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Design, alpha testing, and beta testing of a 3-D printed open-hardware portable cryopreservation device for aquatic species

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Abstract

Efforts in development of germplasm repositories to preserve genetic resources of aquatic species are impeded globally by a lack of standardized, inexpensive, reproducible, and portable cryopreservation technologies. The present work demonstrates a 3-D printed standardizable freezing device that can be used with nitrogen vapor shipping dewars for on-site sperm cryopreservation for aquatic species and be distributed as open-source The SDPCD could hold 22 French straws (0.25-mL or 0.5-mL) and a quick-release ring design could eject straws directly into a canister inside a dewar by pressing a button after freezing. The final prototypes produced cooling rates of 1 to 64 °C/min for 0.25-mL straws, and 3 to 37 °C/min for 0.5-mL straws with material cost of US$3.5 for a single device and US$1,820–2,562 for batch production of 20 replicates. Progressing through design, prototyping, and testing was delineated to help guide development of other open-source devices within cryopreservation user communities.

Keywords

Sperm cryopreservation; germplasm repositories; 3-D printing; alpha testing; beta testing; open source

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Introduction

Currently, there are three major approaches used in cryopreservation by use of liquid nitrogen: computer-controlled programmable freezers, positional freezing in boxes with liquid nitrogen vapor, and positional freezing within shipping dewars (Bernáth et al., 2015; Conget et al., 1996; Jodun et al., 2007). Programmable freezers have the capability to freeze hundreds of samples at a time with precisely controlled cooling rates. These freezers meet the needs for well-funded facilities participating in commercial production, national repositories, or research activities, but are not affordable (US$15,000 and US$60,000) for most users. As such, many laboratories construct non-programmable freezers by using variations of a polystyrene foam box with a raft to freeze samples within liquid nitrogen vapor (Cabrita et al., 2001; Horváth et al., 2005; B. Liu et al., 2015). In this case, cooling rates are determined largely by the distance between the samples and the liquid nitrogen surface (Gwo et al., 1991). This method has limited portability because the insulation provided by common polystyrene boxes is not suitable for transportation of liquid nitrogen for long-distance (> several h) field trips. To address portability issues, shipping dewars, which were originally designed for shipping of frozen samples were adapted for use as field freezing devices (Harvey et al., 1998; W. R. Wayman et al., 1997), with cooling rates determined by the positioning of samples at different heights on canes inside the dewar. However, no aspects of this approach are standardized among user communities, producing unquantified variation which weakens or prevents realistic comparisons of results.

Most previous effort for sperm cryopreservation has focused on research to develop and optimize protocols, while not taking into account how those protocols would be applied in real-world applications. Although many hundreds of protocols have been published, a pervasive lack of standardization, affordable hardware, and reproducibility limits the application of cryopreservation and inhibits repository development (Torres et al., 2016). Open-source technology is a powerful approach to these problems because it allows community users to gain access to standardized technologies with a low cost (Y. Liu et al., 2020). The ‘open-source’ strategy has been applied in software development and now is fueling a new movement in development of scientific hardware (Pearce, 2012). Instead of purchasing expensive proprietary equipment, users can download, fabricate, and assemble designs and devices with low cost. In addition, multiple community members can contribute to design changes for modifications and improvement, facilitating eventual convergence of design and community-level standardization (Y. Liu et al., 2019).

Powerful and widely accessible new technologies such as fused deposition modeling (FDM) three-dimensional (3-D) printing, for example, has begun to be applied in cryobiology to provide standardized, easy to use, and inexpensive cryopreservation devices (C. J. Tiersch et al., 2020; T. R. Tiersch & Monroe, 2016). For example, open-source 3-D printed devices were customized for sperm vitrification, providing opportunities for community-level standardization in real-world applications (N. J. Tiersch et al., 2018; Nolan J Tiersch & Tiersch, 2017). Another example is a 3-D printed rack system with multiple standardized configurations to generate various cooling rates typically used for sperm cryopreservation

(Hu et al., 2017). As such, standardized 3-D printed devices could be developed to address the challenges of sample freezing in the field with shipping dewars.

Although research is emerging in development of 3-D printed devices to assist biological applications (T. R. Tiersch & Monroe, 2016), there has been a lack of strategic guidance for prototype development and testing. We recognize two major testing processes and four prototyping stages during the progression from ideas to user-ready solutions: 1) alpha testing (in-house), including design, component prototyping, operation prototyping, and performance prototyping, and 2) beta testing (external testing), including closed (by experienced users) and open testing (by novice users). In the design phase, computer-aided design (CAD) software is used to facilitate creation, modification, analysis, and optimization of designs (Groover & Zimmers, 1983). During *component prototyping*, computer-aided manufacturing (CAM) is the used (e.g., 3-D printing or computer numerical-control milling) to convert CAD designs into physical prototypes as individual components, and functionalities of these components are evaluated individually. During *operational prototyping*, suitable versions of the component prototypes are integrated and assembled into composite devices. The operation of integrated prototypes ("operational prototypes") is evaluated and multiple "variations" are developed. During *performance prototyping*, prototypes are tested for functionary, including biological utility, reproducibility, reliability, and efficiency, and refinements are made. Together these stages comprise alpha testing which is performed by design team members. For a solution to be considered as "user-ready", closed and open beta testing are performed to further evaluate the overall functionality, ergonomics, and user experience to fully optimize the prototypes before final release.

As such, instead of focusing solely on the design or function of a final device, the goal of this project also included delineation of the progression through design, prototyping, and testing of a 3-D printed standardized freezing device that can be used with nitrogen vapor shipping dewars for on-site cryopreservation and distributed as open-source hardware. The objectives were to: 1) design components of the device using CAD software; 2) fabricate components using 3-D printing and evaluate component prototypes; 3) evaluate operational prototypes; 4) evaluate performance prototypes; 5) evaluate efficiency for batch production of 20 devices, and 6) conduct closed beta testing. The final device was able to produce the range of cooling rates commonly used in sperm cryopreservation for aquatic species with 0.5-mL and 0.25-mL French straws. Modifications can be made in future designs to refine the cooling rates and accommodate other containers, such as Cryo-vials. This work signals great potential for user communities to develop portable standardized devices for cryopreservation and the prototyping process documented can provide strategic guidance for development of open-source scientific hardware.

