic SE | 1,807,799 | Metal |
| Syringe extruder | TOOLCRAFT cylinder head screw M3 30 mm | 7 | 0.77 € | 5.43 € | Conrad Electronic SE | 888,742 | Metal |
| Syringe extruder | TOOLCRAFT 10 × wing nuts M3 | 1 | 4.69 € | 4.69 € | Conrad Electronic SE | 827,784 | Metal |
| Control hardware | Rumba + board | 1 | 67.18 € | 67.18 € | Anzado GmbH | RBS11842 | Semiconductor |
| Control hardware | SilentStepStick TMC2130 V2 | 5 | 46.00 € | 230.00 € | Watterott electronic GmbH | 20160027-002 | Semiconductor |
| Control hardware | SilentStepStick heat sink 9 × 9 × 12 mm | 5 | 5.00 € | 25.00 € | Watterott electronic GmbH | 20,188 | Metal |
| Control hardware | Rumba + shield | 1 | 7.00 € | 7.00 € | Multi CB | Slightly modified, but based on [16] | Slightly modified, but based on [16] | Slightly modified, but based on [16] |

Total 998.41 €
Shorten the trapezoidal shaft to a length of 12.5 cm with a metal saw.

Connect the trapezoidal threaded shaft with the shaft coupler to the Nema 17 motor. Use the 4 mm side of the coupling for the shaft and the 6 mm side for the motor. Mount the trapezoidal threaded nut with three 20 mm M3 screws (s. Fig. 4B number 2) on the trapezoidal shaft and insert with the igus bearing into the ram before fully mounting the motor.

Connect the motor to the modular extruder using two M3 screws with a length of 20 mm, indicated with number 3 in Fig. 4B.

Connect the modular ram to the two linear bearings and tightening with two zip ties. Make sure both bearings are at the same position when connecting to the ram.

Syringe adapter

- Plug the syringe ram and the syringe adapter into the frame and fixate with four 20 mm long M3 screws (s. Fig. 4B number 4) and two 30 mm long M3 screws (s. Fig. 4B number 5).
- Mount the syringe in the syringe adapter and secure it with a 40 mm long M3 screw and a wing nut.

Preparation of the components and conversion of the board

The original board of the Prusa i3 MK3 printer does not have enough unoccupied pins to drive more than one additional printhead in addition to the three motors for the axes. Therefore, a new board had to be installed to turn the printer into a real hybrid bioprinter. In this work, a Rumba + board was used, which provides six slots to control individual motors. Therefore, combining up to three extra motors is possible in addition to the three ports to control the x, y, and z-axis. TMC2130 stepper drivers, which were already installed on the Prusa board, were used to drive the motors. They are quiet and feature sensorless homing. With sensorless homing, the end of an axis is detected without the use of physical sensors. Instead, the stepper driver detects the increased load and sends a signal via the diag1 pin when the axis limit is reached. On the Rumba + board, the stepper drivers are modular and can be replaced so that the entire board does not have to be replaced if a single stepper driver is defective, as is the case for the Prusa board.
Preparation of stepper driver and Rumba + shield

- For the SPI-connection, solder the four pins SDI, SCK, CS, and SDO, and for sensorless homing the Diag1 pin upwards, as shown in Fig. 5A.
- Glue the $9 \times 9 \times 12 \text{ mm}^3$ heat sink onto the driver board. The adhesive film is already applied to the heat sink.
- The Rumba + shield allows a clean and straightforward connection to the SPI pins of the board using the pins of the display interface. Connecting the stepper driver to the board requires a stack of four female/female jumper cables. The cables are easy to make yourself using a simple cable, crimp connectors, and Dupont adapters.
- Mount the sockets for the LCD connectors on the Rumba + shield, indicated in blue in Fig. 5B, with the openings point to the right in the direction of Exp3.
- Use a $2 \times 1$ stack and a $2 \times 7$ stack for the stackable $2 \times 8$ socket to connect the two rows of eight pins on the Exp3-connection, indicated in red in Fig. 5B. A $2 \times 8$ stack will not fit since the distance between the two pins on the right side is a bit wider, as shown in Fig. 6 at Exp3.

