Cryobiology 88 (2019) 64–69

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/cryo

Three-dimensional printing can provide customizable probes for sensing and monitoring in cryobiology applications

Hamed Shamkhalichenar[a], Jin-Woo Choi[a,b], Terrence R. Tiersch[c,*]

[a] School of Electrical Engineering and Computer Science, Louisiana State University, Baton Rouge, LA, 70803, USA
[b] Center for Advanced Microstructures and Devices, Louisiana State University, Baton Rouge, LA, 70803, USA
[c] Aquatic Germplasm and Genetic Resources Center, School of Renewable Natural Resources, Louisiana State University Agricultural Center, Baton Rouge, LA, 70820, USA

ARTICLE INFO

Keywords:
3-D printing
Cryopreservation
Aquatic
Standardized freezing devices
Sensing probe

ABSTRACT

Cryopreservation has been recognized as a powerful tool for long-term preservation of genetic resources. However, the outcomes of cryopreservation by different user groups often vary due to inconsistency in procedures and freezing equipment. Herein, we report on the feasibility of providing customizable sensing probes with three-dimensional (3-D) printing to monitor cryopreservation phenomena. The objectives were to: 1) introduce 3-D printing as a fabrication method for developing customizable probes to be used in cryogenic applications; 2) design and fabricate an example of a 3-D printed sensing probe and multiplexer capable of detecting phase-change phenomena based on quantitative data regarding sample electrical resistance and temperature, and 3) demonstrate the sensing platform in cryopreservation conditions and in combination with a custom-made 3-D printed freezing device. The sensing probe developed was designed to fit within standard 0.5-ml French straws. Phase-transition phenomena were detected by analyzing electrical resistance changes. The quantitative data from this device in conjugation with a 3-D printed freezer rack provided cryopreservation capability with high reproducibility and offered an alternative to expensive programmable freezers. The use of 3-D printing provided flexibility to develop new sensing probes or modify existing designs based on specific needs. After initial prototyping, fabrication, and testing of 3-D printed sensing probes, particularly useful designs can lead to the reduction of variation in performing standardized cryopreservation protocols.

1. Introduction

Cryopreservation is a process in which living cells or tissues are preserved at ultra-low temperatures, while their biological structure and function are maintained. Generally, cryopreservation methods are widely employed for gametes used in artificial insemination and for genetic banking purposes [14]. Expansion of human activities and population growth have produced detrimental changes in natural habitats and raise concerns for increasing food demand. Germplasm cryobanking plays an essential role in preserving genetic resources for conservation of imperiled species and production of improved agricultural animals [1,4]. However, exposure of living cells to low and ultralow temperatures can cause damage; therefore, instrumentation for precise control and measurement during cryopreservation can improve recovery [11].

Due to the considerable biological diversity across aquatic species, hundreds of cryopreservation protocols have been reported [10,20], but most protocols suffer from irreproducible outcomes that originate from a lack of standardized procedural approaches. Multiple factors can be investigated as source of irreproducibility in cryopreservation of aquatic species including variations in rates and modalities of cooling procedures. Therefore, monitoring and control of freezing and thawing can enhance replicability and reproducibility of research efforts, and can improve quality management in commercial-scale cryopreservation facilities [22].

Extensive research has been conducted by scientists from different fields to develop instrumentation for study of the underlying principles involved in cryopreservation and evaluating the influence of physical and chemical processes that can affect cells during freezing and thawing [5,27]. However, most of this instrumentation has been developed for specialized applications such as freezing of human embryos [8], or is intended only for use in research [24]. The high cost of such devices and the necessity for technical skill limit their application in small-scale cryopreservation facilities and agricultural animal production units, especially for aquatic species. Although low-cost freezing devices have been reported to offer inexpensive and portable alternatives to complex

* Corresponding author.
E-mail address: TTiersch@agcenter.lsu.edu (T.R. Tiersch).

https://doi.org/10.1016/j.cryobiol.2019.03.010
Received 17 December 2018; Received in revised form 11 March 2019; Accepted 22 March 2019
Available online 23 March 2019
0011-2240/

freezing instrumentation [9,15], they typically suffer from a lack of consistency due to variations imposed during fabrication and operation by different user groups.

Meanwhile, the emergence of fused deposition modeling (FDM) three-dimensional (3-D) printing has revolutionized prototyping and fabrication in manufacturing and research [6]. The technology provides an inexpensive, simple, and portable fabrication option with high reproducibility, and has been shown to have potential applications in biotechnology and engineering including cryobiology [18,19,21]. Different thermoplastic materials can be used in 3-D printing. Among the commercially available filaments, polylactic acid (PLA) has been shown to have desirable thermal and mechanical properties for use in cryogenic applications [21], and inexpensive 3-D printed freezing devices that can be used in conjugation with conventional polystyrene foam containers have been developed for small-scale cryopreservation purposes [7].