Methods

Design

The overall concept of the design was to produce a device that could: hold containers such as French straws (Fig. 1a), be positioned inside a standard shipping dewar (Fig. 1a–c), provide various straw height options, and eject the straws (Fig. 1d) within the shipping dewar after freezing for short-term storage and transportation. As such, the device was classified as a shipping dewar positional cooling device (SDPCD), and referred to informally as the “Cajun Ejector”. A list of design constraints was established to guide the design process: the device should: 1) fit within the neck and inner chamber of a standard shipping dewar (91 mm); 2) hold 0.25 or 0.5-mL French straws in a radial arrangement without contact between straws to avoid interference with cooling; 3) allow adjustable heights to provide a range of cooling rates commonly used in sperm cryopreservation (4–40°C/min); 4) release the straws directly inside the shipping dewar after freezing, and 5) require only basic 3-D printing skills with entry-level printers (< US$300). Components were designed using freeware CAD software (Fusion 360, Autodesk, San Rafael, CA), and saved as stereolithography (STL) files to be processed by CAM slicer software. As development progressed from initial design through the prototyping stages, prototypes would return to the design stage for revisions based on evaluation.

Fabrication and component prototyping

The CAD files were converted prior to printing by use of a powerful freeware slicer software (Cura 4.0, Ultimaker, Cambridge, MA) to adjust print settings (Table 1) and convert the STL files to G-code files to control operation of the 3-D printers. Consumer-level Ender 3 (Creality3D, Shenzhen, Guangdong, China) printers were used with common polylactic acid (PLA) filament (ZYLtech Engineering, Spring, TX) to fabricate individual components (Table 2). All components were initially prototyped using basic settings such as 25% infill, 2 wall/perimeter layers, and 3 top and bottom layers (specific settings are delineated below). Removable support material was used to assist printing of overhanging structures if necessary. Individual components were evaluated for their functionalities (e.g., the quick-release ring could hold and eject straws under non-cryogenic temperatures). If components were designed to be assembled together (e.g., the inner quick-release ring and positioning rod), the fitting of connection regions were also evaluated. Changes of designs and printing settings were made based of multiple evaluations of components.

Operational prototyping

After the components were deemed to be individually functional and compatible with each other, prototypes of the full device were assembled, and the operation of the assembly at nitrogen vapor temperatures was evaluated. The operational prototypes were loaded with empty French straws and positioned into a fully charged shipping dewar. After 30 min (longer than a typical freezing run to ensure completion), the straws were ejected and structural weaknesses of assemblies were identified. If components cracked or broke during testing, changes of designs or printing settings were made, and evaluation were repeated with the updated prototypes. Multiple versions were tested during this stage and the availability of multiple printers can speeds the process.

Performance prototyping

After repeated operational prototyping, superior designs were chosen for performance testing. Cooling curves produced by different vertical positions inside the dewar were recorded by use of three type-T thermocouples (5SRTC-TT-T-30-36, Omega Engineering, Norwalk, CT) and a multi-channel temperature data logger (UX120-014M, Onset Computer

Corporation, Bourne, MA). Preliminary evaluation was performed to establish the number of height adjustment slots needed to produce a range of cooling rates commonly used in sperm cryopreservation. After the number of slots was established, the cooling rate of each slot was evaluated with two replicated runs.

Prior to each test, a shipping dewar (CXR-100, Taylor-Wharton, Baytown, TX) was filled 3–4 times over several hours until no more liquid nitrogen was adsorbed and was held full for at least 24 hr before testing. Any remaining liquid nitrogen was poured out on the day of testing. The dewar was plugged by the cap for a minimum of 5 min to stabilize the nitrogen vapor. French straws were filled with Hanks' balanced salt solution at an osmolality of 300 mOsmol/kg (HBSS300: 0.137 M NaCl, 5.4 mM KCl, 1.3 mM CaCl2, 1.0 mM MgSO4, 0.25 mM Na2HPO4, 0.44 mM KH2PO4, 4.2 mM NaHCO3, and 5.55 mM glucose, pH 7.2). All straws were sealed using an ultrasonic sealer (Ultraseal 21, Minitube of America, Inc., Verona, WI) except for the three straws used to record temperature.

To ensure thermocouples were positioned at the same location, marks were made at the midpoints of test straws to identify the insertion depth. The open ends of the unsealed straws (with thermocouples) were inserted into specified positions of the ring of the SDPCD. The cotton-plug ends of the sealed straws were inserted into the remaining holes (Fig. 1a). A total of 22 straws were inserted into the ring. For each test cycle, thermocouples were inserted, the data logger was started, the dewar plug was removed, and the SDPCD was lowered (Fig. 1b) into the shipping dewar until the slot bar sat on the dewar opening aiming to keep the device centered inside the dewar chamber (Fig. 1c). It took < 5 s from the start of the data logger until the device was in position inside the shipping dewar. The data logger was stopped when temperatures of all thermocouples reached −80 °C. Thermocouple positioning inside the straws was verified again after straws were removed from the dewar. If noticeable changes in the thermocouple positions (e.g., the thermocouple was pulled out of the straw during operation) were observed, the data were discarded and the testing was repeated. The cooling rate was calculated as the temperature change (i.e., 84 °C) divided by the time used to traverse the temperature range of 4 °C to −80 °C.

Batch Fabrication

To prepare beta testing, multiple units of a prototype (e.g., 10–20, "batch production") need to be manufactured for use by evaluators. Although 3-D printing is effective for prototyping in alpha testing ("craft production"), batch production of devices with a single printer can take days or weeks to produce larger numbers of individual components. In the present study, the startup costs (purchase and assembly of 3-D printers) and production costs (producing prototypes) for printing 20 replicate SDPCD devices (8 individual components per device) was compared at two levels of production. Level one productivity stated that a single printer was used to produce 20 replicates, and level two stated that five printers were used. It was assumed (based on our experience assembling 25+ 3-D printers) that it would require 1 d for inexperienced users to assemble and test a single printer, and 2 d to assemble and test 5 printers (the time for assembly and testing of each printer is not equal because of a learning process of users). Production costs included costs of materials (filament and spring) and labor for the operation of printers. All prices (reported as $US) represent the mean of three prices quotes from different supply vendors in 2020. All labor costs were calculated at $13/hr.

Print time was estimated by use of a feature included in the slicer software (CURA). For each component, the print time for the maximum number of parts that could fit on a build plate was estimated, and the print time for the remaining components required to complete 20 devices was added. The total print time was used to calculate the required number of 8-hr work days. A printing workday consisted of removing completed prints, starting new prints, and performing any post-print processing (e.g., removal of support material).

Closed beta testing

A closed beta testing session was held in 2019 at the annual Aquaculture America conference in New Orleans, LA. A total of 26 people attended the session. There were 12 females and 14 males, 5 participants were <30 yr old, and 14 were native English speakers. Two were in the private sector, 2 were in the federal sector, 3 were in an industrial sector, 1 was from an institute, and 18 were in academia. There were 5 students, 11 faculty, 5 researchers, a facility supervisor, a licensing associate, a vice president, a sales account manager, and a consultant. Five had a bachelor's degree, five had or were pursuing a master's degree, and 16 had or were pursuing a doctorate degree. This testing session was divided into six sections: 1) introduction; 2) part list overview; 3) device assembly; 4) familiarization with operation; 5) calibrating the device, and 6) using the device. After the introduction of the SDPCD, eight groups of 2 to 4 people were formed and tasked with using paper-based or computer-based versions of the instructions to complete Sections 2 through 6. The time to complete sections 3 was recorded and feedback comments were collected from each group.

