3-D Printed Conveyor for Continuous-Mode Cryopreservation of Individual Samples

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3-D Printed Conveyor for Continuous-Mode Cryopreservation of Individual Samples

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Biological and Agricultural Engineering

By
Melissa Eskridge
B.S., Louisiana State University, 2014
May 2017
ACKNOWLEDGEMENTS

I would like to express my sincere thanks to Dr. Monroe for his mentoring and counsel throughout my years at Louisiana State University and this project. In addition, I am grateful for Dr. Tiersch’s advice and instruction during the duration of my graduate work. I would like to thank Amy Guitreau, Jacob Beckham, Megan Chesnut, and Dr. Letitia Torres for their friendship and guidance in the laboratory. For their contributions, both tangible and intangible, I would like to thank Lauren Lilly, Rachel Zeringue, Penny Harrel, Lisa Kriegh, Cong Walker, Yue Lui, Thomas Lee, Nick Totaro, and Dr. Marybeth Lima.

My parents’ support and additional funding helped sustain me and kept me on track during both my undergraduate and graduate years. To my mother: thank you for all the words of wisdom that kept me going. I never would have gotten this far without you pushing me up the hill of life. To my father: thank you for always making my failures seem inconsequential and my successes monumental. Thank you both for believing in me.

To my fiancée and best friend, Alex: I never would have gotten this far without you. All those long days and nights were much easier with you by my side.

I would like to thank Louisiana State University for their funding and the education that I received. I will always be a Tiger at heart and I will always remember my Alma Mater that jumpstarted my research career. I acknowledge support from the National Science Foundation ARI-R² program grant CMMI-096348 and National Institutes of Health grant 5R24OD010441.
PREFACE

This thesis describes a customizable 3-D printed device for continuous-mode cryopreservation of cell samples in French straws. The first chapter discusses the history and parameters of cryopreservation, current cryopreservation device research, and the need for the project. The second chapter describes 3-D printing and how it can be utilized to create devices capable of operating in cryogenic environments. The third chapter outlines the project objective, constraints, and design of the device. This chapter also includes the testing of the device capabilities and a comparison of the device to a controlled-rate freezer. The fourth and final chapter provides conclusions and discussions of future research.
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ABSTRACT

Lack of standardization of terminology and protocols, combined with limited access to freezing equipment due to cost constraints and lack of portability, has created a need for inexpensive cryopreservation devices. Distribution of standardized methods of freezing cells would enable the development and adoption of standards on materials and protocols. Some cryopreservation methods are limited by the amount of cells available and the toxicity of certain cryopreservation agents. These studies would benefit from the ability to cool individual samples. All commercially available and proposed cooling devices operate by cooling batches of samples at the same time. The cryopreservation conveyor was designed to provide a portable, inexpensive, low-throughput means for continuously freezing French cryopreservation straws. The device consists of a 3-D printed conveyor driven by a stepper motor that operates above a reservoir of liquid nitrogen within a polystyrene cooling chamber. The height and speed of the conveyor can be adjusted to provide customizable freezing parameters such as cooling rates (6 to 40 °C/min) and plunge temperature (-30 to -160 °C). Three-dimensional printing is a particularly appropriate means of fabricating the device, as 3-D printed polylactide structures can be created that have low density while still maintaining structural integrity in cryogenic conditions. The cooling rates produced by the conveyor had a standard deviation of less than 4 °C/min. A comparison of freezing koi (Cyprinus carpio) sperm on the conveyor and a commercially available controlled-rate freezer shows similar post-thaw motility. These results suggest that the conveyor device could meet the aforementioned needs to aid in standardization and reproducibility of cryopreservation efforts.
CHAPTER 1. BACKGROUND AND MOTIVATION

1.1. BACKGROUND AND BASIC PRINCIPLES

Current techniques for the cryopreservation of mammalian sperm have been studied and improved since the 1949 discovery of the protective qualities of permeating solutes on cells subjected to cryogenic temperatures (Polge, Smith et al. 1949, Pacey and Tomlinson 2009). Demand for increased livestock quality, coupled with the rise of artificial insemination, created a need for sperm cryopreservation techniques and has produced a field of scientific enquiry focusing on preservation of germplasm. Cryopreservation of aquatic species has also been shown to be a viable method of germplasm storage for biomedical research and aquaculture purposes (Yang and Tiersch 2011). The freezing of genetic material, particularly spermatozoa, allows for efficient storage and transport of important genetic lines of research model organisms (Mandawala, Harvey et al. 2016). Sperm cryopreservation can further influence future genetic studies by making these germ cells available at any point in time. Cryopreservation and storage of germplasm has helped conservation efforts of threatened and endangered species (Blanch, Tomás et al. 2014), enabled creation of genetically superior livestock populations (Woelders, Zuidberg et al. 2007), and created a viable method for long-term storage of genetic material of biomedical models of human health and disease (Yang and Tiersch 2009).

1.2. CRYOPRESERVATION PROCESS

Cryopreservation uses subzero temperatures to cool cells to a state where their metabolic processes are suspended and can be stored indefinitely. As the cell is cooled, the water within the cell freezes, forming crystalline ice that can damage cellular structures. To prevent this, cryopreservation agents (CPAs) are used to lower the cell freezing point. Once the cells are cooled to storage temperature, they can be kept viable until future use. Cryopreservation procedures vary due to the type and species of the cells being frozen and the equipment available. However, in most cases cryopreservation protocol follows these steps: (1) cryopreservation agent is added to the sperm sample; (2) the sample is sealed in a storage container; (3) the sample is subjected to increasingly cold temperatures; (4) the sample is brought to storage temperature; (5) the sample is thawed for use.

1.2.1. Cryopreservation Agents

Cryopreservation agents are used to dehydrate the cell to prevent membrane-disrupting ice crystallization. Although ice can generally form in cells between -5° and -15°, cryoinjury usually occurs between 0 and -40° Celsius (Gao and Critser 2000). Cryoprotectants can be classified as either penetrating (entering cells) or non-penetrating. Penetrating CPAs are small molecules that can pass through the cell membrane, thus displacing the water inside the cell. Penetrating CPAs such as methanol, dimethyl sulfoxide and glycerol are among the most commonly used; however, overexposure to these compounds can cause cell toxicity. Non-penetrating CPAs are typically large molecules that cannot enter the cells and thus create a hypertonic environment outside the cell, thus causing osmotic dehydration (Reed 2008).

There is no consensus on which cryoprotectant is superior for a given cell type. Differences in cell morphology can affect how the cell responds to the cryopreservation method used.
Testing protocols to determine effectiveness of CPAs differ widely, and lack of standardization has limited the ability to compare documentation on cryoprotectant experimentation (Szurek and Eroglu 2011). The cooling rate can have a considerable effect on CPA performance. For example, cryoprotectants that are considered more effective at lower concentrations, such as dimethylacetamide (DMA) (Tselutin, Seigneurin et al. 1999), have been found to produce better results when used for near-instantaneous thermal decrease to storage temperature (vitrification) than with equilibrium cooling (Iaffaldano, Di Iorio et al. 2012). This is due to the uptake time of the cell, or equilibration time. The longer a cell is exposed to cryoprotectant, the more dehydration occurs and the greater the risk of cell death due to toxicity (Fahy, Lilley et al. 1990). The rapid nature of vitrification allows these chemicals, which otherwise would be too toxic, to be used. Fowl sperm vitrified with DMA produced higher quality sperm than that frozen with glycerol; however, when equilibrium cooling was used, glycerol preserved the cells better than DMA (Tselutin, Seigneurin et al. 1999). For CPAs to be evaluated for efficacy in a given species, a consistent cooling rate must be applied to cell samples, described below.

1.2.2. Cooling Profile

An example internal temperature curve for a sample cooled in a French straw is shown below (Figure 1). In this particular example, the straw is cooled to a plunge temperature and submerged in liquid nitrogen. During cooling, aqueous solutions have the capacity to cool to a temperature below their melting point before ice formation occurs (De Santis and Coticchio 2011). Once the process of ice formation begins, the water begins to rapidly crystallize. From 0 to -40°C the sample has the potential to crystallize and subsequently thaw if the cooling chamber temperature should fluctuate (Gao and Critser 2000). Commonly, the sample is cooled to a temperature

![Typical Freezing Curve](image_url)

**FIGURE 1:** Typical cooling curve depicting relevant parameters for cryopreservation. The dotted line denotes the average cooling rate, which is calculated by dividing the change in temperature (e.g. 4 to -40 °C) by the time.
below -40 °C to prevent possible warming during transfer to storage. The sample is then usually either plunged into liquid nitrogen or suspended in nitrogen vapor until later use.

1.2.2.1. Cooling Rates

There are two common methods of freezing, equilibrium cooling and vitrification. Each method presents unique benefits and obstacles to the total process, and each has been shown to be successful in producing viable cells. The success of cryopreservation is dependent upon the type of cryopreserving agent used, the freezing rate the cells undergo, and the temperature at which the cells are instantaneously brought to storage temperature. Equilibrium cooling lowers the temperature slowly (0.3 – 50 °C per minute) until a plunge temperature is reached (Shaw and Jones 2003). Vitrification, or rapid freezing that results in amorphous (noncrystalline) ice, uses small volume samples that are most commonly directly plunged into liquid nitrogen (10⁶-10¹⁰ °C/min) (Franks 1982). Special equipment and cryoprotectants are not needed, making vitrification a more ideal option over equilibrium cooling. However, equilibrium cooling has been shown to produce frozen sperm with higher survival rates (Darvishnia, Lakpour et al. 2013). Vitrification is mainly used for freezing containers that have a high surface-area-to-volume ratio, as it is important to maximize heat transfer to the cells to avoid ice formation (Tsai, Tsai et al. 2015).