Herein, we propose that 3-D printed devices could be used for monitoring cryopreservation procedures and can be designed and printed based on desired applications by different user groups to reduce variations and improve reporting in protocols. We present an example of a 3-D printed sensing probe that can be used to monitor freezing and thawing phenomena inside conventional cryogenic containers such as French straws. The quantitative information obtained from such sensors can be used to validate and standardize cryopreservation protocols. Two sensing mechanisms were incorporated in the prototype 3-D printed platform. The first was measurement of electrical resistance between two conductive electrodes. Also, a thermocouple was incorporated into the probe to monitor temperature profiles.

Our objectives were to: 1) introduce 3-D printing as a fabrication method for developing customizable probes to be used in cryogenic applications, 2) design and fabricate an example of a 3-D printed sensing probe and multiplexer capable of detecting phase-change phenomena based on quantitative data regarding sample electrical resistance and temperature, and 3) demonstrate the sensing platform in cryopreservation conditions and in combination with a custom-made 3-D printed freezing device. Development of such 3-D printed platforms with multiple sensing mechanisms and existing or modified structural designs of devices by different user groups from the aquatic and other cryopreservation communities could facilitate standardization at research and industrial scales and can have utility among other taxa and applications.

2. Materials and methods

2.1. Working principle of the sensor

The customization capability of 3-D printing makes it possible to incorporate various sensing mechanisms in printed probes to satisfy user-specific needs. To study phase-change phenomena during cryopreservation procedures, measurement of electrical conductivity and temperature were chosen for incorporation in the current probe. Analysis of the electrical conductivity of aqueous solutions has been extensively studied in the area of physical chemistry [26]. Conductivity depends on the amount of electrical current that can pass through a solution with ions as charge carriers. Increases in the concentration and mobility of ions typically result in higher conductivity (or lower resistivity) of a solution. Because the mobility of ions can drastically affect resistance, it can be employed to monitor phase-transition and ice formation process during cryopreservation.

In general, for precise and long-term resistivity measurement in solutions, alternating current (AC) is employed to avoid accumulation of ions at the surface of the electrodes which can cause measurement error due to the additional resistance imposed by polarization. However, by measuring the variation of resistance (not the precise resistivity of the solution) during phase-transition, direct current (DC) can be utilized to simplify electronic circuitry. By applying DC current, an electric field is induced inside the solution and consequently ions, as charge carriers, move according to the field direction. Depending on the concentration and mobility of the cations and anions, a potential difference is generated between the electrodes. By measuring this potential difference, the resistance between the electrodes can be calculated using Ohm's law ($V = IR$, where V is the applied electrical voltage, I is the current, and R is the electrical resistance) [12].

Our premise was that, with the occurrence of phase-transition, the mobility of the ions would decrease drastically, resulting in an increase in measured resistance. Reverse phenomena would occur during the thawing process. Based on the cooling conditions, various ice crystal morphologies and consequently ion mobilities could be obtained; therefore, monitoring the resistance between the sensing electrodes would provide information to evaluate the cryopreservation procedure with consistent and reproducible outcomes.

2.2. Sensing probe design and fabrication

The sensing platform we chose to test was configured to fit within a 0.5-ml French straw which is a conventional container in aquatic species sperm cryopreservation [22] and presents design challenges due to size constraints. The printed sensing probe essentially comprised a stick with dimensions of $100 \times 2 \times 1.5$ mm (L × W × H) with a flat widened end to facilitate handling of the device and provide sufficient area for the necessary electrical connections. To set the distance between electrodes at 1 mm, a spacer was designed along the probe by increasing the printed height of the stick portion by 0.3 mm at the center. Sample solutions filled the gap between the electrodes in the sensing area. The electrodes were exposed to samples only at the detection zone which was a cavity with dimensions of 10×1 mm (L × W) on the probe. Two holes were fabricated on the device for passage of the thermocouple from the backside of the probe, which made it easier to fit the probe inside a French straw. The 3-D printed platform was designed by use of Autodesk Fusion 360 software (San Rafael, CA, version 2.0.3706) (Fig. 1A). A 3-D printer (Replicator 2, MakerBot Industries, Brooklyn, NY) and PLA filament (True White 1.75 mm, MakerBot Industries, Brooklyn, NY) were employed to fabricate the probes. The PLA filament was chosen because of its desirable thermal and mechanical