Results

Design of individual components

The final device had a total of eight separate 3-D printed components (Fig. 2): 1) dewar cap (which reduced heat exchange between inside and outside of the dewar, and stabilized stratification); 2) cross bar (suspended the device in dewar at selected slot number); 3) ejector cap (pressed to eject straws); 4) ejector cap locking bar (attached ejector cap to device); 5) positioning rod (connected to inner quick-release ring and provided nine slots for height variation); 6) upright support (aided in ejecting straws.); 7) inner quick-release ring with thermocouple port (held and released straws), and 8) outer quick-release ring with thermocouple port (held and released straws). Each part underwent multiple design changes with most changes necessary for satisfactory functioning of the inner and outer quick-release rings (~45 design versions). French straws with 0.25-mL or 0.5-mL volumes were held by use of two different sizes of inner quick-release rings.

Evaluation of component prototypes

Each component progressed through multiple versions. The mechanism to secure and release the straws offered a typical example of this progression. The preliminary versions of the quick-release feature relied on small pegs to eject straws from the ring (Fig. 3). This version reliably held straws, but the pegs often snapped or failed to eject completely. To reduce the weakness of the pegs, the percent infill for the pegs was increased from 25 to 100 and the straw slots were given a fillet (rounded edge) to assist ejection, but snapping still occurred after these changes. A split-ring design was used in subsequent versions, where the inner ring could be depressed by pressing the ejector cap to reliably eject all straws into the bottom of a shipping dewar. The positioning rod was attached to the inner quick-release ring using a T-shaped connection. This type of connection was chosen because it was easier to print compared to a snap-fit or screw connection.

Evaluation of operational prototypes

Ultimately, the split-ring design was chosen for operational prototyping, and five design variations were made to improve assembly, stability, and operation (Fig. 4). Early variations of the overall device design were complicated, requiring excessive support material to print, and the use of two hands to eject the straws (one to hold the outer ring and one to push down on the positioning rod). Subsequent designs included an upright support and ejector cap, allowing straws to be ejected with a single hand. Some variations of the split ring were simple to print, but components failed (e.g., cracked or separated) during operational testing in cryogenic temperatures (Fig. 5). Early versions of upright support also failed during testing. Alterations were made to the design and print settings to balance fabrication ease and functional strength (Table 3).

Evaluation of performance prototypes

The overall dimensions of the final assembled device used for performance testing was 197 mm tall and 65 mm wide at the widest point. It featured 9 height slots (earlier versions had 8) with 13.3 mm intervals to produce various cooling rates. Slot 1 positioned the device at the lowest point inside the dewar resulting in the highest cooling rate, and Slot 9 positioned the device at the highest point, resulting in the lowest cooling rate (earlier prototypes had a reversed numbering system). These slot numbers could be printed directly on the upright support to provide permanent labeling and easy identification. The device produced a range of cooling rates of 1 to 64°C/min for 0.25-mL French straws, and 3 to 37°C/min for 0.5-mL French straws (Fig. 6). The target temperature of −80°C was not reached for 0.25-mL French straws at Slot 9. During late testing, a metal compression spring (Product number SP-9711, Prime-Line, Redlands, CA) was added to the positioning rod between the upright support and ejector cap. These springs were originally 89 mm long, but were cut into 35 mm sections for use. This spring added functionality by allowing the inner quick-release ring to be automatically rejoined with the outer quick-release after straws were ejected into the bottom of the shipping dewar.

Batch fabrication

Startup costs for Level-one production were estimated to be $298 and startup costs for Level two were $1,180 (Table 4). A complete device including support materials weighed 110 g, requiring three 1 kg rolls of filament at $20 per roll to produce 20 devices. While single-printer production required three rolls, five-printer production required five individual rolls to utilize all five printers. Each spring package included 2 spring at $3.76 per package. One package could outfit four devices after being cut into the appropriate lengths, requiring

5 packages for 20 devices. It was estimated to take 21 d to print 20 devices with a single printer and 5 d with five printers, resulting in production labor costs of $2,184 for a single printer and $520 for five. Total cost (startup + production) was $2,562 for a single printer, and $1,820 for five.

Closed beta testing

The goal of the closed beta testing session was to gather user performance information and feedback evaluations to guide design changes to improve usability of the device. The average assembly time for four individual steps was 55 ± 43 s (mean ± SD), with an overall assembly time for the completed device of 3.6 ± 2 min. All groups completed the assembly by reading the instruction materials without assistance from outside their groups. The two most frequent comments on usability were: 1) the dewar caps did not rest stably on the cross bar, and 2) the straws easily fell from the holes. To address the first problem, a new design was developed with the cross bar integrated into one of the dewar caps with a snap-lock system to reliably connect the two dewar caps during freezing (Fig. 7). For the second problem, the thickness (distance between top and bottom surfaces) of the inner and outer quick-release rings was increased to provide more holding surface area, and the instructions were modified to ensure complete insertion of straws into the holes.

Discussion

To address challenges of the lack of low-cost and reproducible freezing devices, 3-D printing has begun to be applied for cryobiology. To provide guidance for potentially increasing and standardizing development among research communities, the purpose of this study was to not only introduce a 3-D printed shipping dewar cryopreservation device, but more importantly to document the overall process from initial designs to final user-ready solutions. As such, it is recommended that prototypes reported in manuscripts should be tested and documented in a standardized way, and thus readers can appropriately reproduce and apply these solutions in their own research. This concept is embodied in collective efforts such as the open-source hardware movement (Oberloier & Pearce, 2018) and is based on our longstanding interdisciplinary collaboration with multiple engineering fields.

The cost of 3-D printers has decreasing markedly over the past 10 yr, and they have become widely available for use at home and in the workplace (Attaran, 2017). In addition to the increasing accessibility of 3-D printers, CAD software has become more user-friendly and available for entry-level users. With programs such as Tinkercad (Autodesk, San Rafael, CA), novice users can begin to design objects from ideas for free without an engineering background. Scientists have begun to use CAD/CAM technologies for basic labware (e.g., centrifuge tube holders), and to create custom devices for use in research (Coakley & Hurt, 2016). For example, electronics and components made from different manufacturing techniques (e.g., milling) can be combined with 3-D printing to create low-cost research devices ranging from water turbidity measurement to Western blot processing (Bravo-Martinez, 2019; Kitchener et al., 2019; Shamkhalichenar et al., 2019). In addition, 3-D scanners are being used to capture profile data and have been used to print life-size decoys for animal behavior studies (Bulté et al., 2018).