1.2.2.2. Plunge Temperature

The plunge temperature is the temperature at which the cooling rate ends and the cells are immersed at storage temperature. The plunge temperature is usually at least -40° C, but is often between -60° and -80°, and can be as warm as 30° (Pegg, Hayes et al. 1973). The temperature at which cells are subjected to storage temperature also depends upon the transit time from the cooling device to the storage container; to prevent cellular damage due to warming, the sample is cooled to below the cryoinjury threshold so that warming during transit does not threaten the cells.

1.3. Freezing Devices

Equilibrium cooling requires specialized equipment to precisely control the temperature decrease of the samples. These instruments range in complexity, price, and reproducibility. The main types of equilibrium cooling devices are commercial devices, or those available for purchase, and novel devices, or those that have been described and tested in literature but are not manufactured for sale.

1.3.1. Commercial

Several types of devices that can achieve desired cooling rates are currently available commercially. These devices vary in price, size, accuracy, complexity, and portability, and are marketed towards large research organizations and independent users. Although commercial devices are usually designed to process large amounts of samples quickly, there are portable options available for purchase as well.

1.3.1.1. Controlled-Rate Freezers

To achieve a controlled and reproducible decrease in temperature, equipment such as controlled-rate freezers (CRFs) are often used. These devices are computer controlled and pre-
programmed for standard freezing rates, and provide documentation of the freezing process through the use of thermocouples to measure sample and chamber temperatures. However, they are often bulky and expensive, making them unsuitable for freezing in the field and unfeasible for mobile freezing. Controlled-rate freezers most commonly use vapor from liquid nitrogen to control the cooling chamber’s internal temperature, although some use stirling cycle engines to create a cold environment without the need for stored or on-hand liquid nitrogen (Creemers, Nijs et al. 2011).

Computer controlled freezers are often composed of a computer, liquid nitrogen tank, regulator, and cooling chamber (Figure 2). The computer is connected to the regulator, which controls the flow of liquid nitrogen and the cooling chamber. The cooling chamber is an insulated metal container with a hinged lid (Figure 3). The computer program determines the necessary internal temperature of the cooling chamber during operation. The flow of liquid nitrogen into the cooling chamber is controlled by the regulator, which opens and closes a valve on the liquid nitrogen nozzle. When the nozzle is open, pressurized liquid nitrogen is released onto a continuously rotating fan on the bottom of the cooling chamber. The liquid nitrogen vaporizes upon

FIGURE 2: Common setup of a computer-controlled cryopreservation device.

contact with the fan, and the resulting nitrogen vapor is directed towards the samples that are suspended in the center of the cooling chamber by an aluminum sample rack. The changing chamber temperature is measured by the regulator and communicated to the computer for feedback and control, resulting in potential differences between the programmed and sample temperatures (Figure 4). Computer-controlled freezers can create complex freezing profiles and provide feedback to the user to minimize sample loss through cooling failure (Shu, Kang et al. 2010). They can also process multiple types of cryopreservation containers such as bags, vials, and tubes.

Computer-controlled freezers provide high precision in freezing protocols and are capable of freezing large numbers of samples at a time (>1000 straws). Most commercial freezers require a large supply or tank of liquid nitrogen to operate. Computer-controlled freezers are expensive, usually costing tens of thousands dollars, and are not considered portable because they contain several large, bulky components. Maintenance and repair of freezers most often have to be provided by the company of origin, which requires repair professionals to travel to the site of the freezer; thus, any damage or wear to the freezer consumes additional resources and time while the freezer is not operational.

1.3.1.2. Portability
Portable devices have been developed to enable the freezing and transport of cryopreserved specimens. The most extensively used portable cryopreservation method utilizes the stratified vapor layers above the surface of liquid nitrogen to cool samples. Samples are either suspended vertically or placed horizontally on racks at specified heights based on calculated

![FIGURE 4: Illustration of the differences in programmed, sample, and chamber temperature in a computer-controlled freezer.](image-url)
freezing rates that are dependent upon the shape and space of the freezer (Figure 5). These devices often produce inconsistent results due to the inability of users to monitor or control the temperature fields inside the freezing chamber, and are considered inferior to the more expensive computer-controlled freezers (Hubbard, Penrose et al. 2013). Disagreement as how to achieve the most viable cells cooled with nitrogen-vapor techniques has also limited further progress in portable devices (Santos, Sansinena et al. 2013).

The two most common types of portable devices are hanging-sample and floating-sample freezers. Hanging-sample freezers suspend the samples vertically over the surface of liquid nitrogen. Suspending the straw this way provides maximum surface area of the sample access to nitrogen vapor. Floating-sample devices consist of a buoyant piece of material, usually expanded polystyrene foam that acts as a raft for the straws. Samples are laid horizontally on the raft and are cooled at a rate dependent upon several factors, including the size of the raft, the geometry of the cooling chamber, and the surface area of the sample exposed to nitrogen vapor. The floating-sample method is considered superior in effectively cooling samples due to the vertical differences in the temperature field surrounding the sample. The temperature of nitrogen vapor surrounding the sample furthest from the liquid nitrogen will be higher than that closest to the liquid nitrogen, resulting in uneven cooling of the sample.

1.3.1.3. Freezing Containers

One of the major costs associated with cryopreservation is that of the storage of liquid nitrogen. Cryopreservation companies have developed cryopreservation containers to provide freezing methods that do not require liquid nitrogen or expensive equipment. These containers, such as ThermoFisher Scientific Mr. Frosty and Corning CoolCell, are essentially small enclosed storage devices that are filled with a cooling agent and are designed to be loaded with samples
and cooled in -80 °C freezers. Each container operates at one cooling rate, which is determined by the geometry and chemical composition of the cooling container material, and so cannot provide customizable options.

1.3.2. Published Devices
Efforts have been made to produce inexpensive and more reliable cryopreservation devices that satisfy user needs in small-sample freezing. These devices seek to address the cost-prohibitive nature of traditional standard cryopreservation freezers as well as providing consistency to freezing rates for successful cryopreservation.

1.3.2.1. Cryopreservation Elevator
The cryopreservation elevator device consists of a 16 cm$^2$ platform attached to a pulley controlled by a stepper motor attached to a cooling chamber filled partially with liquid nitrogen (Figure 6) (Medrano, Anderson et al. 2002). The “straw elevator” was fitted with thermocouples to measure the temperature of the nitrogen vapor surrounding the platform. The stepper motor was controlled by a microcontroller so that the platform was raised and lowered depending upon the desired cooling rate. This device could cool up to twenty French straws at a time and addressed the need for precision cooling with feedback from the nitrogen environment and created a real-time display of the cryogenic sample temperature. The device allowed users to determine if temperature change during freezing affected the outcome of the cryopreservation process.

FIGURE 6: Illustration of the cryopreservation elevator (Medrano, Anderson et al. 2002)
1.3.2.2. Positional Cooling Platform Device

The Positional Cooling Platform Device (PCPD) was developed by Louisiana State University Agricultural Center (LSUAC) Aquatic Germplasm and Genetic Resources Center to be a self-contained platform for batch freezing and was designed to fulfill the needs of inexpensive customizable cryopreservation. It is composed of a cooling chamber made out of polystyrene foam boxes, a 3-D printed straw rack, and a polystyrene raft (Figure 7). The rack accommodates cryovials, half-milliliter straws, and quarter-milliliter straws. The device can be assembled by the user to produce different cooling rates. It differs from the traditional floating-sample methods in that the straws are not directly placed on the polystyrene raft, but are suspended horizontally in the nitrogen vapor. The distance of the straws from the liquid nitrogen surface is determined by the rack configuration, as opposed to the thickness of the polystyrene raft itself.

The PCPD effectively addresses need for portable, inexpensive cryopreservation with customizable freezing rates. In addition, the nature of the 3-D printed rack allows researchers to “download” copies or new formats of the device and inexpensively produce the thermoplastic parts. The ability of the device to be recreated in any place that a 3-D printer is present could decrease wait times for broken or lost parts.

1.4. Challenges

There are several challenges to successful and standardized cryopreservation. Firstly, the morphological differences between cell types and the cells of different species may require different cryopreservation protocols to successfully preserve viable cells. Due to the sheer number of different species and cells that are being studied, this requires a substantial amount of research on these cell lines (Prieto, Sanchez-Calabuig et al. 2014). Secondly, some cryopreservation terminology has not been standardized. For example, the range of temperatures from which a cooling rate is calculated has not been agreed upon. The temperature range from which the cooling rate is calculated may be determined as those from the introduction of the sample to an arbitrary temperature. Thirdly, the freezing equipment available varies in cost, customizability, reliability, and feedback. The lack of reliable, precise freezing equipment results in more uncertainties in the freezing process and fewer laboratories able to cryopreserve cells.
1.5. Significance

The number of protocols that need to be developed for specific cell cryopreservation requires access to reliable freezing equipment to develop and adapt cryopreservation standards. However, in the case of low-throughput cryopreservation, individual samples must be frozen using high-throughput or batch equipment. This process can take more time than a continuous cooling method would, and thus prolongs the research timeline to produce and compare cryopreservation variables. A device is proposed that could produce customizable cooling rates and process individual samples to provide a cost-effective method of low-throughput cryopreservation.
CHAPTER 2. 3-D PRINTING FOR CRYOPRESERVATION

2.1. OVERVIEW

Prototyping and product development present two major challenges: time consumption due to fabrication and small part customization to meet customer needs. Although Computer-aided design (CAD) and computer-aided manufacturing (CAM) have significantly helped traditional product development, CAD and CAM research have not been able to address the need for complex patterns and rapid prototyping (Yan and Gu 1996). Additive manufacturing, and in particular fused deposition fabrication (FDF), has provided a form of manufacturing that allows the creation of complex parts without the need for molds or patterns. Three-dimensional thermoplastic printing directly converts computer drawings of objects to functional, physical parts and has made broad impact in fields ranging from household goods to medical devices (Patra and Young 2016, Rayna and Striukova 2016).