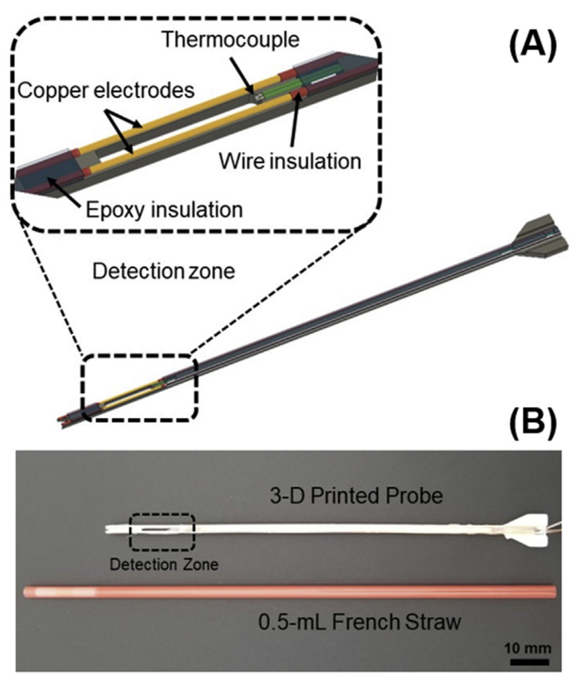

Fig. 1. (A) Schematic representation of the sensning probe, and (B) the actual fabricated 3-D printed sensning probe in comparison to a 0.5-mL French straw.

properties in cryogenic temperatures [21]. A pair of insulated copper wires (Remington Industries, Johnsburg, IL) with a diameter of 0.202 mm (AWG 32) were placed across the sides of the spacer. The wire insulation was removed by heat at the detection zone to provide electrical contact with sample solutions. To attach the wires to the probes and to set the active electrode surface area, the probes were covered with epoxy resin (Bob Smith Industries, Atascadero, CA) except at the sensing area. In addition, a T-type thermocouple (5 TC-TT-T-40-36, Omega, Norwalk, CT) was placed on the probe to record sample temperature. The thermocouple tip was oriented to ensure direct contact with the sample solutions in the detection zone. All of the parameters listed above can be controlled and customized as needed.

2.3. Measurement unit and multiplex circuitry

Different measurement capabilities can be designed according to the sensing mechanisms adopted. In this work, a digital multimeter (Keithley 2110, Keithley, Cleveland, OH) was used to measure the resistance between the two electrodes. Measured data were transferred to a PC using a USB cable and commercial software (KI-Tool, Keithley, Cleveland, OH, version 2.04). Typically, digital multimeters apply different test currents for different resistance value ranges to ensure that the measured voltage across the resistance is in range with the analog-to-digital converter unit in the multimeter. The resistance range in this work was fixed at 12 MΩ maximum to avoid errors originating from test current switching during the measurement.

For measurements at this range, a 0.1 μA test current was applied to the electrodes, which was low enough to minimize the electrochemical reactions at the surface of the electrode. To record output from multiple probes, a multiplexer circuit was designed and fabricated (Fig. 2). The Arduino platform (Arduino Uno Rev3, Arduino, Somerville, MA) was employed as the controller for the circuit due to its simplicity, compatibility with Arduino Software (IDE) (Arduino, Somerville, MA, version 1.8.7) and universal availability [3]. The digital multimeter input was switched among multiple sensors by utilizing a 16-channel analog multiplexer (CD74HC4067, Texas Instruments, Dallas, TX) controlled by the Arduino microcontroller. After each measurement, the digital multimeter sent a completion signal to the Arduino to switch the multiplexer output channel to the next sensor and a triggering signal to the digital multimeter to start a new measurement. Each measurement cycle required 25 ms; therefore, the sampling rate of the device was 40 Hz.

2.4. Characterization of sensing probes at cryopreservation conditions

Probe performance at ultra-low temperatures was assessed with various sample solutions that are regularly used in cryobiology applications. French straws (0.5-ml) were filled with sample solutions, and the sensor was placed inside each French straw (displacing 0.17 ml of sample). To monitor phase-transition and ice formation phenomena, straws were placed in liquid nitrogen at −196 °C for 30 s. After exposing samples to ultra-low temperatures, the straws were transferred to room temperature (27 °C) to monitor thawing. For measurements, electrical resistance between the electrodes and temperatures inside the detection zone were recorded while the procedure was repeated three times. To evaluate sensor performance across different conductivity ranges, deionized water, sodium chloride (NaCl), and glycerol (used in cryogenic applications as a cryoprotectant) solutions were prepared. The concentrations of NaCl solutions were 0.1%, 0.3%, 0.5%, 0.7% and 0.9% (w:v), and glycerol solutions were prepared at 20% (v:v) concentration.