For the exchange of the Prusa board against the Rumba + board, all screws from the housing have to be removed, and the Prusa board has to be dismounted entirely. Afterward, all connections on the Prusa board must be connected to the new Rumba + board. Some connections can be transferred directly to the new board without modification. These connections are shown in Fig. 6, indicated with blue arrows. The connections are indicated in bold in the following text.

The z-axes are mounted in two places, both on the left and right of the printer. Therefore, two z-stepper motors are used to move the axis. Since the two z-axis motors are always controlled synchronously, it is preferable to control the motors via a single stepper driver. The four stepper motor drivers for $X$, $Y$, $Z$, and $E_0$ can be seen in Fig. 7A. The single $Z$ stepper driver must be connected in parallel to the motors. Use a simple solder grid board to connect the pins in parallel, as shown in Fig. 7B.

Connection of Rumba + board

- For the use of sensorless homing, connect the Diag1 driver x-axis with the endstop X pin (D37) and the Diag1 driver y-axis with the endstop Y pin (D35) on the board.
- The fans consist of a red (+), black (-), and yellow cable. The yellow cable reports the status and speed of the fans, which are not connected in this setup.
- Connect the fans to FAN0 and FAN1, shown in Fig. 6 as Fan Extruder and Fan Print.
- Each of the fans operates with a supply voltage of 5 V. Several pins on the board provide a constant 5 V and can be used as a voltage source. As shown in Fig. 6 with black squares, the fans can be powered by Supply Fan0 (D36) and Supply Fan1 and are connected to Fan extruder and Fan print, respectively.
- For this purpose, remove the marked green jumpers on the board.
- In the original state of the printer, the PINDA probe is in addition to the z-alignment also used as a thermistor to minimize the temperature effect. Since there are only three pins on the Diag1 driver PINDA-probe, the white cable is not connected in this case, and the function is not used, as shown in Fig. 8A.
- Place the Rumba + shield on top of Exp1, Exp2, and Exp3, as shown in Fig. 8B.
- Connect the SPI connection of each stepper driver to the board using the four-stack female/female jumper cable. The positions of $X$, $Y$, $Z$, $E_0$, and $E_1$ as well as the LCD1 connector, which is plugged into the box on the right and the LCD2 connector on the left, are shown in Fig. 8B.
Fig. 6. Pin assignment and placement of the components on the Rumba + board. Blue arrows indicate connections that can be transferred from the Prusa to the Rumba + board, and red arrows indicate connections that need to be modified. Figure based on [17]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 7. Mounted stepper drivers on X, Y, Z, and E0 on the Rumba + board to provide the same functionality as on the original board. The two motors for the z-axes must be connected in parallel to be controlled by a single z-stepper driver.

Fig. 8. Connection of the PINDA probe with not connected thermistor A). Mounted Rumba + shield on top of the Rumba + board B).
When connecting the Rumba + board to the 3D-printer, some components cannot be easily connected to the board. Either due to their connector shape or size. These components are marked with red arrows in Fig. 6. The possibilities to connect one of those components are either using an adapter to connect them or change the connector of the wires. In this setup, all connectors were replaced with matching connectors. Therefore, the old connectors were cut off, and a crimp connector was added to the cables’ wire end sleeves, as shown in Fig. 9A and B for the Heater E0 and PSI connection to board. The connection of Heater E1 is not shown in the Figure but is the same as for Heater E0.

The Filament sensor and the Powerpanic connector are no longer used in the new setup and should not be connected, as shown in Fig. 9B. Either there is no function in the Marlin firmware yet, or the component is not essential to the setup.