Design of Individual Components

Previously, shipping dewars have been used by researchers for sperm cryopreservation in field applications. In these cases, samples were placed in cryopreservation goblets positioned at the bottom (fast cooling) or top (slow cooling) of an aluminum cane inside shipping dewars (Carolsfeld et al., 2003; William R Wayman et al., 2008). Although it was feasible to use this method to cool samples, it was difficult to report and replicate the cooling rates because of unstandardized variables, such as shipping dewar model, straw size, positioning of straws, number of straws being cryopreserved, and the amount of liquid nitrogen present (W. R. Wayman & Tiersch, 2011). A major advantage with the design of the SDPCD was the radial arrangement of straws, preventing straws from contacting each other or the inner wall of the dewar. As such, the undesired interference of cooling rates because of thermal contact was greatly reduced.

In addition, the quick-release mechanism enabled direct release of straws after freezing, eliminating the risk of sample damage due to handling outside of dewars. Dropping straws directly into the dewar canister (or a large goblet) allowed for samples to be frozen in the field and easily transported or shipped back to a central facility for sorting (under liquid nitrogen) and long-term storage. The standardized adjustable height slots ensured various cooling rates were reproducible. The SDPCD was specifically designed to fit inside the neck of a standard (Taylor-Wharton CXR-100) shipping dewar, but can be customized easily if necessary to fit other dewar models (validation of cooling rates should always be performed and reported for use of any shipping dewars).

Component prototyping

It is important to consider the utilization of the individual components and the device as a whole to identify the most appropriate type of thermoplastic filament. To date, 3-D printed devices for use in cryogenic temperatures have been printed using PLA and ABS (acrylonitrile butadiene styrene), two of the most commonly used filaments (Shamkhalichenar et al., 2019; N. J. Tiersch et al., 2018). For novice 3-D printer users, PLA is easiest to use because of its lower melting temperature (153 °C) and the reduced likelihood of parts warping during printing, but there are disadvantages (Squires & Lewis, 2018). It is more brittle after cooling compared to ABS, making it less suitable for high stress applications, and has a lower glass transition temperature (60°C compared to 113°C for ABS) which can result in 3-D printed parts becoming soft and warped in hot environments (e.g., in the field or on the dashboard of a vehicle). The ABS material is stronger but is more suited to intermediate or advanced users because it is more difficult to use as the printing bed must be heated to at least 110 °C (PLA can be printed without a heated bed), and the higher thermal expansion coefficient (100×10^{-6} °C compared to 70×10^{-6} °C for PLA) causes parts to easily warp during printing.

Just as FDM technology has expanded, so has the development of new filament types. One filament type gaining popularity is PETG (polyethylene terephthalate with glycol). Components printed in PETG are as strong as ABS, but the printing process is similar to PLA as it has a lower thermal expansion coefficient (59×10^{-6} °C). The PETG filament would also be more suited over PLA for devices used in hot environments as it has a glass transition temperature of 75°C. Future studies should test the performance of multiple filament types for cryogenic applications.

Operational prototypes

Operational evaluation in cryogenic temperatures of the initial designs (e.g., using pegs to eject straws) resulted in broken pieces and incomplete ejection. Moisture buildup on straw surfaces and pegs due to condensation also caused straws to seize after freezing. To address these problems, split-ring designs were developed to reliably hold straws under cryogenic temperatures, and completely eject them by the separating the two pieces of the rings.

During early evaluations, the upright support, inner quick-release ring, and outer quick-release ring could fail due to cryogenic temperatures. Three-dimensional printed components are typically not fabricated as solid (100% infill) pieces, because it would require unnecessarily large amounts of filament and longer printing times. Instead, components are printed with various layer numbers for walls, tops, and bottoms, and various infill percentages and patterns to achieve adequate strength with less materials and time. This work focused on optimizing the design, number of wall and perimeter layers, and infill percentage, with three top and bottom layers being sufficient for all parts.

For the upright support, the square pegs that connected to the outer quick-release ring, and the slot for the positioning rod each failed. To address this, the number of wall layers was increased from 2 to 3, and the designs were modified to increase the thickness of the positioning rod slot. For the inner quick-release ring, the T-shaped connection point separated from the base. To address this, surface areas of the connection point to the base were increased in designs, infill percentage was increased to 50, and the number of wall layers was increased to 3. For the outer quick-release ring, the upper part of the component separated from the ring. To address this, the design was modified to increase the surface area of this connection point, infill percentage was increased to 75, and the number of wall layers was increased to 3.

Performance prototyping

Cooling rates are important to post-thaw quality. Slow cooling rates may lead to cell damage due to solution effects (e.g., exposure to high concentrations of solutes for extended periods of time), whereas fast cooling rates can damage cells because of increased intracellular ice formation (Kumar & Betsy, 2015). Adjustment of cooling rates is essential in studies aiming to investigate optimal cooling for development of cryopreservation protocols and practical applications. This is especially important for aquatic species because they are extremely diverse, often resulting in drastic differences in response to the same cooling rate. Although commercial programmable freezers can control cooling rates accurately, they are not affordable to most researchers. This situation greatly impedes extensive community-level repository development and application of cryopreservation technologies.

With the SDPCD, nine different cooling rates ranging from 1 to 64°C/min for 0.25 mL straws and, 3 to 37°C/min for 0.5 mL straws were achieved. These ranges cover cooling rates typically used for cryopreservation research of most animals, such as mammals (Sherman, 1962), fishes (Childress et al., 2019), and bivalves (Hassan et al., 2015). When different models of shipping dewars are used, the cooling rate produced by each slot of the SDPCD could be different from the results in the present study. However, cooling rates can be easily calibrated by use of a thermocouple, a date logger (or temperature meter), and the methods provided in the present study with an extra cost of < $50. In future studies, components to accommodate cryopreservation vials can be designed and tested. Modifications could also be made to further refine the cooling curves for 0.25-mL French straws.

Batch Processing

The batch production of prototypes falls between custom production (1 or 2 replicates) and mass production (thousands replicates) (Wang et al., 2019). In operational and performance prototyping, one or two units of a prototype are often produced for each variation in design change. At this stage, design improvement needs could be identified by one or several internal developers with a small number of prototype replicates. In the past, 3-D printing technology has mostly been utilized until this stage in the development process because of the ability to rapidly change and prototype single designs. As the testing phase moves forward to closed beta testing, dozens of devices can be required to send to potential users for evaluation. When producing dozens of devices, machinery such as injection molding could be used, but 3-D printing technology can still be a good option in production settings in lieu of this traditional machinery (Hopkinson & Dicknes, 2003). Three-dimensional printing does have lower cycle times (e.g., number of devices produced per hour), but with 3-D printing, new molds do not need to be produced for every small or substantial design change in the early stages of device development (Griffiths et al., 2016). There is also less material waste with 3-D printing compared to other manufacturing processes. This helps lower the overall production costs and gives an advantage to 3-D printing.