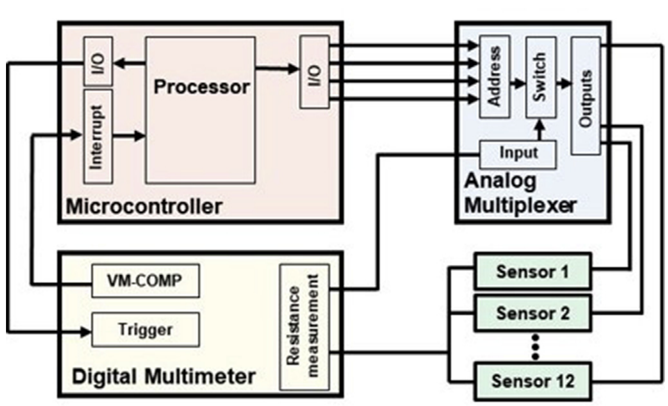

Fig. 2. Wiring diagram for the custom electrical resistance measurement unit used for switching signals with a 12 channel analog multiplexer. Each measurement cycle required 25 ms(40 Hz).

2.5. Combination of the sensing probe with a 3-D printed freezing device

To investigate sensor functionality in an applied format, the demonstration probes were tested with a 3-D printed freezing device [7]. Briefly, the device was based on a platform capable of holding cryopreservation containers including 0.5-ml French straws at specific heights above liquid nitrogen on a floating rack inside an insulating box, and depending on the gap between the raft and the wall of the box, and the proximity of the sample to liquid nitrogen, different freezing rates were achieved. Sensing probes were placed inside 0.5-ml French straws filled with deionized water and were transferred to the freezer rack. The device was placed inside the Styrofoam box with 5 cm of liquid nitrogen, and after freezing, was removed from the box and left at room temperature to thaw. Electrical resistance and temperature were measured during this process.

3. Results

3.1. Characterization of sensing probe at cryopreservation conditions

Electrical resistance and temperature were recorded before, during and after placing various samples and the sensor under liquid nitrogen (Fig. 3). The measured resistance inside the DI water sample increased as the temperature of the samples decreased until reaching a maximum measurable value (12 MΩ, limited by the digital multimeter) (Fig. 3A). After the samples were removed from the liquid nitrogen and left at room temperature to thaw, the temperature began to increase and the measured resistance decreased.

A similar pattern was observed when this procedure was repeated with solutions containing glycerol or NaCl. During freezing of 20% glycerol (Figs. 3B) and 0.9% NaCl (Fig. 3C) solutions, resistance increased rapidly after exposure of samples to liquid nitrogen, which was similar to the DI water. During thawing, solution resistance decreased at a lower rate in glycerol compared to DI water and NaCl. In 0.9% NaCl solution resistance decreased at a higher rate and reached a minimum value at the melting temperature. Electrical resistance was measured with respect to sample temperature for solutions containing different concentrations of NaCl during thawing at room temperature (Fig. 4).

3.2. Characterization of the sensing probe in combination with 3-D printed freezing device

Electrical resistance and temperature of a DI water sample were recorded during freezing with a 3-D printed freezing device (Fig. 5). The freezing device provided a slower cooling rate compared to direct exposure of samples to liquid nitrogen, and cryopreservation related phenomena such as phase change were observable in the recorded temperature profile [13]. The electrical resistance increased during precooling, remained relatively constant during the latent heat removal and increased again while the sample temperature decreased further.