Firmware modification

The firmware is designed in a modular way so that parts of the firmware can be activated only when the second extruder setup is used. This allows setting up multiple user profiles to use only parts of the printer’s features (for example, basic and advanced users) or using the firmware for other modified 3D-printers with different motors or multiple extruders.

The configurable base firmware is available from Marlin under the terms of the GPLv3 License [18]. This section presents the modifications that enable the alternating use of both printheads within a single printing process. The changes to the configurable firmware that enable the control of the motors for basic 3D-printing functions are described in the appendix. Changes that must made in the configuration file are for example:

- A dummy thermistor is used in the firmware to set the syringe extruder temperature since no real thermistor is mounted in the syringe printhead. A constant temperature of 200 °C is set to not impair the cold extrusion safety feature.
- Due to the increased printhead size after modification, the usable print bed size is reduced after the modification. The new maximum length in the x-direction is 110 mm starting from the \( x = 80 \) mm. Therefore, \( x = 80 \) is defined as the new \( x = 0 \) position. The new maximum length in y-direction was reduced from 210 mm to 170 mm because the offset between the two printheads was 40 mm. This allows that both printheads reach every point on the new defined print bed.
- To include the steps per mm for the additional syringe printhead, the distinct e-factors must be activated.
- General parameters as the maximum feed rate and applied current for the syringe extruder must be defined.
- The offset between the syringe tip and the FDM printhead, which can later be altered at the calibration step, must be defined.
- The pin assignment on the board of the SPI of the second extruder is defined.

Changes to allow printing with two extruders

At the start of the configuration file

```c
#define HYDROGEL
```

Then for every modification, the parameter is changed with the following structure:

```c
#if ENABLED(HYDROGEL)
    #define PARAMETER
#endif
```

```c
#endif
```

Fig. 9. Crimp connector added to the PSI connection and the heater extruder cables. The filament sensor and the Powerpanic connector remain unconnected for the new Rumba + board.
The complete modified firmware used for this project is available online and can be found here: https://doi.org/10.17632/ywb5zdjk5x.1

Operation instructions

The Rumba + board can be connected via USB to a computer running the open-source 3D printing host software Pronterface [19]. Pronterface is used for printing and the calibration of the printheads. Before connecting to the computer for the first time, a USB-driver for Windows must be downloaded and installed. The driver is available online on the RepRap Rumba homepage [20]. The modified 3D-bioprinter is working like an ordinary gcode-based 3D-printer that controls the extrusion rate and the movement of the printhead. However, using the new Rumba + board, some functions of the original 3D-printer are limited or missing, while the control of two separate printheads (syringe-based microextrusion printhead and original Prusa FDM printhead) is provided as a new feature.

Limitations

- Filament sensor and powerpanic functions are missing, which do not affect the printing itself and are not necessarily needed.
- Prusa software that helps with filament changes and z-height calibration is not available for the new board.

Some functions are limited for the modified printer.

- The extrusion rate of the syringe extruder is limited to 0.09 mm/s due to the hardware components used.
The base area of printed structures with both printheads is limited to a maximum of 110 × 170 mm² due to a smaller print bed accessible for both printheads.

Preparation of the syringe before printing

- Fill the syringe with material and avoid the formation of air bubbles.
- Raise the ram in relation to the filling level of the syringe and place the piston of the syringe into the ram adapter and the body of the syringe in the syringe adapter.
- Tighten the screws on the bottom of the adapter to further secure the syringe in place.

From design to printed construct

- Design the construct with a CAD program and export it as an stl-file as it is compatible with the PrusaSlicer used.
- Create a gcode-file from the stl-file with PrusaSlicer with the desired parameters depending on the specific application. Alternatively, write the gcode-file manually for simple structures.
- If two materials were not assigned in the gcode, modify the gcode manually or use a slicer that supports two materials respectively extruders.
- Select T0 for the FDM printhead and T1 for the syringe extruder.
- A standardized gcode template, as shown in Fig. 10, can be used for switching between the FDM printhead and the syringe extruder. Priming of the FDM printhead after extrusion with the syringe extruder is not required.