The present study demonstrated that for batch production with 3-D printing, multiple printers compared to single printer could reduce the production time (21 d to 5 d) and overall cost (US$2,566 to US$1,958). This time and cost efficiency is even more important when multiple batch productions are expected. Development teams often have multiples different prototypes being evaluated at the same time. An understanding of increased productivity resulting from increased numbers of fabrication machines can help development teams make project management plans, coordinate staff, and optimize budget. In addition, for research institutes, the possession of multiple 3-D printers can also allow education, training, and development of tools to assist other research when the printers are not being used for rapid prototyping.

Closed Beta Testing

Beta testing plays an important role in ensuring that products are ready to be released to public users (Dolan & Matthews, 1993). Elements that are not evaluated in the earlier prototyping process can also be evaluated in beta testing, such as user’s manual, training materials, websites, and computer-based Learning Management Systems (LMS). In addition to basic operation and performance of prototypes, other useful aspects, such as perceived functionality, user demand, and ergonomics, can usually evaluated in beta testing. All these considerations are critical to translate research innovation into practical applications for users in the real world. In the present study, based on the instructions provided, prototypes were assembled by testers in several min without assistance, indicating that the performance prototypes and instruction materials were comprehensible by users who had no previous knowledge of the prototypes.

Refinement design changes were made based on feedback of beta testers. These design problems were not recognized by the internal developers prior to beta testing, indicating that beta testing can provide useful information to modify designs and instructional materials. In the present study, potential users who had basic knowledge and cryopreservation experience participated in the closed beta testing. It is possible that their comprehension of the instruction materials and the prototypes was linked to their previous knowledge. In further development, open beta testing would be needed to collect input from users with little knowledge or experience.

Conclusions

In the present study, an open-source 3-D printed device used with standard shipping dewars was developed to generate cooling rates typically used in sperm cryopreservation. This device is low-cost, portable, standardized, and customizable, providing a solution for sperm cryopreservation for various user groups, such as conservation, agriculture, and biomedical research. In contrast to patenting and commercializing products, an open-source strategy allows development outcomes of prototypes or solutions shared for free for any users. As such, individual users not only can gain access to technology with low cost but they can also contribute to the design improvement as a development community.

With the contributions from a larger number of community members, useful new creations and solutions can be developed in a relatively short time and community-based standardization can be achieved (Y. Liu et al., 2019). This work was also intended to demonstrate a generalized process of prototype development from design to closed beta testing. This concept can be used by individual or institutional users to guide their own development. For designers and testing teams, a different focus is needed in the different prototyping stages (e.g., major version changes during component prototyping vs. refinement during performance prototyping). For project managers, different resources and expertise would need to be allocated for different stages of prototyping and testing. In future studies, open-source motoring, automation, and data transfer and management systems could be added to the present work to produce smart cryopreservation devices that can record cooling rates, automatically calibrate, control sample positions, and transfer data to smart devices via Wi-Fi or Bluetooth. These innovations would be motivated by solving problems in biological research and could be accomplished by interdisciplinary collaborations between biologists and engineers.

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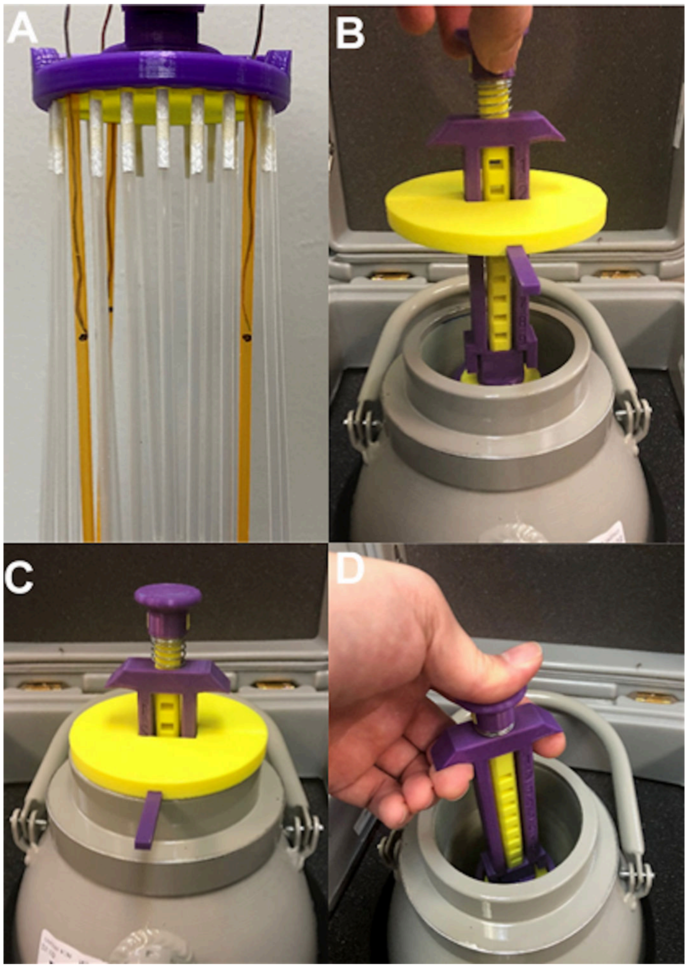

Fig. 1.

Overview of the application process of the SDPCD. (A) Sealed straws were inserted at the cotton-end inserted into holes in the quick-release rings, and three straws with thermocouples for temperature recording were also inserted. (B) Lowering of samples into the shipping dewar. (C) Position of the SDPCD during the cooling process. (D) Ejecting the straws into the bottom of the shipping dewar by pressing of the ejector cap.