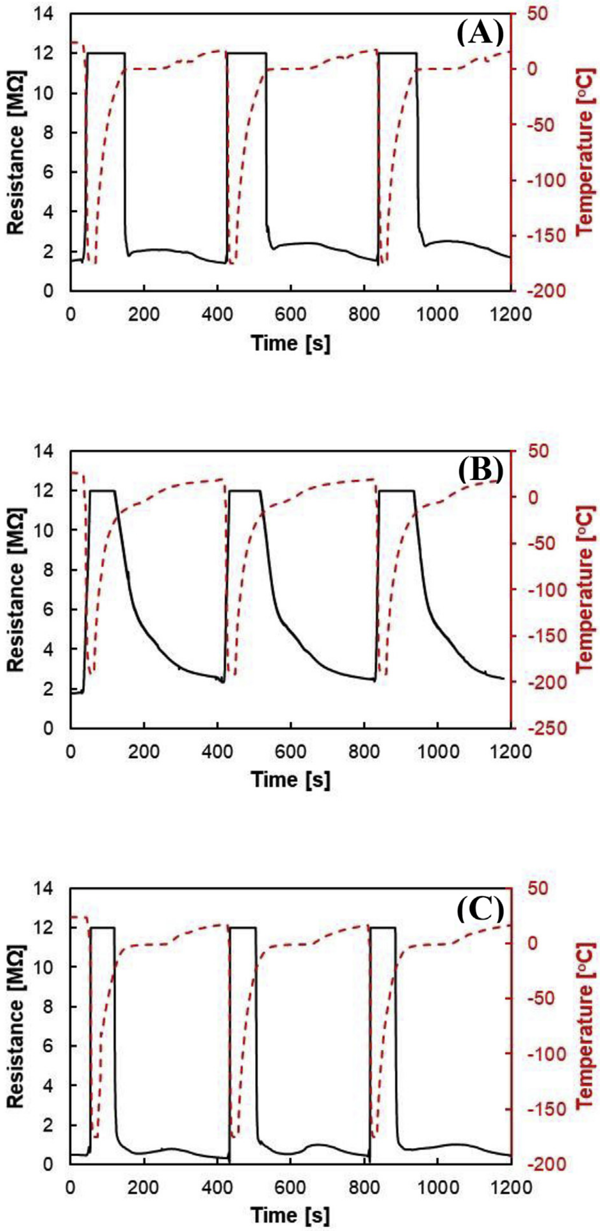

Fig. 3. Measurement of electrical resistance (solid line) and temperature (dashed line) made during three cycles of rapid cooling produced by plunging straws and sensors into liquid nitrogen, followed by thawing at room temperature (27 °C) of (A) DI water, (B) 20% glycerol, and (C) 0.9% NaCl.

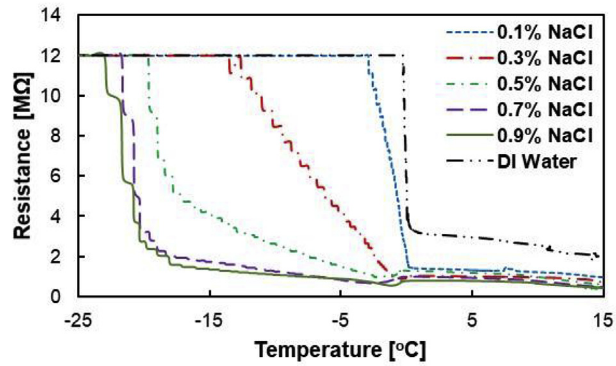

Fig. 4. Electrical resistance measured with respect to temperature of solutions containing different concentrations of NaCl during thawing at room temperature (27 °C).

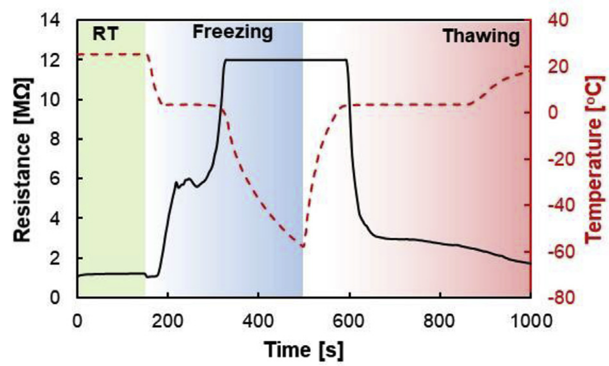

Fig. 5. The recorded resistance (solid line) and temperature (dashed line) of DI water during freezing and thawing in 0.5-mL French straws held horizontally above liquid nitrogen on a 3-D printed freezing device and thawed at room temperature (RT = 25 °C).

4. Discussion

4.1. Sensing probes produced with 3-D printing for monitoring cryopreservation procedures

Although additive manufacturing techniques have been used for more than 30 yr, 3-D printing has gained attention during the past 10 yr due to increased availability of advanced FDM printers combining high resolution and printing speeds with low cost [2]. Sensing devices fabricated with 3-D printing technology can be customized according to specific applications in cryobiology, and customization of sensing probes is achievable at low cost. This is important in cryopreservation of aquatic species because the sensitivity of cells to cryopreservation parameters can be species specific and complicates development of generic monitoring systems for the more than 25,000 extant fish species [1].

Our use of the narrow 0.5-ml straw was a demanding constraint compared to other containers such as 2 or 5-ml tubes. This reinforced the point that platform designs can be made to order for compatibility with different containers, or in the case of this work, various sensing mechanisms with corresponding sensing areas can be incorporated within specific geometries on custom probes.