Calibration routine

FDM printhead

- The referencing of the printer’s position in z-direction is based on a proximity sensor that triggers at a certain distance from the print bed. However, every printer has a slightly different distance between the proximity sensor and the nozzle. To obtain the correct distance between the nozzle and the print bed, an offset value needs to be calibrated before the first use of the printer.
- The offset value can be determined by using a sheet of paper. As a rule of thumb, the paper should be slightly pinched between the nozzle and printing bed to define a height of 0. Therefore, the offset must be manually varied, saved, and tested to find the correct offset for the setup.
Syringe printhead

- Switching between the printheads requires a well-known offset between the nozzles in x- and y-direction and no offset in the z-direction.
- The z-height of the syringe extruder can be calibrated with two wing nuts on the left and right sides of the modular extruder.
- Two plastic structures with identical height are placed below the syringe and FDM printheads. The printer is manually moved down until the FDM printhead touches the structures. The syringe printhead is moved down until it also touches the structure, and then the wing nuts are screwed tight.
- For the offset in x- and y-direction, specified offset values from the design of the printheads are defined in the firmware. With the gcode commands T0 and T1 (see Fig. 10), one can switch between the two extruder nozzles and the printhead is then moved relative to the defined offset values.
- For printing that involves both printheads, the exact value of the offset between the two printheads is significant to achieve good printing results. Therefore, the x- and y-offset must be manually fine-tuned by printing a test structure consisting of two FDM-printed lines perpendicular to each other. One line is printed in the x-direction and thus enables the y-adjustment of the syringe extruder, and the other line is printed in the y-direction and enables the x-adjustment.
- The offset in the x- and y-direction can be set with the command M218 T1 X... Y... Increasing the x-value shifts the position of the syringe printhead to the left side during printing, increasing the y-value shifts the position of the syringe printhead forward.

Validation and characterization

A wider range of materials can be printed with the heating syringe adapter compared to the standard 2 ml and 5 ml syringe adapters without temperature control. In addition to materials printed at room temperature (such as silicone or alginate/gelatin-based hydrogels), materials printed at elevated temperatures for example 37°C (such as Pluronic or GelMA) or temperatures above 50°C (e.g. agarose) are also of interest for bioprinting. To provide processability for these materials, the heating syringe adapter features an aluminum holder that ensures uniform heat distribution. In the aluminum holder, a heater cartridge was implemented that provides a heating power of up to 40 W and a thermistor that measures the temperature in the aluminum holder.

The heating syringe adapter was characterized for temperatures between 25°C and 80°C. The temperature was measured with the thermistor embedded in the aluminum block as well as in the water-filled 2 ml syringe. Fig. 11 shows the measured temperature profiles in the aluminum holder measured with the thermistor (blue) and in the syringe (red). The temperature in the syringe was measured by removing the piston and inserting a digital temperature gauge.

Three temperatures were set as target temperature and the increase in temperature was recorded as a function of time. It can be seen that a preheating time of 300 s should generally be observed for printing at elevated temperature so that uniform heat distribution in the syringe can be assumed. A slight overshoot can be seen the lower the target temperature is selected. Overall, the differences between the measurement in the aluminum block and the measurement in the water-filled syringe are not significant, so that only the measurement of the temperature in the aluminum block is used for the following experiments.