2.2. INTRODUCTION

2.2.1. 3-D Printing

Computer-aided design programs create virtual three-dimensional drawings. These drawings can be utilized by 3-D printing software to create a set of commands for a 3-D printer. The software reduces the CAD images to vertical layers of singular thickness plots, and further breaks down each layer into curing or binding paths. These paths direct the machine’s 2-D movement to create a solid linepath which make up a singular layer (Yan and Gu 1996). A three-dimensional printer converts this rendering into a physical object by creating layers of melted plastic consecutively in the z-direction.

2.2.2. Fused Filament Fabrication

Fused Deposition Fabrication (FDF), also commonly referred to as fused filament fabrication (FFF) or fused deposition modeling (FDM), is a method of rapid prototyping that uses spools of filament (most commonly thermoplastics) and creates solid objects by layering two-dimensional sheets of plastic in the shape of the object (Matta, Raju et al. 2015).

The fused deposition 3-D printer operates by using a toothed gear to draw filament from the spool to a heated nozzle (Figure 8). As the nozzle melts the plastic, the gear continues to feed filament

FIGURE 8: Diagram of the major parts of 3-D printing.
through the nozzle, forcing the melted plastic through a small diameter hole at the tip of the nozzle. The ejected plastic is firmly deposited onto a flat heated or non-heated build plate. The machine creates a bottom or original layer that is temporarily adhered to the build plate. After the first layer, subsequent solid layers are added to the original layer to create a 3-dimensional object. The thermoplastic rapidly cools so that each layer retains the shape in which the printer extruded it.

2.2.3. Terms and Parameters

Objects created by FDM printers are formed using a set of common guidelines. Each printed layer consists of shells and infill, and the thickness of the layers determines the resolution. A shell is the outline of the layer and help determine the strength of the outside surface of the object. Infill is the amount of material that is printed within the shells, and is often a honeycomb pattern that can range from 0 to 100 percent. As the infill percentage is increased, the stronger the overall part is in all directions. The base layer and the next several subsequent layers are printed as solid pieces with 100 percent infill. After the base layers are printed, the rest of the object is constructed using the user-determined infill (Figure 9). Sections of the object that do not have layers beneath them (holes, overhanging features, etc.) need to be structurally supported so the melted plastic does not sag before cooling. Support material, a porous network of thin strands of plastic—usually created using consecutive overlapping x and y lines of material—is printed in place of the normal layers of plastic.

2.3. Printing Materials

2.3.1. Introduction

Aluminum is traditionally the material used for the sample racks and cooling chambers in cryogenic conditions because its thermomechanical properties undergo less change than other metals exhibit at temperatures below freezing (Glazer 1988) and it is relatively inexpensive and easy to manufacture. However, aluminum requires expensive and specialized equipment to mill and shape it, which renders it unsuitable for rapid prototyping. The creation of thermoplastics
that can be melted and cooled quickly revolutionized the manufacturing industry in regards to 3-D printers, and the ability to quickly and easily produce customizable devices is ideal in laboratory applications (Baden, Chagas et al. 2015). The thermoplastics used in 3-D printing have shown promise for use in cryogenic environments due to their low thermal conductivity and density (Tiersch and Monroe 2016). Additionally, certain fused deposition modelling polymers have been show to possess high strength and stiffness in the presence of liquid nitrogen (Cruz, Shoemake et al. 2015). However, traditional 3-D printing materials have not been analyzed for used in these condition. PLA, ABS, and aluminum samples were tested for temperature change over time after exposure to liquid nitrogen to assess the thermal properties of these materials in relation to aluminum.

2.3.1.1. Poly(lactic) Acid and ABS

The two most popular and commonly used thermoplastics for 3-D printing filaments are Polylactic acid and Acetyl Butadiene Styrene. Polylactic acid (PLA) is synthesized from sugar crops such as beets or sugarcane, and is a commonly used printing material due to its low glass transition temperature, nontoxic nature, and biodegradability (Afrose, Masood et al. 2016). Polylactic acid does not require a heated build plate and can be printed at room temperature. Acetyl butadiene styrene (ABS) is a printing material that is regarded as a more versatile and less brittle material than PLA (Jo, Ryu et al. 2012).

2.3.1.2. Aluminum

Although metal printers capable of creating aluminum objects are available, they are expensive and require extensive post-print processing to achieve a finished part. Metals have also been used to seed cryopreservation straws; touching a straw with a piece of metal initiates ice crystal formation (Songsasen and Leibo 1997). For this reason, aluminum racks holding cryopreservation straws use minimal surface contact (> 1 mm²) to hold the straws. Fused filament fabricated parts lose structural integrity when printed at such small sizes. When aluminum parts are removed from cryogenic conditions, the water vapor in the air tends to condense on the surface of the aluminum which consequently freezes when returned to subzero temperatures. Often, this causes the aluminum racks or the straws to freeze together, making it difficult to smoothly transition from a computer-controlled freezer to permanent storage.

Creating cryogenic equipment using rapid prototyping methods such as 3-D printing could expand access and use of cryopreservation equipment. The cryogenic properties of 3-D printing filaments have not been explored, although parts created from PLA have been anecdotally observed to retain strength at subzero temperatures and warm more quickly than aluminum after retrieval from liquid nitrogen. Additionally, although thermal properties of polylactic acid and acetyl butadiene styrene have been discovered, the filaments used by 3-D printers are composite polymers. Although aluminum appears to warm undergo slower heat transfer than PLA and ABS filaments, the higher density of aluminum may be the cause of this phenomenon. To determine if the heat transfer properties of ABS and PLA are primarily dependent upon material density, cubes of aluminum, ABS, and PLA were cooled using liquid nitrogen. The temperatures at the centers of these cubes were recorded during cooling and subsequent warming.
2.3.2. Methods and Materials

Solid cubes of PLA and ABS were printed at 100% infill with a layer thickness of 0.1 mm (Figure 10). Two PLA cubes were created using Makerbot natural PLA filament (filament diameter = 1.75 mm) and printed by a Makerbot Replicator 2 3-D printer. The volumes of the cubes were of 4.74 cm$^3$ and 2.05 cm$^3$. A 2.05 cm$^3$ cube of ABS was created using Makerbot natural ABS filament (filament diameter = 1.75 mm) and printed by a Makerbot Replicator 2X 3-D printer. Aluminum (6061, OnlineMetals) was milled into a 2.05 cm$^3$ cube. One millimeter holes were drilled into each cube to a depth of 0.635 cm. Thermocouples (Type K, Omega) were inserted into each cube so that the wire junction tip made contact with the inner surface (Figure 11). The cubes were submerged in liquid nitrogen until an internal temperature of -180 °C was reached, and removed and allowed to warm to 0 °C at an air temperature of 22 °C.

2.3.3. Results

The warming rates were significantly different for each cube of different material and mass (Figure 12). The cubes of matching mass (aluminum and PLA cubes of 5.82 g) did not have a significantly different warming rate ($P = 0.96$).

<table>
<thead>
<tr>
<th>Material</th>
<th>Mass (g)</th>
<th>Cube Side Length (cm)</th>
<th>Warming Rate ($^\circ$C/min) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>2.26</td>
<td>1.27</td>
<td>38.7 ± 0.5</td>
</tr>
<tr>
<td>ABS</td>
<td>1.67</td>
<td>1.27</td>
<td>45.3 ± 2.3</td>
</tr>
<tr>
<td>Aluminum</td>
<td>5.82</td>
<td>1.27</td>
<td>22.0 ± 1.3</td>
</tr>
<tr>
<td>PLA</td>
<td>5.82</td>
<td>1.68</td>
<td>22.0 ± 1.4</td>
</tr>
</tbody>
</table>
The heat transfer properties of PLA and ABS appear to be mainly determined by their densities. Low-percentage infill structures of 3-D printed parts can further reduce the thermal mass of the object while still maintaining structural integrity. This low thermal mass makes 3-D printed thermoplastics a viable option for cryogenic devices which are regularly subjected to changing temperatures. The ability of a 3-D printed object to possess the same external geometry of an aluminum object, yet be able to cool and warm much faster, could beneficial property of cryogenic equipment. However, further studies are needed to ascertain the thermomechanical properties of these materials at conditions suitable for cryopreservation.

2.4. Considerations for cryopreservation

Three-dimensional printing may be useful for creating cryogenic devices, but there are several factors to consider when designing parts that will be in a liquid nitrogen environment. The physical design, printing method, and interactions with other parts can affect the design capability to withstand the temperature changes that arise from cryogenic conditions.
2.4.1. Printing Orientation

The structural properties of objects created using 3-D printing are heavily reliant upon the printing orientation on the build plate. Most often, the point of failure occurs between printed layers where the shells and infill are weakest, and this is emphasized even more in cryogenic conditions due to temperature stresses. Therefore, care should be taken to minimize the amount of layers on the printed object. This can be accomplished in several ways: increased layer thickness, reduction of support material, and orientation on the build plate that reduces height in the z-direction.