The tight control of 3-D printing enhances reproducibility of fabricated devices by reducing human-induced variation. Consistent information provided by such devices can facilitate standardization and replication of cryopreservation methods. In addition, on-demand and decentralized manufacturing of such devices can eliminate the necessity of transporting animals or samples to a cryopreservation facility while maintaining the reproducibility of common cryopreservation protocols. Transfer of fishes or samples to a cryopreservation facility is not always favorable or possible, especially in the case of imperiled species [25]. The computer-assisted design (CAD) files used to direct the printer can be shared over the internet in stereolithography (STL) or other file formats. Sharing of design files digitally among user communities can eventually lead into application-specific standardization.

Although many thermoplastic materials can be used to print sensing probes, their mechanical properties should be investigated at cryogenic temperatures. Sensing platforms printed with PLA did not show deformation after exposure to ultra-low temperatures and had enough mechanical flexibility to tolerate tension imposed by thermal expansion and contraction of the metal electrodes. The presence of electrodes in straws could influence the temperature profile of the sample due to the high thermal conductivity of copper wires. However, the conductive electrodes were only directly exposed at the detection zone (10 mm), and because of the small surface area of electrodes in contact with sample and low thermal conductivity of the insulating material and PLA, potential temperature changes caused by heat transfer through the wires were thus minimized.

The flexibility of 3-D printing fabrication, in terms of design and manufacturing makes it possible to incorporate multiple sensing approaches such as electrochemical [16,23] or optical [17] methods on sensing probes. Electrical resistance measurement as a sensing mechanism can provide more information about phase transition than temperature measurement alone. The simplicity and integrability of the circuitry required for electrical resistance measurement, and information that this variable can provide regarding ice formation and ionic content fluctuation, make it a promising candidate for monitoring and quality control of cryopreservation processes.

4.2. Characterization of sensing probe at cryopreservation conditions

Several factors can affect the electrical resistance of solutions during freezing and thawing such as liquid-to-solid ratio, ion concentration fluctuations, and mobility of ions [21]. Analysis of the detailed physical processes affecting electrical resistance during freezing and thawing is beyond the scope of this report, especially due to the random nature of the nucleation process; however, using the data measured we were able to detect phase-change phenomena and evaluate the influence of sample solution composition on cryopreservation.

The rapid change in electrical resistance recorded after placing DI water samples and sensing probes under liquid nitrogen occurred because of the rapid phase change at this low temperature. During the freezing of 20% glycerol and 0.9% NaCl solution under liquid nitrogen, resistance variations were similar to DI water, and phase transition could be detected by a rapid increase of resistance. Further differentiation between samples using recorded electrical resistance would require higher sampling rates than used herein because of the expected near-instantaneous phase transition after placing samples under liquid nitrogen. During the thawing process at room temperature, resistance changes provided information relevant to cryopreservation conditions and sample composition. For example, electrical resistance recorded during thawing of saline solutions decreased at a higher rate compared to glycerol, which was expected due to the higher concentration of ions inside the NaCl solution.

In addition, electrical resistance measurement can provide information about additives such as the cryoprotectants and salts in samples. Sample solutions containing higher levels of NaCl melt at lower temperatures due to the impediment of water molecules re-entering solid phase. Accordingly, the electrical resistance measured during thawing of sample solutions containing higher levels of NaCl began to decrease at lower temperatures.

Given that changes in resistance value are related to physical or chemical processes occurring during phase transition, studying and analyzing electrical resistance can assist researchers by providing additional information leading to a better understanding of the mechanisms involved in thawing and freezing processes. Specifically, the electrical resistance values reported herein using the presented probe, were the resistance measured between two electrodes with a DC excitation current and should not be confused with the specific resistivity of the actual samples. Conventionally, for determining sample resistivity, an alternating excitation signal is used in conjunction with specific electrodes with known conductivity cell constants (K). As such, measurement of this sort would benefit from multidisciplinary or interdisciplinary approaches combining the fields of electrical engineering and cryobiology.

4.3. Characterization of the sensing probe in combination with 3-D printed freezing device

Generally, programmable freezers can provide well-controlled temperature profiles for cryopreservation procedures, and they could be utilized for commercial and research applications that require high-throughput sample processing. However, due to the high cost and complexity of these systems, they are generally not available for low-throughput applications. Although the simplicity and portability of user-constructed (low-technology) devices make them good candidates to be utilized in low-throughput applications, they suffer from a lack of consistency due to differences in design and lack of control capabilities. The use of 3-D printing as a fabrication method can overcome some of those problems. Suitable 3-D printers are available around the world at relatively low cost, and design files can be shared over the internet [7]. In addition, by monitoring the freezing process using 3-D printed probes, it is possible to characterize the variation among freezing protocols in relation to desired standards, and 3-D printing would enable users to print complete systems and not just components.