To validate the syringe-based microextrusion printhead for bioprinting applications, the viability of cells was characterized after extrusion-based bioprinting. A live/dead assay (L3224, Invitrogen) based on calcein AM and ethidium homodimer-1 was used to assess the viability of immortalized mesenchymal stem cells (iMSCs) after printing. Hydrogel consisting of 2%
alginate and 8% gelatin was heated up to 37 °C and a bioink was prepared by gently mixing cells in a concentration of 500,000 iMSCs/ml into the hydrogel. The bioink was filled into 2 ml BBraun syringes, cooled down to room temperature, and a conical 410 μm nozzle was used to extrude filament with a total volume of 200 μl for each sample. After printing into the bottom of 6-well-plates, the bioink was crosslinked with 10% CaCl₂ solution, washed three times with PBS, and incubated in cell culture medium for 2 h. Live/dead staining was performed after 2 h of incubation according to the protocol recommended for this kit. Three samples were evaluated with the live/dead assay, 1. manually pipetted bioink, 2. bioink extruded with 3.64 μl/s (corresponding to an extrusion rate of 0.05 mm/s), and 3. bioink extruded with 3.64 μl/s and PCL printed with 140 °C on top of the crosslinked bioink. Each sample was prepared 4 times and each replicate was evaluated with at least 6 representative image sections, giving a total of at least 24 evaluated images for each sample.

Viability was very high for all samples, ranging between 79.9% and 85.3%, with a standard deviation between 8.8% and 14.1%, typical for biological assays. No significant differences were observed between the three samples, but a slight decrease in viability for extruded bioinks and even more for bioinks covered with hot PCL compared to the pipetted sample, as shown in Fig. 12B. Representative microscope images under bright-field and fluorescence were selected for the three samples and are shown in Fig. 12A. In bright-field, the cells are clearly visible, as is the PCL filament, which forms a dark, elongated shadow on the image. Green cells indicating a viable cell, red cells indicating free DNA and thus a damaged or dead cell.

The hybrid bioprinter was characterized in two steps, first only using the newly built syringe-based microextrusion printhead, second using both printheads to print examples of hybrid structures with relevance in 3D-bioprinting. The extruded volume can be controlled via the stroke of the syringe-based printhead. The larger the diameter of the syringe, the higher the dispensed volume. For the characterization, a 2 ml syringe from BBraun was filled with water, and the piston was moved with different strokes between 1 and 5 mm. The dispensed material was weighted with a balance LPWG 213i from VWR International (Darmstadt, Germany), the corresponding volume was calculated and then compared with the theoretical volume calculated from the stroke and nominal diameter of the syringe. Both calibration curves are shown in Fig. 13A.
The measured volume showed linear dependence on the stroke of the piston. Slight deviations between the theoretically
determined volume and the experimentally measured volume were found. This might be caused by a deviation of the real to
the nominal syringe diameter, evaporation from the balance during measurement, or other measurement errors. Character-
ization using a linear fit (dotted line) resulted in an average extruded volume per stroke in mm of 72.9 \mu l/mm with low vari-
ance ($R^2 = 0.999$).

The line width of the extruded filaments was characterized for two representative materials, as shown in Fig. 13B. Hydro-
gel and silicone are widely used in 3D-bioprinting applications. The hydrogel can be used as a bioink by mixing it with cells
or as a substrate for cell growth. For this experiment, a hydrogel consisting of 90% water, 8% gelatin (G9391), and 2% alginate
(A0682) both from Sigma-Aldrich (St. Louis, Missouri, USA) was used for extrusion-based bioprinting due to its well-
characterized properties [6]. “Obi Universal Silicone” from OBI E-Commerce GmbH (Wermelskirchen, Germany) is used as
sealing material for the microperfusion chamber that could be used for dynamic culture of in vitro tissue models. Both mate-
rials can be filled into the syringes quickly and without trapped air bubbles. For extrusion-based printing, conical-shaped
dispensing nozzles from Globaco GmbH (Rödermark, Germany) with 410 \mu m inner diameter were used. Example images
of extruded lines of silicone with different flow rates between 0.73 \mu l/s and 3.65 \mu l/s are shown in Fig. 14.