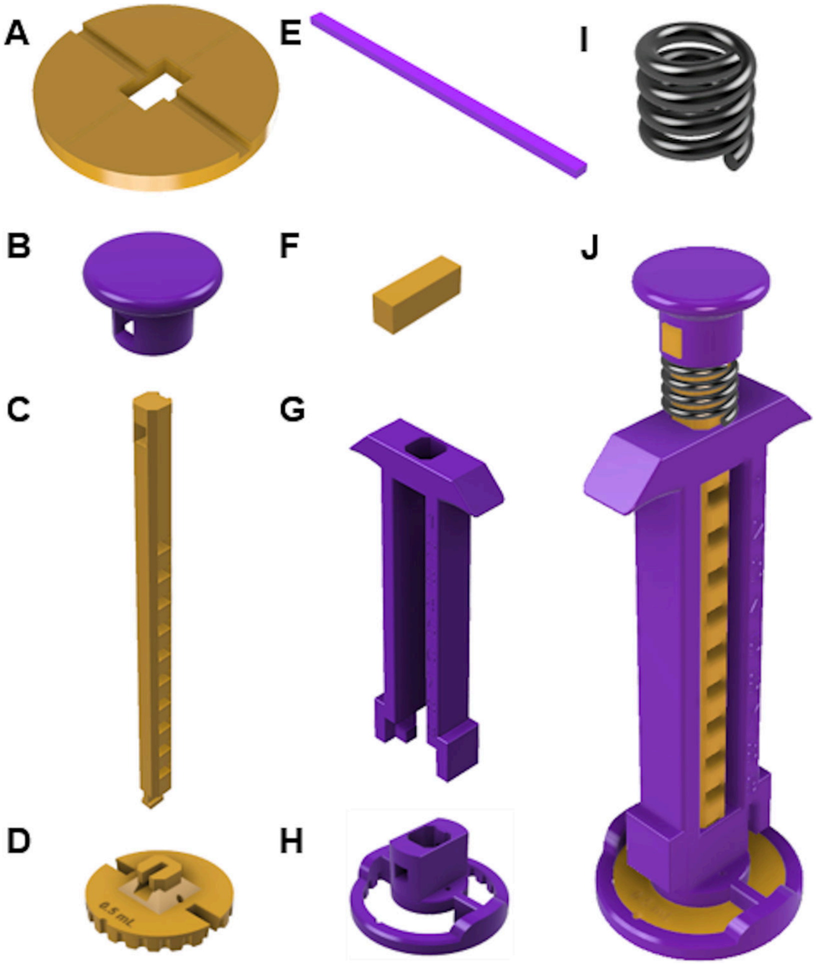

Fig. 2.

Individual components of the SDPCD used for beta testing: (A) Dewar collar (pre-beta test version); (B) Ejector cap; (C) Positioning rod; (D) Inner quick-release ring with thermocouple port; (E) Cross bar; (F) Ejector cap locking bar; (G) Upright support; (H) Outer quick-release ring with thermocouple port; (I) Spring; (J) Assembled device without dewar collar and cross bar.

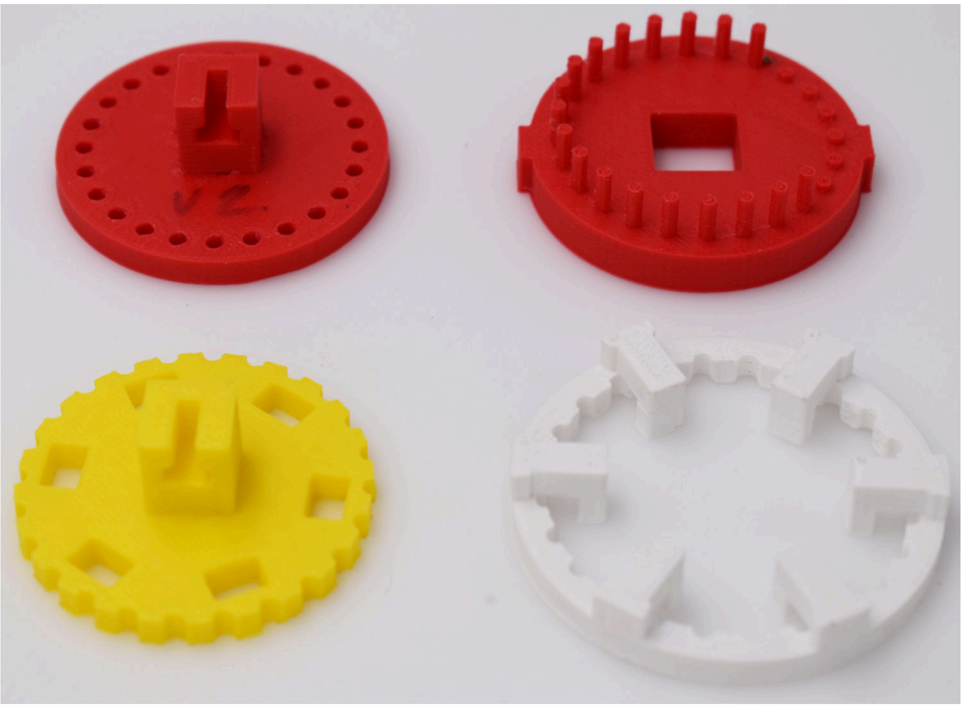

Fig. 3.

Two versions of the straw quick-release ring. Top: Early versions relied on small pegs to eject the straws from the ring. Bottom: An example of the split-ring designs which required use of two hands for ejection.

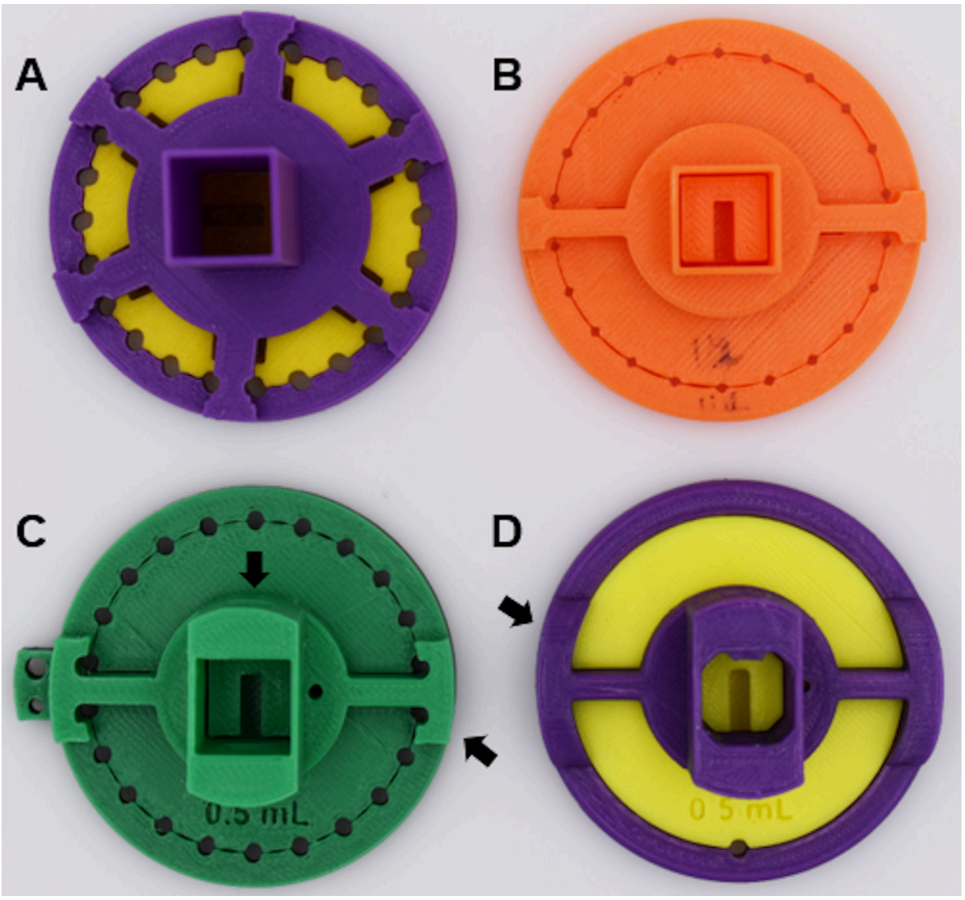

Fig. 4.