Our testing which combined a custom probe with a 3-D printed freezing device demonstrates the possibility of manufacturing complete monitoring and freezing systems with 3-D printing. Custom-made freezing devices would provide slower freezing rates compared to direct contact of samples with liquid nitrogen, and therefore the measured resistance would show more detailed information during freezing and cooling. Considering the observed changes of resistance during nucleation and latent heat removal and despite our relatively low sampling rate (40 Hz) it appears evident that nucleation can be considered as a process rather than an event, and can be monitored using inexpensive customized user-constrained probes.

Study of the freezing and thawing processes with electrical resistance measurement is a relatively unexplored area, and multiple physical and chemical phenomena must be taken in to account. The propose of this report was to document the potential of 3-D printed sensors, by demonstrating a probe capable of monitoring DC resistance and temperature in combination with a 3-D printed freezing device, and opening new avenues for research and application.

5. Conclusions

In summary, 3-D printing offers unexplored utility as a fabrication method to design, prototype, test, and implement sensing platforms for monitoring of cryopreservation procedures and phenomena. Such devices can provide quantitative data regarding temperature profiles and phase-transition events, and can be used to minimize the variations in implementing standardized cryopreservation protocols. Given that 3-D printing is increasingly common around the world, and specific devices can be designed based on user needs, customization leading to eventual standardization could be an expected outcome of applying this technology. By sharing design files, different user groups can become micro-manufacturers and perform reproducible cryopreservation procedures regardless of their location.

These advantages of 3-D printing make it possible to develop various standardized monitoring devices for cryopreservation applications with consistent outcomes. In this work, we report a custom 3-D printed sensing probe capable of detecting electrical and thermal changes associated with phase-transition phenomena inside a French straw. This shows the potential of such devices for research and in implementing standardized freezing protocols with reproducible results. More complex sensing mechanisms can be incorporated into 3-D printed probes, and eventually can be miniaturized using printed circuitry. This could even offer options for control of freezing or thawing. It should be noted that such devices can also be equipped with WiFi and Bluetooth connectivity to transfer data and expand utility.

Acknowledgments

This research was supported in part by funding from the National Institutes of Health, Office of Research Infrastructure Programs (R24-OD010441 and R24-OD011120), with additional support provided by the National Institute of Food and Agriculture, United States Department of Agriculture (Hatch project LAB94231), and the Louisiana State University Research & Technology Foundation (AG-2018-LIFT-003). We thank Y. Liu for technical assistance and W. Childress for providing 3-D printed freezing devices, and T. Monroe and T. Gutierrez-Wing for discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cryobiol.2019.03.010.

References

[1] J.F. Asturiano, E. Cabrita, Á. Horváth, Progress, challenges and perspectives on fish gamete cryopreservation: a mini-review, Gen. Comp. Endocrinol. 245 (2017) 69–76, https://doi.org/10.1016/j.ygcen.2016.06.019.

[2] M. Attaran, The rise of 3-D printing: the advantages of additive manufacturing over traditional manufacturing, Bus. Horiz. 60 (2017) 677–688, https://doi.org/10.1016/j.bushor.2017.05.011.

[3] A. D'Ausilio, Arduino: a low-cost multipurpose lab equipment, Behav. Res. Methods 44 (2012) 305–313, https://doi.org/10.3758/s13428-011-0163-z.

[4] D.E. Wildt, W.F. Rall, J.K. Critser, S.L. Monfort, U.S. Seal, Genome resource banks, Bioscience (1997) 689, https://doi.org/10.2307/1313209.

[5] K.R. Diller, Engineering-based contributions in cryobiology, Cryobiology 34 (1997) 304–314, https://doi.org/10.1006/cryo.1997.2012.

[6] B.C. Gross, J.L. Erkal, S.Y. Lockwood, C. Chen, D.M. Spence, Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences, Anal. Chem. 86 (2014) 3240–3253, https://doi.org/10.1021/ac403397r.

[7] E. Hu, W. Childress, T.R. Tiersch, 3-D printing provides a novel approach for standardization and reproducibility of freezing devices, Cryobiology 76 (2017) 34–40, https://doi.org/10.1016/j.cryobiol.2017.03.010.

[8] M. Kuwayama, Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method, Theriogenology 67 (2007) 73–80, https://doi.org/10.1016/j.theriogenology.2006.09.014.