Hybrid printing of hydrogel and thermoplastics was evaluated by printing PCL at 140 °C in a single layer with a pitch be-
tween 2.40 mm and 0.80 mm. The gaps were filled with hydrogel printed with a pitch between 1.975 mm and 0.90 mm.
The pitch was measured from the middle of the filaments. The average distance between PCL and the average distance
between hydrogel filaments was evaluated by measuring the shortest distance between two PCL and two hydrogel filaments,
respectively, as indicated in Fig. 15A. To compare the distance between the filaments as a function of the pitch, five struc-
tures were printed and evaluated at four representative positions, resulting in 20 analyzed distances for each pitch. One posi-
tion with the defined pitch and measured distances is shown in Fig. 15A.

For printing PCL filaments, a nozzle with 0.40 mm diameter was used. Therefore, a filament width of around 0.40 mm was
expected. Fig. 15B shows the measured distances as a function of the pitch down to 0.80 mm. It can be seen that the distance
between the PCL filaments scales linearly and shows very little deviation below 100 \mu m over 20 measurement points.

The deviation of the distance between the hydrogel filaments is larger than for printing PCL. With a large pitch, the hydro-
gel can be placed between the PCL filaments without contact to the PCL filament. With a pitch down to 0.80 mm, resulting in

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**Fig. 14.** Example images of printed silicone lines with different extrusion rates between 1 and 5 mm/s or flow rates between 0.73 and 3.65 \mu l/s respectively, with a feed rate of 300 mm/min. Scale bar 500 \mu m.

**Fig. 15.** Hybrid printed pattern of individual filaments of PCL and hydrogel with different pitches. The distances between filaments of PCL and hydrogel
were measured and compared with the pitches.
a distance between filaments of 0.40 mm, the hydrogel can be printed with little wetting of the PCL in this area. Since a 410\,\mu m diameter conical nozzle was used for hydrogel extrusion, smaller pitches than the nozzle size were not investigated.

Exemplarily, two designs related to relevant applications in the field of 3D-bioprinting were realized with the new hybrid bioprinter (The gcodes used for both designs are available online and can be found here: https://doi.org/10.17632/ywb5zd- jk5x.1). The first design, which is of potential use for mechanically stable constructs in regenerative medicine, consists of a biodegradable thermoplastic material, polycaprolactone (PCL), printed as a porous cube of 10 × 10 × 10 mm³ with a porosity of about 75%.

PCL is a widely used material in the biomedical field, as it allows to combine high mechanical strength with compatibility to surrounding cells. Hutmacher [21] and Zein [22] improved the mechanical stability of cell culture systems by embedding FDM printed porous PCL scaffolds already two decades ago. If the spaces between the PCL filaments were filled with cell-loaded hydrogel in a way that a residual porosity remains, tissue necrosis could be further reduced due to a better diffusion of oxygen and nutrients during in vitro and in vivo cultivation [23]. Therefore, when embedding PCL with a residual porosity, it is a trade-off that an increase in PCL content leads to an increase in mechanical strength, but also a decrease in porosity and hydrogel content [24].

The printer presented here offers the possibility to fabricate thin PCL and hydrogel filaments and incorporate porous channels in the micrometer scale. For this application hydrogel consisting of alginate and gelatin was printed as a thin filament (diameter 445\,\mu m ± 61\,\mu m) between each filament of PCL, leaving a residual porosity of about 50%. For in vitro or in vivo applications, different kinds of hydrogels and cell types can be used to address various tissue types for regenerative medicine. Among others, PCL/hydrogel structures of such composition have already been successfully used for the replication of bone [25], cartilage [26,27] and skin [28]. An exemplary design, the printed structure, and an x-ray image from inside the structure can be seen in Fig. 16 A. X-ray images were obtained from a CT-scan with a \mu CT Skyscan 1272 from Bruker Corporation (Billerica, USA).