2.4.2. Part Interconnections

Complex objects can be created by separating the features into individual parts that can be connected together. Although this overall increases the structural integrity of the object, the points of connection tend to be the sites of failure, especially if the connections are significantly smaller than the object. During exposure to cryogenic temperatures, the plastic may shrink, causing separation between parts. This result can be minimized by attaching the components in a way which constrains movement in all axes of direction (Figure 13). Connections that require friction between the surfaces of the components are not recommended due to shrinkage, which can result in separation of parts and ultimately mechanical failure. Shrinkage may also increase friction depending on the part geometry, in the case of moving parts can cause either motion failure or increased stresses on the object, which can lead to breakage.

![Figure 13: Examples of part connections. Threaded connections (left) create stronger attachments than simple pin connections (right) when exposed to cryogenic conditions because of the plastic deformation due to shrinkage.](image)

2.4.3. Moving Parts

Three-dimensional printed objects which have moving components often encounter problems due to the frictional force produced by the interaction between articulating plastic surfaces. These surfaces, under cryogenic conditions, can accumulate condensation from the laboratory or testing environment between uses and the water freezes when subjected to cold temperatures. Components that are required to move should be fabricated to minimize contact surfaces to prevent addition of heat to the system through friction and the possibility of device failure from frozen condensation.
2.5. Conclusions

Rapid prototyping, and in particular 3-D printing, has given rise to the ability to create prototypes and goods in various settings, from homes to hospitals. Polylactic acid, the most common filament used due to its biodegradability and ease of printing, has promise in cryogenic applications based on its thermomechanical properties. Three-dimensional printers, unlike traditional molding methods, are capable of producing objects with honeycombed infill structures. These partially hollow objects do not have the same mass as aluminum or solid plastic parts, and therefore have a higher rate of heat transfer. Furthermore, the mechanical strength of polylactic acid enables it to withstand the temperature change and thermal stresses that arise from cryogenic environments. This is particularly useful in designing portable cryopreservation devices, as the capacity to create customizable prototypes can be utilized to develop standardized inexpensive freezing devices.
CHAPTER 3. DEVICE DESIGN

3.1. INTRODUCTION

3.1.1. Device Justification

Cryopreservation of germplasm, and in particular sperm, has been shown to be effective in storing genetic material. The procedures and equipment needed for cryopreservation depends upon the species and cell type. Typically, equilibrium cooling equipment is bulky and expensive, and so is only available to large laboratories or repositories. Cryopreservation research and testing of protocols for each species is limited by the number of laboratories with access to reliable freezing equipment. Furthermore, most computer-controlled freezers are designed to handle large batches of samples at a time. Research using limited quantities of genetic material, such as in cases of endangered species or microfauna, is conducted using the same high-throughput equipment. Not only does this waste energy and resources, but the amount of time needed to freeze individual samples using batch equipment limits the amount of samples that can be processed within a certain time frame. The creation of a device that can accurately and cost-effectively handle individual samples would provide a means of efficient low-throughput cooling. Batch processing cools a group of samples at one time, and subsequent groups must wait until the previous group has been completely processed. Currently, there are no cryopreservation devices capable of continuously freezing individual samples. The objective of this project was to create an inexpensive, portable device that can cool sperm samples in 0.5-ml and 0.25-ml French straws at predetermined cooling rates continuously.

3.1.2. Device Objectives and Constraints

To be considered an effective portable cryopreservation tool, the device must be able to fulfill three objectives: 1, cool straws at different freezing rates; 2, produce internal sample temperature curves comparable to those produced by a computer-controlled freezer; and 3, cool samples that remain viable after thawing. Objective 1 is important to ensure that the device can be used for multiple cryopreservation protocols, while objectives 2 and 3 validate the ability of the device to successfully cool samples.

An important element of experimental equipment is customizability, or the capacity to produce different variables with the same device. The most crucial parameter that the user should be able to control is the cooling rate. The device should be able to accommodate cooling rates between 5 and 40 °C per minute. Additional capacity of the device to obtain different plunge temperatures is preferable.

The device must meet certain criteria commonly considered necessary for cryopreservation devices (Pegg, Hayes et al. 1973). Creation of internal temperature fields of the device must be reproducible to establish reliable cooling rates of the samples. The device should be capable of cooling samples below -40° C. A mechanism to carry the straws horizontally over the surface of liquid nitrogen inside a box could be designed to accomplish this. The straws should remain horizontal during cooling to ensure even thermal change throughout the entirety of the sample. Minimal contact between the device and straws is desirable to allow the entire straw access to nitrogen vapor and to decrease the area of contact in which straws may freeze to the device.
The entire device must be able to be transferred to work areas either in the laboratory or field. A suitable size and weight for the total equipment used should be within a single user’s ability to carry and move the assembly. The equipment used for the device should be able to fit within the cooling chamber for ease of storage and transportation.

3.2. METHODS AND MATERIALS

3.2.1. Device

3.2.1.1. Modeling and Fabrication
The device was modeled using Autodesk Inventor 2015 and printed using a Makerbot Replicator 2. All components of the device, except for the external chain links (described in 1.2.1.2), were printed using Makerbot natural PLA filament at a layer height of 0.2 mm. The external chain links were printed with purple Makerbot PLA filament because the resultant printed links, after post-print contraction, were larger than the natural internal links and produced less friction between the surfaces of the two parts.

3.2.1.2. Overview and Components
The device consists of nested polystyrene foam boxes, a stepper motor, and a 3-D printed chain conveyor and loading mechanism (Figure 14). The internal polystyrene box is filled with a reservoir of liquid. The straw samples are inserted using the printed loading device and deposited onto the continuously moving conveyor chain. Samples are added to the system until the freezing process is completed, and the frozen straws remain in the reservoir until transferal to storage. The straws are added to the conveyor through a horizontal tube (Figure 15). The straws are inserted into the outside hole on the tube and inserted to the interior of the box using a straw.

FIGURE 14: The device consists of: 1, an external polystyrene container; 2, an internal polystyrene box; 3, a loading mechanism; 4, a conveyor chain; and 5, a stepper motor.
When the straw is located in the opening at the end of the tube, the tube is rotated 180 degrees until the straw is deposited onto the conveyor chain.

The device operates within an assembly of nested polystyrene boxes. The system is comprised of a small (12"x12",9.5"x9.5") cube shaped internal polystyrene box, which is inserted into the bottom of a larger (17"x17"x19") T-shaped polystyrene container that is fitted with a lid (Figure 16). Both boxes are situated inside a cardboard box that doubles as extra insulation and as a shipping container. This nesting box system is the same that is used by the PCPD, and has been shown to provide a reliable cooling chamber.

The conveyor is composed of two axles with two double-single sprockets per axle. A geared 12V stepper motor (Sparkfun, ROB-09238) turns the axles, and the attached sprockets drive two toothed chains. Each axle is supported by two arms that attach to the sides of the polystyrene box. The arms are interchangeable so that the height of the conveyor from the bottom of the box can be adjusted according to the freezing protocol. The teeth on the chains are spaced 3.175 mm apart to accommodate a single cryopreservation straw can fit between them. Each cryopreservation straw is supported on both ends.

FIGURE 15: Orthogonal (left) and top (right) views of the conveyor assembly with the internal nitrogen reservoir container. The loading mechanism creates a path for the straw to travel from the external environment to the cooling chamber.

FIGURE 16: The box assembly consists of: 1, a polystyrene lid; 2, a smaller internal polystyrene box; 3, a larger external polystyrene box; 4, a cardboard box.
by the chains above the reservoir of liquid nitrogen. The middle of the straw where the sample is located is exposed freely to the nitrogen vapor for effective cooling.

The motor used to drive the conveyor is a bipolar stepper motor (Sparkfun, ROB-09238). The stepper motor was verified to operate within cryogenic temperatures without stalling or freezing over the course of 1 hr and is contained within a printed mount (Figure 17). A gear is attached to the shaft of the motor to allow the motor to turn the axle from a greater distance above the liquid nitrogen than the conveyor and to apply rotation to the center of the axle as opposed to one side. The motor is controlled by an EasyDriver stepper motor control board (Sparkfun, ROB-12779) which is powered by an Arduino Uno Microcontroller attached to a 12V wall power adapter. Microcontroller code and electronic layout designs are included in Appendix B.

Gears were designed to provide a means for the stepper motor to turn the front axle of the conveyor (Figure 18). One gear is attached to the stepper motor shaft and secured using metal clamps (Figure 19). The other gear tightly fits the axle on the proximal side of the conveyor. The gear diameter used depends on the height of the conveyor. For the neutral height setting, the diameters of the gears are 6.35 cm; for the height setting 1.905 cm below neutral, the diameter of the gears are 7.62 cm; and for the lowest height setting, the diameter of the gears are 8.89 cm. Each gear is 0.635 mm thick. These gears were designed using Autodesk Inventor Design Accelerator feature, which creates gear profiles based on design constraints such as center distance, gear ratio, and number of teeth.

Double-single sprockets were created to drive the chains (Figure 20). The notches in the circular toothed sides of the sprockets fit the side-posts of the chain. Each chain link is supported on both sides by the sprocket, which stabilizes the chain horizontally. The sprockets at the beginning of the conveyor are supported on the axle between the two sprockets. The ending sprocket assembly is supported on the outsides of the sprockets. This creates a space between the sprockets and the support structure large enough so that the cryopreservation straw is not hindered from dropping into the liquid nitrogen.
The axles were designed to fit the sprocket assemblies without any protruding features (Figure 21). This reduced printing time, eliminated the need for support material, and strengthened the method of connection between the sprockets and axle.