Design variations of the quick-release ring. (A) An early variation that had a complex split-ring design that was challenging to print. (B) A simpler split-ring design that showed structural weakness during testing. (C) A design variation of (B) with added cross support to the outer quick-release ring and integrating with the upright support that allowed the straws to be ejected with one hand. (D) the beta-tested version of the split-ring design with further changes to the outer quick-release ring to provide structural strength. The slots for the straws (except for the single thermocouple slot) were also closed at the top to prevent straws from being pushed through. Final versions of the parts also had integrated printed text to identify the 0.5 mL and 0.25 mL inner quick-release rings. Other information such as batch or serial number could be included in future designs.

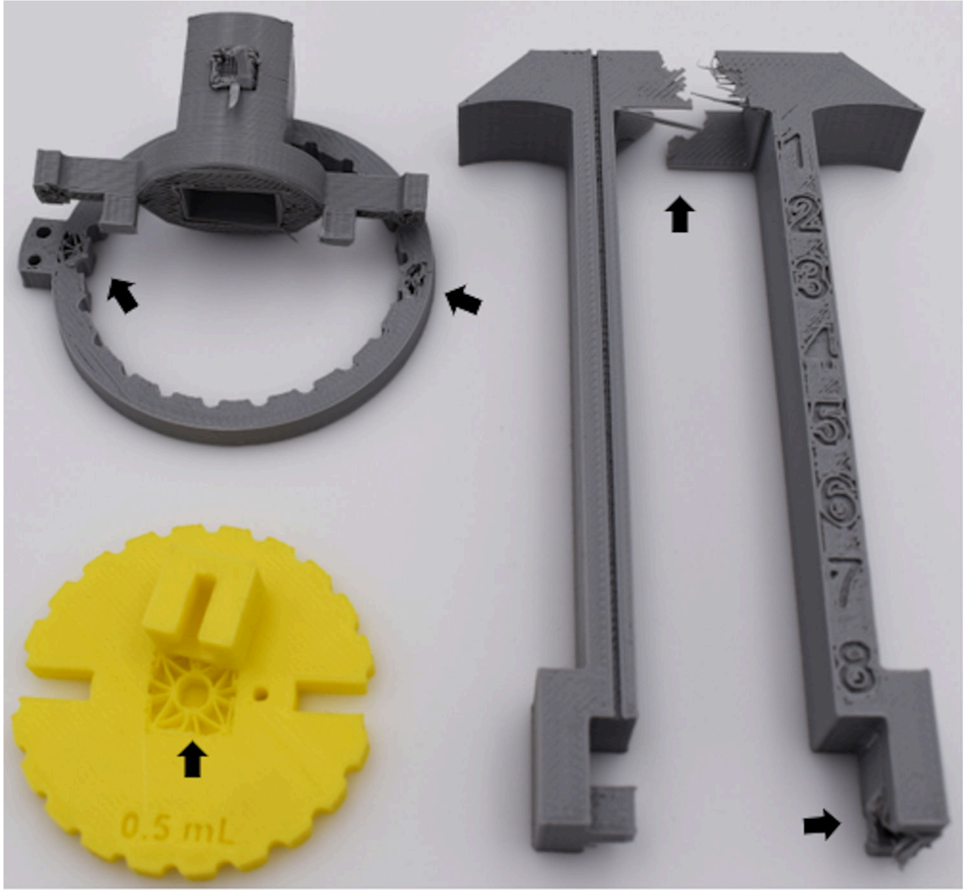

Fig. 5.

Examples of structural weaknesses in 3-D printing including those identified in the quick-release bar, outer quick-release ring, and inner quick-release ring. Multiple changes were made to the designs and the printing parameters to strengthen these parts. Arrows indicate location of structural failures. The lower infill percentage can be seen in the inner quicker-release ring.

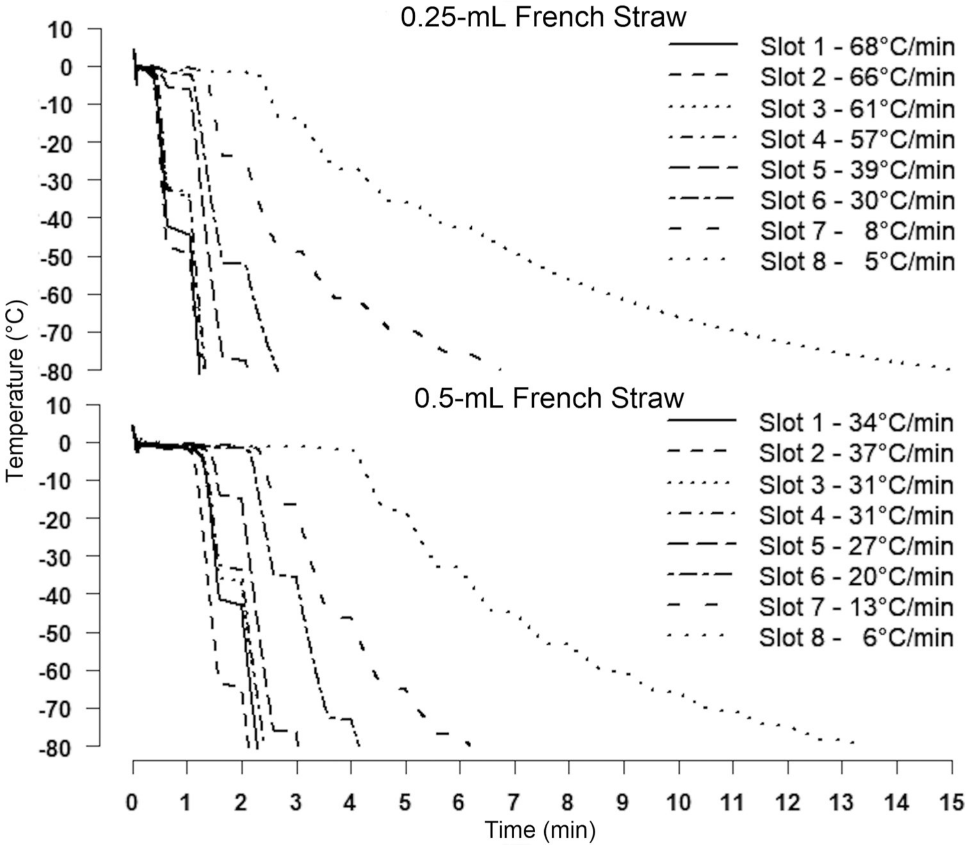

Fig. 6.