The design, the printed structure, and a \mu CT-scan of a transparent in vitro microperfusion chamber can be seen in Fig. 16B. The chamber was designed with the objective to provide a closed cartridge for tissue-culture featuring a transparent housing for microscopic investigations. This was achieved by printing the body of the perfusion chamber and adding a fine line of

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Fig. 16. Example applications for hybrid 3D-bioprinting. The design, printed porous structure, and a \mu CT image of the inside of the porous structure are shown in A). PCL (white) was FDM-printed as a 10 × 10 × 10 mm³ scaffold with a porosity of about 75% to enhance mechanical properties. The hydrogel (red) was printed layer by layer as single filaments in the spacing between the PCL filaments. Inside the structure, hydrogel and PCL can be easily distinguished in the \mu CT image. The remaining porosity of about 50% provided an excellent possibility for diffusion for further in vitro cultivation or in vivo application. The design, the printed construct, and a \mu CT scan of an FDM-printed perfusion chamber equipped with a silicone sealing and a glass window are shown in B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
silicone as sealing and adhesive for embedding a microscope glass coverslip on the top and bottom to close the 3D-printed cavity tightly. Through the glass, the inside of the chamber remains accessible for both transmitted and reflected light. From the two Luer-Lock connectors on the left and right side, three microfluidic channels pass through the interior of the chamber and allow fluid to flow homogeneously through the space between the two glass plates. This enables the permanent supply of a tissue sample with fresh cell culture medium under controlled perfusion conditions, for example, for long-term in vitro incubation of 3D-bioprinted tissue samples as well as for the controlled supply of growth or differentiation factors.

In the current state of the art, the perfusion chambers are either manufactured separately from the bioprinted constructs [29] or purely made of silicone [30, 31]. In contrast, the here presented FDM printing of the chamber body from stable thermoplastic offers increased mechanical strength and the integration of connection points, as shown here for example by printed Luer-Lock connections. Furthermore, 3D printed silicone sealing offers the possibility to embed the entire perfusion chamber fabrication into one continuous fabrication process, which can be particularly important for the production of a microenvironment for cells.

In conclusion, it was shown that the presented 3D-bioprinter has retained its full functionality as a conventional 3D-printer as far as the FDM-printing is concerned. By the addition of the syringe-based microextrusion printhead, as described in detail above, one can now also use the printer to incorporate hydrogel, silicone, or other materials simultaneously into the FDM-printed parts at any position within the design, as shown in the examples. This opens up the opportunity for low-cost 3D-bioprinting using multiple materials and technologies to produce complex hybrid structures that are not feasible using conventional manufacturing technologies.

CRediT authorship contribution statement

Fritz Koch: Methodology, Project administration, Validation, Visualization, Writing – original draft. Ole Thaden: Data curation, Investigation, Software, Writing - review & editing. Kevin Tröndle: Formal analysis, Writing - review & editing. Roland Zengerle: Conceptualization, Writing - review & editing. Stefan Zimmermann: Formal analysis, Resources, Writing - review & editing. Peter Koltay: Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by funding of the Bundesministerium für Bildung und Forschung (03VNE1034B) and of the Deutsche Forschungsgemeinschaft (KO3910/1-1 and KO3910/1-2). The article processing charge was funded by the Baden-Wuerttemberg Ministry of Science, Research and Art and the University of Freiburg in the funding programme Open Access Publishing.

Human and animal

The work did not involve human or animal subjects.

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Fritz Koch studied from 2011 to 2014 microsystems engineering (B.Sc.) at the University of Freiburg and finished his studies during an internship at XaarJet AB in Stockholm, Sweden. In his bachelor thesis he developed a non-contact printing and sintering process of highly conductive copper inks. In 2016 he finished his studies in microsystems engineering (M.Sc.) majoring in Materials and MEMS processing. In his master thesis at the Laboratory for MEMS application in the “Non-contact microdosage”-group of Dr. Peter Koltay he developed a technique for an in-line viscosity measurement in a disposable flow sensor for medical applications. Since 2016 he is a member of the scientific staff of the Laboratory for MEMS application of Prof. R. Zengerle and started his PhD in 2017 in the field of 3D-bioprinting. He focusses on the development and quantitative assessment of generic processes for 3D-printing of artificial tissue.