The arms were designed so that they directly attached to the edge of the inner polystyrene box and were held in place using arm stabilizers. Three different arm heights were printed (Figure 22). Height 0 (neutral) arms are at the height of the original design of the conveyor. Height 0.75 arms hold the sprocket axle 1.905 cm (0.75 in) lower than the height 0 arms. Height 1.5 arms hold the sprocket axle 3.81 cm (1.5 in) lower than the original arm height. The stabilizers hold the arms at a set distance apart and keep them from moving during device operation (Figure 23). They can accommodate any arm used and attach to the polystyrene edge.

The chain is comprised of two types of links: internal and external (Figure 24). Each chain link has three teeth located on the top surface of the link. These teeth create two channels for either side of the cryopreservation straw to fit into. The cylindrical posts located on the lateral sides of the links

FIGURE 19: Gears attached to the motor and axle and secured using shaft clamps.

FIGURE 20: Sprocket with spacers (left) and sprocket cap (right).

FIGURE 21: The axle connects the two sprockets on the sides of the conveyor and holds the sprocket halves together.
act as both the pins for attaching chain links and as the surface which comes in contact with the sprocket teeth.

![FIGURE 22: (From left to right) neutral arm, 1.905 cm arm, and 3.81 cm arm.]

**3.2.2. Variable/Cooling Rate Determination**

For each experiment, the conveyor was assembled inside the box system, followed by liquid nitrogen addition and box closure. The assembly reached thermal equilibrium within 15 minutes. The liquid nitrogen was refilled to testing weight. Each straw was filled with Hank’s Buffered Salt Solution (HBSS) and a type-K thermocouple wire (Omega) was inserted into the straw. The straws were placed on the conveyor and each straw was allowed to fall into the liquid nitrogen reservoir before another straw was added.

![FIGURE 23: Supports for the arms and motor mount (left) and arms on the end of the conveyor where the straws are dropped into the reservoir (right).]

![FIGURE 24: Internal chain link (top left), external chain link (top right), and chain assembly (bottom).]
3.2.2.1. Sample Distance from Liquid Nitrogen Surface

Three different conveyor heights with different amounts of liquid nitrogen were tested for cooling rates (Figure 25). For a conveyor height of approximately 21 cm from the bottom surface of the reservoir box, masses of 6.8, 7.3, 7.8, 8.3, and 8.8 kg of liquid nitrogen were tested. For a conveyor height of approximately 19 cm from the bottom surface of the reservoir box, masses of 5.0, 6.0, and 7.0 kg of liquid nitrogen were tested. For a conveyor height of approximately 17 cm from the bottom surface of the reservoir box, masses of 3.5, 5.0, and 6.0 kg of liquid nitrogen were tested. The conveyor was programmed to run at 0.34 mm/s, or the equivalent to one straw cycle every 10 min.

3.2.2.2. Conveyor Slope

The internal sample temperatures were measured for three different slopes of the conveyor: 0°, 7°, and 14° (Figure 26). These slopes were obtained by using the neutral arms (sample height of 21 cm from the bottom of the reservoir box) and using the 21 cm, 19 cm, and 17 cm arms on the non-motor end of the conveyor where the straws are dropped into the liquid nitrogen.

FIGURE 25: Illustration of testing configurations of different nitrogen amounts (top) and different conveyor heights (bottom).
3.2.2.3. Conveyor Speed

Sample internal temperatures were measured for cooling using three different conveyor speeds: 0.34, 0.49, and 0.68 mm/s. These speeds were determined by delay function in the Arduino code for the motor (Appendix B). The delay values were 4150 for 0.34 mm/s, 3075 for 0.49 mm/s, and 2000 for 0.69 mm/s.

3.2.3. Benchmark Comparison

Standard 0.5-ml French straws (IMV Technologies) were filled with 0.5 ml of Hanks’ balance salt solution. A type-k thermocouple wire (Omega Engineering, Norwalk, CT) was inserted into the open end of the straw until the tip of the thermocouple was in the middle of the straw to monitor the internal temperature of the sample (Bwanga, de Braganca et al. 1990). The thermocouple wire was wrapped around the open end of the straw to restrict movement of the thermocouple and to prevent it from being pulled out of the straw during testing. The straw was not sealed prior to testing.

The Micro-Digitcool (IMV Technologies, Maple Grove, MN) and Ice Cube computer-controlled freezers (Sy-Lab, Neupurkersdorf, Austria) were programmed to begin sample freezing at a chamber temperature of 4 °C. After the samples were loaded into the cooling chambers, the freezers were programmed to cool the chambers from 4 to -80 °C at a rate of 20 °C/min. The samples would be maintained at -80 °C until the user removed the samples from the chambers.

The conveyor configuration had a slope of 0° and a sample height of 21 cm. The amount of liquid nitrogen used to achieve the cooling rates were 6.8 kg for 5 °C/min, 8.0 kg for 20 °C/min, and 9.0 kg for 30 °C/min. The differences between the cooling rates produced by the conveyor and Micro-Digitcool were compared by dividing the absolute value of the difference in temperatures by the time.

3.2.4. Viability Testing

Sperm (concentration = $1.67 \times 10^{10}$/ml) were stripped by hand from three adult (approximately 3-6 years old, 30-36 cm long, 0.75-1.0 kg) pond-reared koi (Cyprinus carpio) and suspended in Hanks’ balanced salt solution to make 10% diluted samples. Samples from each fish were separated into two beakers. Dimethyl sulfoxide was added to the samples to be cooled by the computer-controlled freezer. The equilibration time for the samples was 10 min. Thirty 0.5-
ml French straws were filled and frozen by the freezer at a rate of 20 °C/min, with an introduction temperature of 4°C and a final temperature of -80°C.

The samples to be cooled by the conveyor were divided into thirty 250 microliter aliquots. At thirty second intervals, the samples were combined with DMSO and inserted into straws. After a 10 min equilibration time, the straws were added to the conveyor. The conveyor was prepared by assembling the cardboard and polystyrene boxes and attaching the conveyor to the inner box. The neutral arms and corresponding gears were used. The assembly was filled with 8.25 kg of liquid nitrogen and allowed to cool for 30 minutes. Prior to addition of cryoprotectant to sperm samples, the liquid nitrogen was refilled to a weight of 8.25 kg. At thirty second intervals, the samples were combined with DMSO and inserted into straws. The 0.5-ml straws were added to the conveyor consecutively. Straws were stored in liquid nitrogen. The samples were thawed and motility was analyzed using a Makler counting chamber (Appendix C).

3.3. RESULTS

3.3.1. Variable/Cooling Rate Determination

3.3.1.1. Sample Distance from Liquid Nitrogen Surface

The liquid nitrogen amount and height of the conveyor produced cooling rates dependent upon the distance from the liquid nitrogen surface (Table 2, Table 3, Table 4). Furthermore, the height of the conveyor also affected the deviation between cooling rates of different samples. The lower the conveyor was inside the cooling chamber, the greater the standard deviation became.

<table>
<thead>
<tr>
<th>Liquid Nitrogen (kg)</th>
<th>Cooling Rate (°C/min) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8</td>
<td>5.2 ± 0.36</td>
</tr>
<tr>
<td>7.3</td>
<td>9.0 ± 0.08</td>
</tr>
<tr>
<td>7.8</td>
<td>18.7 ± 0.22</td>
</tr>
<tr>
<td>8.3</td>
<td>22.3 ± 1.18</td>
</tr>
<tr>
<td>8.8</td>
<td>27.5 ± 1.40</td>
</tr>
</tbody>
</table>

TABLE 2: Corresponding cooling rates at various liquid nitrogen amounts at a conveyor height of 21 cm.
3.3.1.2. Conveyor Slope

The different slopes of the conveyor (0, 7, 24 degrees) produced different freezing rates (Figure 27). For the same variables, a flat conveyor produced a cooling rate of 9 °C/min, a conveyor with a slope of 7 degrees produced a cooling rate of 13 °C/min, and a conveyor with a slope of 24 degrees produced a cooling rate of 15 °C/min.
3.3.1.3. Motor Speed

As the speed of the conveyor increased, the cooling rate did not show any significant changes. The plunge temperatures of the straws increased as the speed was increased (Figure 28).

FIGURE 27: Comparison of average cooling curves of samples when cooled at different conveyor slopes.

FIGURE 28: Comparison of cooling curves of samples when frozen at different conveyor speeds. The arrows denote the plunge point of the sample.
3.3.2. Benchmark Comparison

The conveyor displays cooling curves that were consistent with those of the CCF. Most notably, the cooling curves of the conveyor and the CCF were most similar at higher (20 and 30 °C/min) cooling rates (Figure 29). Measured sample cooling rates of the computer-controlled freezer were: 4.7, 19.3, and 32.8 °C/min for the Micro-Digitcool freezer and 16.9 °C/min for the Sy-Lab freezer. The cooling rates of the conveyor were 32.4, 19.4, and 8.6 °C/min when compared to the Micro-Digitcool and 20.0 °C/min when compared to the Sy-Lab freezer (Figure 36). The average differences between the curves for the Micro-Digitcool and the conveyor were 2.28 °C/s for 5 °C/min, 1.93 °C/s for 20 °C/min, and 2.09 °C/s for 30 °C/min.