[9] B. Liu, Y. Liu, S. Liu, T. Xu, Q. Liu, X. Li, Cryopreservation of strip spawned sperm using non-programmable freezing technique in the blue mussel Mytilus galloprovincialis, Aquacult. Res. 47 (2016) 3888–3898, https://doi.org/10.1111/are.12839.

[10] S. Martínez-Páramo, Á. Horváth, C. Labbé, T. Zhang, V. Robles, P. Herráez, M. Suquet, S. Adams, A. Viveiros, T.R. Tiersch, E. Cabrita, Cryobanking of aquatic species, Aquaculture 472 (2017) 156–177, https://doi.org/10.1016/j.aquaculture.2016.05.042.

[11] T.K. Nannou, H. Jouhara, J. Trembley, J. Herrmann, Cryopreservation: methods, equipment and critical concerns, RET (2016) 247–258.

[12] G.S. Ohm, The Galvanic Circuit Investigated Mathematically, D. Van Nostrand Company, 1905.

[13] L. Otero, A.C. Rodríguez, M. Pérez-Mateos, P.D. Sanz, Effects of magnetic fields on freezing: application to biological products, Compr. Rev. Food Sci. Food Saf. 15 (2016) 646–667, https://doi.org/10.1111/1541-4337.12202.

[14] P.H. Purdy, Review article: a review on goat sperm cryopreservation, Small Rumin. Res. 63 (2006) 215–225, https://doi.org/10.1016/j.smallrumres.2005.02.015.

[15] G.F. Richardson, C.E. Wilson, L.W. Crim, Z. Yao, Cryopreservation of yellowtail flounder semen in large straws, Aquaculture 174 (1999) 89–94, https://doi.org/10.1016/S0044-8486(98)00509-2.

[16] H. Shamkhalichenar, J.-W. Choi, An inkjet-printed non-enzymatic hydrogen peroxide sensor on paper, J. Electrochem. Soc. 164 (2017) B3101–B3106, https://doi.org/10.1149/2.0161705jes.

[17] Y.-H. Shin, J.Z. Barnett, M.T. Gutierrez-Wing, K.A. Rusch, J.-W. Choi, A hand-held fluorescent sensor platform for selectively estimating green algae and cyanobacteria biomass, Sensor. Actuator. B Chem. 262 (2018) 938–946, https://doi.org/10.1016/j.snb.2018.02.045.

[18] N.J. Tiersch, W.M. Childress, T.R. Tiersch, Three-dimensional printing of vitrification loop prototypes for aquatic species, Zebrafish (2018), https://doi.org/10.1089/zeb.2017.1520.

[19] N.J. Tiersch, T.R. Tiersch, Standardized assessment of thin-film vitrification for aquatic species, N. Am. J. Aquacult. 79 (2017) 283–288, https://doi.org/10.1080/15222055.2017.1339153.

[20] T.R. Tiersch, C.C. Green, Cryopreservation in Aquatic Species, second ed., Baton Rouge, 2011.

[21] T.R. Tiersch, W.T. Monroe, Three-dimensional printing with polylactic acid (PLA) thermoplastic offers new opportunities for cryobiology, Cryobiology 73 (2016) 396–398, https://doi.org/10.1016/j.cryobiol.2016.10.005.

[22] L. Torres, T.R. Tiersch, Addressing reproducibility in cryopreservation, and considerations necessary for commercialization and community development in support of genetic resources of aquatic species, J. World Aquac. 49 (2018) 644–663, https://doi.org/10.1111/jwas.12541.

[23] R.P. Tortorich, H. Shamkhalichenar, J.-W. Choi, Inkjet-printed and paper-based electrochemical sensors, Appl. Sci. 8 (2018) 288, https://doi.org/10.3390/app8020288.

[24] H.-Y. Tseng, S. Malfesi, N. Tehranchi, M. Rahbar, J. Jones, B. Gray, On-board array for multiplexed semi-active cooling-rate-controlled cryopreservation of living cells, J. Med. Biol. Eng. 36 (2016) 206, https://doi.org/10.1007/s40846-016-0123-2.

[25] M. Vanderzwalmen, L. Eaton, C. Mullen, F. Henriquez, P. Carey, D. Snellgrove, K.A. Sloman, The use of feed and water additives for live fish transport, Rev. Aquacult. (2018) 1–16, https://doi.org/10.1111/raq.12239.

[26] W. Xu, C.A. Angell, Solvent-free electrolytes with aqueous solution-like conductivities, Science 302 (2003) 422–425, https://doi.org/10.1126/science.1090287.

[27] G. Zhao, J. Fu, Microfluidics for cryopreservation, Biotechnol. Adv. 35 (2017) 323–336, https://doi.org/10.1016/j.biotechadv.2017.01.006.