Average cooling curves for the different height increments for 0.25-mL (top) and 0.5-mL (bottom) French straws. In general, Slot 9 provided the slowest cooling rates as the straws were positioned in the top of the shipping dewar and Slot 1 provided the fastest as the straws were positioned at the lowest point in the shipping dewar. Data for Slot 9 was excluded from this graph for scaling purposes because cooling rates of 0.25-mL French straws did not reach the −80°C target temperature after 1 hr inside the shipping dewar.

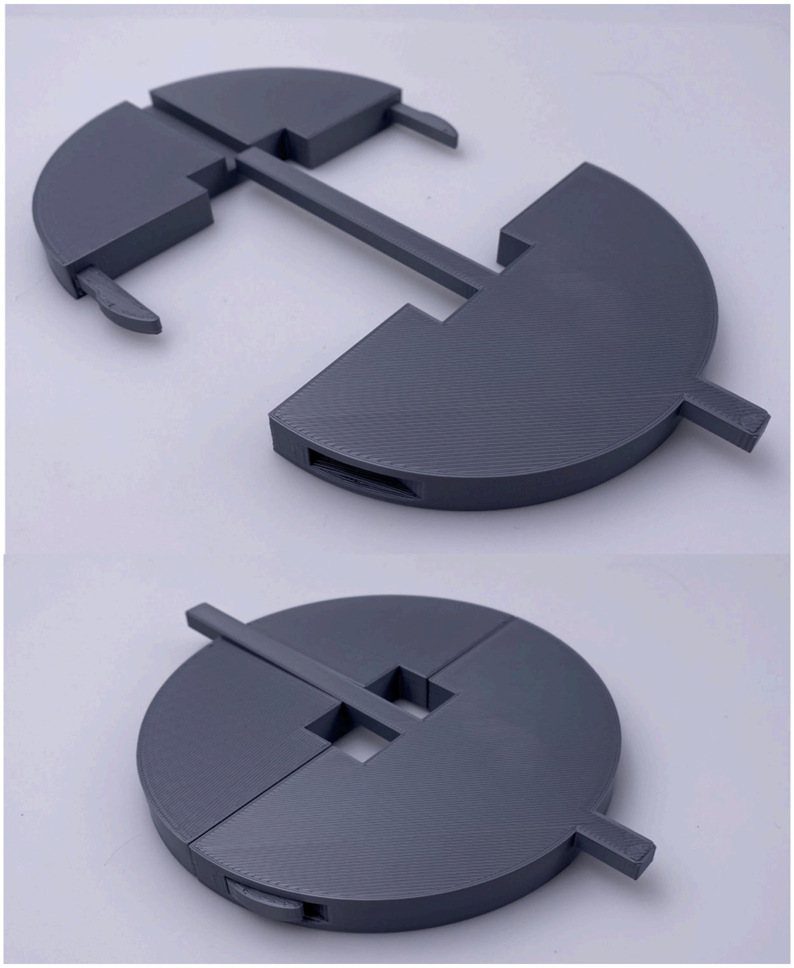

Fig. 7.

Redesigned dewar cap (based on user comments from beta testing) with the integrated cross bar, buckle, and snap-locking system.

Table 1.

Routine 3-D slicer software settings used to control the printing of all components. These features are universal and can be found in most slicer software. They should be included in any reports of open-source hardware to enable proper replication.

Feature	Value
General settings	
Hotend temperature	200°C[1]
Print speed	60 mm/s[1]
Nozzle type	Brass
Nozzle diameter	0.4 mm
Extrusion/line width	0.45 mm
Nominal layer height	0.2 mm
Retraction distance	6 mm[1]
Retraction speed	40 mm/s[1]
Printer bed temperature	60 °C[1]
Part cooling fan speed	90%[1]
First layer settings	
Extrusion/line width	0.45 mm
Layer height	0.18 mm
Print speed	20 mm/s
Hotend temperature	205 °C[1]

[1] These settings can change based on the printer and filament type being used.

Table 2.

Technical specifications for a consumer level (> US$250) Ender 3 printer. Specifications such as these should be included in any reports of open-source hardware to enable proper replication.

Component/function	Value
Power supply voltage	24 V
Extrusion	Bowden
Filament size	1.75 mm
Filament supplier and type	PLA
Filament storage conditions	63-L plastic bin
Build surface size	235 × 235 mm
Build surface	Stock Magnetic
Cooling fan	Blower
Cooling fan size	40 × 40 × 10 mm
Cooling fan voltage	24 V
Auto bed leveling sensor	None

Table 3.

Advanced, part-specific infill percentages, infill pattern, wall and perimeter layers, top layers, bottom layers, support placement, support overhang angle, and support density for each component. These settings assist in the ease of printing and part strength.

Part	Infill (%)	Infill pattern	Wall/perimeter layers	Top layers	Bottom layers	Support placement	Support overhang angle (°)	Support density (%)
Dewar cap	25	Grid	2	3	3	None	None	None
Cross bar	25	Grid	2	3	3	None	None	None
Ejector cap	25	Grid	2	3	3	None	None	None
Ejector locking bar	25	Grid	2	3	3	None	None	None
Positioning rod	25	Grid	2	3	3	None	None	None
Upright support	25	Grid	3	3	3	Build plate only[1]	65	20
Inner ring	50	Line	3	3	3	Everywhere[2]	65	20
Outer ring	75	Line	3	3	3	Everywhere[2]	65	20

[1] Support material is only printed from the build plate

[2] Support material is printed everywhere that is needed

Table 4.

Batch fabrication capital costs (US$) for producing 20 devices (each with 8 separate parts) at two levels of production. Purchase of five printers increased start-up costs, but reduced overall printing time from 21 to 5 days.

	Production level	
Items	**1 Printer**	**5 Printers**
Start-up		
Printer (purchase cost)	$194	$972
Assembly labor	$104	$208
Production		
Spring	$19	$19
Filament	$61	$102
Production labor	$2,184	$520
Totals		
Startup	$298	$1,180
Production	$2,264	$640
Overall	$2,562	$1,820