FIGURE 29: Internal sample temperature during cooling using the IMV Micro-Digitcool, Sy-Lab Ice Cube, and the cryopreservation conveyor.
3.3.3. Viability Testing

Motility analysis performed after thawing for the sperm frozen by the computer-controlled freezer and conveyor (Figure 30) shows the motility results of the sperm frozen by the conveyor, with straws at the beginning (1-10), middle (11-20), and end (21-30) of the experiment separated into groups (Figure 31). There was no significant difference (koi fish 1, p=.60427; koi fish 2, p=.08023; koi fish 3, p=.05866) between the motility of the samples frozen by the conveyor and the

![Figure 30](image-url): Motility of koi sperm frozen by the computer-controlled freezer (dark bars) and the conveyor (light bars). There was no statistical significance between the two freezing methods.

![Figure 31](image-url): Motility of koi sperm frozen by the conveyor.
computer-controlled freezer. The average motility of the samples of the beginning, middle, and end of the freezing process did not show significant differences.

3.4. DISCUSSION

In the present work, the conveyor device was analyzed for four parameters: reliability, customizability, cooling profiles versus a computer-controlled freezer, and viability of koi sperm frozen in the conveyor. The cooling curves produced using the conveyor were consistent for each parameter, with deviation remaining below 5 °C/min. However, the precision of the cooling rate was highest between rates of 10 and 30 °C/min. The cooling rates near the limits of the conveyor’s capabilities, 5 and 40 °C/min, are not suggested for use, due to the high variability and inconsistency of freezing rates at these temperatures; instead, cooling rates between 10 and 35 °C/min are recommended. Conditions required to reach a cooling rate of -40 °C/min require the stepper motor to be partially submerged in liquid nitrogen, which can lead to mechanical failure and eventual deterioration of the motor. Additionally, each customization variable provided a different freezing curve, which enables users to create distinct cooling profiles. The results of the motility analysis indicate that the cooling rates over the course of a 30-min freezing do not decline enough to affect post-thaw sample viability.

3.4.1. Variable/Cooling Rate Determination

Control of the cooling rates of sperm, particularly in relation to equilibrium cooling, has been regarded as important to the survival and retention of motility of samples. Computer-controlled freezers have more precise control of the rate of freezing when compared to floating straw protocols, due the computer-controlled freezer feedback and control mechanisms. Floating-straw methods often produce less motile thawed cells (Oliveira, Duarte et al. 2015) which may be caused by temperature fluctuations due to changing liquid nitrogen levels. Previous portable devices addressed this need by either providing feedback during freezing (Medrano, Anderson et al. 2002) or by ensuring consistent freezing conditions within the cooling chamber. However, these devices address batch freezing and do not efficiently process single samples. Portable freezers, which require less liquid nitrogen than computer-controlled freezers to operate, and thus make them the current efficient option for single-sample freezing, have been shown to produce unevenly cooled samples with inferior motility due to the evaporation of liquid nitrogen from the storage reservoir (McLaughlin, Ford et al. 1990, Oliveira, Duarte et al. 2015).

The device is designed to enable the user to change the freezing process to accommodate different species and experimental practices. The conveyor height, motor speed, liquid nitrogen height, and slope of the conveyor can all be adjusted to produce different freezing results. The height of the conveyor and amount of liquid nitrogen change the temperature field around the conveyor. Moving the conveyor towards the bottom of the internal polystyrene box can change the amount of liquid nitrogen needed to achieve a certain cooling rate. The height of the conveyor also determines how concentrated the nitrogen vapor is in the vicinity of the sample; the evaporated nitrogen is colder and heavier than air and so will settle inside the small polystyrene box. The larger polystyrene box allows the nitrogen vapor to disperse across a larger area, and so the temperature field available within the larger box is much warmer than those temperatures available within the smaller box. Addition of liquid nitrogen decreases the distance between the straw and liquid nitrogen surface, which lowers the temperature field around the conveyor. Users can
utilize this to be able to freeze in a greater range of temperatures. If the distance between the straw and the liquid nitrogen is reduced during transit of the sample within the cooling chamber, the cooling rate of the sample can be increased mid-cycle. The slope of the conveyor enables the sample to move through multiple temperature fields during transit and therefore expose it to different freezing rates. The speed of the motor effects the plunge temperature of the sample. If the conveyor speed is increased, users can plunge at a higher temperature. This is useful in combination with changing the liquid nitrogen level or the slope of the conveyor. For example, a higher liquid nitrogen level will increase the freezing rate. If the conveyor speed is increased also, the straw can be plunged at a higher temperature than otherwise allowed. Each of these customization options allow the user to tailor the freezing protocol to each experiment.

3.4.2. Benchmark Comparison

Mechanical freezers, particularly computer-controlled freezers, are favored for cryopreservation due to their high-reproducibility of cooling rates and temperature feedback and display (Babiak, Fraser et al. 1999). Recent developments of inexpensive cryopreservation devices, such as the LSUAC Positional Cooling Platform Device and the cryopreservation elevator have proposed different methods of increasing cooling rate precision. The PCPD uses the geometry of the cooling chamber and sample platforms to achieve reproducible cooling rates, while the elevator uses the thermocouple feedback to control the cooling of the sample.

The cryopreservation conveyor produces cooling curves consistent with those of the computer-controlled freezer at 5, 20, and 30 °C/min. Although the chamber temperature of the computer controlled freezer is normally displayed as a linear curve, due to the ability of the freezer to precisely control the chamber temperature using the thermocouple feedback, the actual cooling rates of the samples are not linear. Because the cooling curves of the conveyor are similar to those of the computer-controlled freezer, the conveyor most likely will produce comparably viable cells when the cryopreservation variables are kept the same. This assumption was supported by the results of the viability testing.

3.4.3. Viability Testing

The cells cooled by the conveyor showed approximately the same post-thaw motility of those cooled by the computer-controlled freezer. This would indicate that the cooling rate or the effectiveness of the system does not change as samples are added and the conveyor produces consistent results. Furthermore, the motility of the samples cooled by the freezer did not decrease as more samples were added; the motility of the samples at the beginning of the experiment were not significantly different to those at the end of the experiment. Future temperature collection of the cooling chamber during sample addition could be used to determine the proper range of operating time and when the user would need to add more liquid nitrogen to prevent a change of cooling rates.

3.5. CONCLUSION

Standardization is key to developing and adopting optimized cryopreservation protocols. Reducing user error, providing detailed feedback, and the dissemination of cryopreservation data can accelerate the process of collecting and comparing relevant freezing information. To enhance
and develop the capabilities of the cryopreservation conveyor, it is essential to continue to mechanize and increase its operation potential. Implementing an electronic feedback and control system, using thermocouple wires to measure internal temperatures and infrared break beam sensors to assess straw count, in combination with a display screen and continuous data acquisition, could enable the conveyor to operate more similarly to a computer-controlled freezer. Furthermore, mechanizing the loading device and creating a straw hopper capable of timing sample input would remove user error from the tedious monitoring of equilibrium time and manual addition of straws into the device. Although the conveyor was created using only PLA, the full potential of 3-D printing filaments has not been determined. Additional characterization of the strength, brittleness, and deformation of PLA objects subjected to liquid nitrogen may allow the optimization and creation of new cryogenic devices that can be customized and fabricated by individual laboratories and repositories. In summary, the cryopreservation conveyor can achieve continuous processing of samples while maintaining consistent freezing rates and providing customizable parameters to users. This device represents an initial attempt to improve reproducibility and standardization of cryopreservation protocols, particularly in settings where computer-controlled freezers are not available or appropriate.
CHAPTER 4. CONCLUSIONS AND FUTURE WORK

4.1. CONCLUSIONS

4.1.1. Future Directions
Cryopreservation studies can only benefit from the standardization of collecting and reporting freezing data and access to consistent freezing equipment. The cryopreservation conveyor addresses the needs of those who require the successive freezing of samples, but does not provide feedback to the user. Continued improvement of the conveyor should address data collection, areas of user error, and additional customization options. The standardization and creation of inexpensive products for other parts of the cryopreservation process, including CPA loading, thawing, and motility analysis, can provide higher quality data and form a more complete picture of which methods result in preserved cells of higher quality. The combination of these products and the cryopreservation conveyor would enable smaller laboratories and repositories to reliably collect and report freezing data.

4.1.2. Device Goals and Proposed Research
Standardization of freezing protocol is key to optimizing successful cryopreservation of different species. User error can occur when non-feedback systems are used, as a change in the system usually cannot be detected by the user until the sample has already been frozen and thawed. Portable systems, which are the most rudimentary of cryopreservation devices, are the most prone to temperature fluctuations and user error. The continuous-mode cryopreservation conveyor is designed to standardize portable small-sample freezing and reduce user error; however, the system could be improved to reduce the possibility of unsuccessful freezing or device failure. The loading mechanism, which relies on the timing and ability of the user to quickly and effectively insert straws at regular intervals, is the most likely point of failure for the conveyor device. Mechanizing the loader would remove the need for the user to monitor the input of straws. Adding feedback equipment, such as temperature sensors to the device would also enable the user to better monitor internal temperatures and straw progression along the conveyor.

4.1.2.1. Electronic feedback and monitoring
Feedback and control is useful in monitoring system variables and recording data output for analysis and storage. Most commercial freezers utilize thermocouple feedback that is displayed in real-time graphic user interfaces (GUIs) so that in the event of system failure or insufficient or inconsistent temperature fields within the cooling chamber, the user can cancel cooling or attempt to alter the freezing protocol. In preliminary studies conducted by an undergraduate research team, several sensors controlled by an Arduino microcontroller were successful in capturing and storing data in a cryogenic environment. This system recorded straw numbers, measured the temperature of the cooling chamber, and counted the number of straws that fell off of the conveyor. The data recorded was displayed on a digital LCD screen and stored in a memory chip which could be uploaded to a computer. Implementation of this system could greatly improve the user’s ability to monitor the freezing process and share freezing data.

Standardization does not only benefit individual laboratories or repositories, but can allow the comparison of protocols to analyze results from changes in freezing variables.
Compilation of and the electronic sharing of freezing data could promote discussion and standardization of variables such as cooling rates, plunge temperatures, and equilibrium times that have not always been included in research reports.

### 4.1.2.2. Automated Straw Loader

Although the straw loading mechanism on the conveyor provides a relatively reliable means of inserting straws without the need for opening the cooling chamber, and thus introducing external thermal energy to the system, the user still must monitor the amount of time the straws need to reach the proper equilibrium time (time of cryoprotectant uptake) and insert the straw correctly. By introducing a straw hopper that would hold the straws during equilibrium time and mechanically load them onto the conveyor, the possible user error would be removed. Therefore, an automated straw loader is proposed (Figure 32). The loader would consist of a cylindrical straw hopper that is driven by a single stepper motor. The straws processed by the hopper would be deposited on a ramp that is attached to the box. A spring-loaded door would keep the entrance to the cooling chamber closed until the straw would be pushed through via a loading piston. After the straw would enter the cooling chamber and be deposited on the conveyor belt, the spring door would automatically close until the next straw was inserted. This device could be constructed using only 3-D printed components and a stepper motor.

![Figure 32: Proposed design of an automated straw loader.](image-url)
4.1.2.3. **Cryogenic Elasticity**

Fused deposition fabrication is a relatively new field, and so the full applications of 3-D printed parts and materials have not yet been identified. Recently, PLA has been identified as a suitable plastic for use in cryogenic applications due to its apparent durability in the presence of liquid nitrogen (Tiersch and Monroe 2016). Polylactic acid also undergoes less temperature deformation, commonly noted after printing, than ABS. The lower glass transition temperature of PLA slower cooling rate allows the layers of the print to have greater layer adhesion, which results in a less porous surface than a part printed with ABS. It is hypothesized that temperature-induced stresses therefore affect the structural integrity of PLA objects less so than those of ABS. The exact difference between these materials has not been documented in regards to cryogenic efficiency, and so a comparison of the strengths of PLA and ABS is needed to further investigate their abilities in cryopreservation applications.

Further research is proposed using stress/strain analysis on parts printed from PLA and subjected to liquid nitrogen during testing. Plastic springs, designed using Autodesk Inventor 2016, could be used in a tension experiment using an Instron (5960 Dual Column Tabletop) Testing System (Figure 33). Previously, 3-D printed objects were tested in a cryogenic environment through the use of hollow wire, filled with liquid nitrogen, wrapped around the printed part during stress analysis. However, this setup is not feasible for testing which requires thorough submersion in liquid nitrogen. A vessel is proposed that can be attached to standard Instron tension equipment that can simultaneously hold the springs and contain liquid nitrogen at the same time so as to not damage the instrumentation (Fig. 34).
4.1.2.4. Production and Deployment

For the cryopreservation conveyor to have the greatest impact, it must be able to be used by a large number of users. This can be accomplished in two ways: (1) the conveyor could be printed and constructed at one location and shipped to purchasing laboratories, or (2) the printing files for the conveyor could be obtained by laboratories electronically and printed at local 3-D printers. Due to the complex infill structures of the parts, which allows them to be strong while still maintaining a low mass, these objects cannot easily be molded and mass-produced through normal plastic fabrication methods. The costliest factor of the conveyor device is the person-hours needed to print the entirety of the parts (Table 5). Although a central location could purchase and utilize large number of 3-D printers, this would still not be as cost-effective as laboratories printing their own conveyors or contracting out the printing work. In the case of parts breaking or being lost, the laboratories could print replacements without relying on a production center. It is suggested that the conveyor be made as a for-purchase package that includes hard copies of the part files, the polystyrene and cardboard box assembly, and the motor and microcontroller components.

FIGURE 34: Proposed design of a vessel capable of securing a spring in place (top). The spring can be removed and replaced with samples of different geometries and the vessel can hold liquid nitrogen (bottom).
TABLE 5: Conveyor part printing times and costs. The cost is calculated using the price of Makerbot Natural PLA filament ($0.053/kg, 2016).

<table>
<thead>
<tr>
<th>Part Name</th>
<th>Print Time (min)</th>
<th>Material Use (g)</th>
<th>Cost (Total)</th>
<th>Print Time- Total (min)</th>
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<td>45</td>
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<td>$ 29.35</td>
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</table>
REFERENCES


APPENDIX A. DEVICE DESIGN DEVELOPMENT

1. INITIAL DESIGN

a. Design Overview
The initial design was created to have as few components and connection points as possible to require minimal setup and printing time as possible. The conveyor attached to the walls of the inner polystyrene box and was comprised of two support structures, two chains, and two sprocket structures.

b. Components

i. Supports
The support structures consist of two arms and a connecting piece that ensures that the arms remain at a set distance from each other (Figure 35). The support structures are designed to attach to the edge of the Polystyrene box.

ii. Chain
The chain links were created by modifying a pre-existing Inventor rendering of a chain link from a K‘nex© plastic construction set (Figure 36). The rendering was simplified, the round posts on the sides of the chain were lengthened, and rectangular posts on the top surface of the link were added. The rectangular posts create slots for the cryopreservation straws to fit into during transit.

FIGURE 35: Arms designed to attach the sprockets to the polystyrene reservoir. The arms at the motor-end of the conveyor were made to hold the axle between the sprockets (top), and the arms at the other end of the conveyor were designed to hold the sprockets in a way that would not impede the straws from falling.

FIGURE 36: Original design of chain links. The teeth on the top of the links would hold straws in place.
iii. Sprockets

Double-single sprockets were created to drive the chains (Figure 37). The notches in the circular toothed sides of the sprockets fit the side-posts of the chain. Each chain link is supported on both sides by the sprocket, which stabilizes the chain horizontally. The sprockets at the beginning of the conveyor are supported on the axle between the two sprockets. The ending sprocket assembly is supported on the outsides of the sprockets. This creates a space between the sprockets and the support structure large enough so that the cryopreservation straw is not hindered from dropping into the liquid nitrogen.

![FIGURE 37: Double-single sprocket structure designed to drive the conveyor chain.]

FIGURE 37: Double-single sprocket structure designed to drive the conveyor chain.

c. Design Challenges

This design showed promise in that the PLA printed chain could effectively still remain flexible while submerged in liquid nitrogen and not freeze or shatter under stress. However, there were several major problems with this design.

The chain links did not flow smoothly and provided fair resistance to turning and maintaining contact with the sprockets. The singular link design was too large and required a larger sprocket to accommodate the distance between sprocket posts. The amount of chain links that interfaced with the sprockets was also insufficient to provide enough force to smoothly and effectively move the chain.

The components were large and required a massive amount of support material. The printing times of the parts were several hours, and if the print failed before completion, the entire object would have to be reprinted. The support material for PLA printing can only be PLA, and so removal of the support material often took hours. Any support material that could not be removed by hand would need to be sanded off, or the leftover material would provide friction between moving parts.
2. SECOND DESIGN

a. Design Overview
The second design sought to fix the initial design and procure a working prototype. The parts were broken down into components that were easy to print and assemble. The chain was converted from a singular link style to a bike chain style. A servo motor was used to operate this design.

b. Components

   i. Supports
   The supports were altered so that the arms would be interchangeable (Figure 38). The arms and connecting piece were secured using cable ties, which provides a stronger and more secure attachment than pin connections. Cable ties also ensure that the parts do not become loose due to thermal expansion in the presence of liquid nitrogen.

   ii. Chain
   New chain links were created. Like bike chains, these links have alternating inside and outside connections. This structure decreased the amount of surface area of contact between links and decreased the overall link size. This chain design is the most recent design and is described in Chapter 3.
iii. Sprockets
Sprockets were designed to print as separate pieces to reduce printing time. The sprocket assemblies (Figure 39) were altered so that they printed as five separate pieces, and each piece would not require support material. Each assembly is comprised of two flat sprocket sides, two sprockets with spacers, and an axle. The parts connect through cross-shaped pin connections.

iv. Motor
A continuous rotation servo was substituted for the DC motor. The continuous rotation servo can operate at a slow enough speed so that it can directly drive the front axle of the conveyor. A mounting piece was created that replaced one of the arms on the support structure at the end of the conveyor (Figure 40).

c. Design Challenges
In the second design, the cryopreservation conveyor was kept at a fixed height in the cooling chamber. As the nitrogen evaporated, the distance between the straws and liquid nitrogen increased, potentially decreasing the cooling rate of the straws. To prevent the possible problems that may arise from the changing height, a floating conveyor was designed.
3. THIRD DESIGN

a. Design Overview

The floating design (Figure 41) consists of a frame that attaches to the walls of the cooling chamber, arms that can move up and down the frame, a polystyrene raft for each sprocket assembly, and interchangeable posts that determine the height of the conveyor from the surface of the liquid nitrogen.

b. Components

i. Frame

The frame attached to the edges of the polystyrene box so that the assembly did not shift during operation (Figure 42). The polystyrene attachment pieces held the guide rail segments in place, and the bottom piece connected the two guide rail assemblies together. The middle piece
acted at a means to ensure that the conveyor was horizontally aligned and could also be used as an air dispersion device if liquid nitrogen aeration was desired.

ii. Supports

The floating supports consisted of a polystyrene holder, two posts, two arms, an arm attachment piece, and the sprocket assembly from the second design. The polystyrene holder fit a rectangular piece of Polystyrene that kept the arms and sprockets floating above the liquid nitrogen (Figure 43). The removable posts could be altered to different lengths to determine the height of the straws above the liquid nitrogen. Each arm attached to the connection piece using a post with pin fitting connection.

iii. Chain

The chain that was used for this design was the chain that is used for the most current version of the device, as is described in Chapter 3.
iv. Motor

The servo motor fit into an attachment designed to fit into the end floating support assembly (Figure 44). The attachment acted at a support arm, but the sprocket directly connected to the servo motor shaft. The servomotor could be secured to the attachment by screws.

FIGURE 44: The servo motor holder (left) was connected to the support structure to directly drive the sprockets (right).

c. Design Challenges

The connecting parts had a much shorter printing time than those of the original design, but the t-shaped connector proved to be weak. The gradual wear on the connectors made the connection between the parts weak over time, and the connector that protruded from the surface of the sprocket would break if any non-axial pressure was applied to it.

The most concerning challenge with this design was the discovery that the continuous rotation servomotor could not operate correctly in the presence of liquid nitrogen. The potentiometer inside the servomotor that calibrated the motion of the servo shaft was subject to the changing temperatures inside the cooling chamber. These temperatures affected the capacitive properties of the potentiometer, and ultimately rendered the servomotor unsuitable for cryogenic use. A stepper motor, which was determined to be the best choice for driving the conveyor at a precise and slow movement, is too heavy to float on the surface of liquid nitrogen without a large raft. Furthermore, the final design of the conveyor demonstrated a negligible change in cooling rate over a 30 min period.
APPENDIX B. CONVEYOR BOX INTERNAL TEMPERATURE TESTING

1. INTRODUCTION

Cooling rates that could be obtained by the device were dependent upon the temperatures within the cooling chamber. The amount of liquid nitrogen, placement of the device, amount of material within the cooling chamber, and heat output by the motor could affect the temperature field inside the polystyrene boxes. Thus, a study was performed to determine the temperature field of the cooling chamber so that the user can select sample cooling rates, plunge temperatures, and throughput speed of the device for their cryopreservation protocol. The nitrogen vapor above the liquid nitrogen reservoir exponentially decreases in temperature as it approaches the surface of the liquid nitrogen. The aeration of liquid nitrogen would increase its evaporation rate and create vapor movement within the cooling chamber. To determine if the aeration of liquid nitrogen can minimize stratification of air layers of differing temperature above the surface, an air-bubbler system was created to provide a form of aeration (Figure 45).

2. METHODS AND MATERIALS

A 6.6 GPH aquarium pump (Whisper 10, Tetra) was attached to a 3-D printed air dispersion device as shown in Figure 27. Initially, liquid nitrogen was added to the cooling chamber, and after fifteen minutes, the device reached thermal equilibrium and the liquid nitrogen was replaced. Type-K thermocouple wires were attached to a 3-D printed thermocouple holder. The holder was designed to position four thermocouples vertically within the box at 2.54 cm intervals (Figure 46). Temperatures were recorded for 2 hours.

![Static Liquid Nitrogen and Nitrogen Vapor](image_url)

FIGURE 45: Illustration of static vapor within cooling chamber assembly (left) and aerated liquid nitrogen (right).
3. RESULTS

Pumping air into the liquid nitrogen box assembly increased the temperature of the nitrogen vapor and made the overall system warmer than the non-aerated system (Fig. 47). For example, at one hour following liquid nitrogen addition, at a 10.2 cm height, the temperatures were -49 °C in the box with air introduced and -55 °C in the static box. Temperatures at each height in the box increased with the introduction of the outside air from the bubbler. In addition, the water vapor from the external environment condensed within the tubing inside the box, causing tube freezing and blockage.

FIGURE 46: Illustration of the vertical thermocouple holder attached to the inner box (left) and the thermocouple holder (right).

FIGURE 47: Comparison of temperatures inside cooling chambers containing static and aerated liquid nitrogen at 2.5, 5.1, 7.6, and 10.2 cm above the bottom of the internal reservoir box. The star symbol denotes the addition of liquid nitrogen.
APPENDIX C. USER MANUAL

1. PRINTING

a. 3-D Printer Setup

The 3-D printer should be calibrated and operated within a temperature- and humidity-controlled environment. For best results, the printing chamber or room should be dehumidified and kept within the recommended temperature guidelines suggested by the printer manufacturer. The Makerbot Replicator 2 was used to fabricate all of the printed parts of the conveyor. The suggested print settings for the conveyor parts may be different depending on what fused-filament printer and what color PLA filament is used.

Standard resolution, or a layer thickness of 0.2 mm, was used for the part resolution setting. A layer thickness of 0.3 mm may be used with success, but a higher resolution (layer thickness ≤ 0.1 mm) would greatly increase the print time and would not necessarily improve the quality of the prints. The percent infill of each part can also be altered, but an infill lower than 10% is not recommended due to the decreased internal strength. The chain links should be printed at 100% infill because they have small protruding features.

TABLE 5: Suggest conveyor part print guidelines.

<table>
<thead>
<tr>
<th>Number Required</th>
<th>Name</th>
<th>Print Time (min)</th>
<th>Material Use (g)</th>
<th>Shells</th>
<th>Infill %</th>
<th>Raft</th>
<th>Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SGLow1</td>
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<td>11.66</td>
<td>2</td>
<td>25</td>
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2. ASSEMBLY INSTRUCTIONS
a. Motor Assembly
b. Arm Assemblies

5. The components needed for the motor sprocket assembly are:
   - 1 axle gear
   - 1 axle
   - 2 arms
   - 1 arm holder
   - 2 sprocket caps
   - 2 sprocket spacers

6. Insert the axle into the gear. The gear should fit tightly on the axle and should not rotate freely.

4. Insert the axle into the openings on the arms. The axle should be positioned so that the sprockets will be rotated towards the side of the box the arm is attached to.

3. Slide the second half of the sprocket onto the axle. Hold the axle in place and rotate the sprockets towards the side of the box the arms are attached to until the teeth on the sprocket pieces are firmly pressed against the tooth wall.

1. Inserted the arms into the slots on the arm support.

2. Slide the first half of the sprocket onto the axle (this can be either the sprocket cap or sprocket spacer. Each sprocket piece has a tooth that must be inserted into the sprocket path.

1. The components needed for the non-motor arm assembly are:
   - 1 axle
   - 2 arms
   - 1 arm holder
   - 2 sprocket caps
   - 2 sprocket spacers

2. Slide the first halves of the sprockets onto the axle. The sprocket teeth should fit into the sprocket path as described in the Motor Arm Assembly.

3. Slide the other halves of the sprockets into place and lock the sprockets to the axle using the method described in the Motor Arm Assembly.

4. Slide the arms onto the axle. For ease of assembly, attach the assembled arms and sprockets to the edge of the reservoir box prior to step 5.

5. Insert the arms into the arm support slots.
c. Overall Assembly

1. The conveyor components are assembled according to the desired cooling rate parameters.

2. Insert the motor assembly into the opening on the arm support and attach it to the box.

3. Attach the arm assembly on the motor-end of the conveyor to the box.

4. Attach the arm assembly at the non-motor end of the conveyor to the box.
3. MOTOR WIRING

The motor wiring configuration for the Arduino Uno microcontroller and Sparkfun EasyDriver stepper motor control was designed by the blog Bildr for use with their Arduino code (http://bildr.org/2011/06/easydriver/). This design required two power supplies to operate. One 12V power supply powered the motor and another 12V power supply powered the Arduino Uno.
4. PROGRAMMING

The Arduino code used was created by the blog Bildr for use with the Arduino Uno microcontroller and the Sparkfun EasyDriver stepper motor driver. The rotate function (line 21) can be changed to change the speed of the stepper motor, although for this project the motor speed and length of operation per iteration of code was kept as (-2,1000). The delay command (line 22) was changed to alter the delay time between motor movements (Table 6). This created a “step pattern” instead of continuous rotation, which allowed the samples to be transported by the conveyor slowly without the need to use the slowest possible speed of the stepper motor. The change in ‘motor speed’ was actually caused by the length of the pause between rotations.

5. FAILURE ANALYSIS

a. Box Assembly

The low temperatures of the internal boxes during use causes condensation to form and freeze on the external cardboard box, especially in humid environments. Over time, this repeated dampening of the cardboard will warp the box and allow mildew to form. This can be minimized by removing all liquid nitrogen from the box after freezing and allowing the components to dry separately. If the cardboard box has degraded substantially, it should be replaced to ensure proper insulation of the conveyor environment.

b. Liquid Nitrogen Displacement

Because the liquid nitrogen is poured into the small polystyrene box after the entire system has been assembled, the user may accidentally pour liquid nitrogen into the space between the inner and outer boxes. If the height of liquid nitrogen is measured, the excess liquid nitrogen

<table>
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<th>Approximate Cycle Time (min)</th>
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will create a colder environment and produce faster freezing rates. If the liquid nitrogen weight is measured, the weight measured will include the nitrogen not located in the smaller box, which will result in lower cooling rates. Possible user error should be checked before each freezing run. The user should visually confirm that there is no excess liquid nitrogen present in the box system.

c. **Conveyor Failure**

The conveyor is attached directly to the polystyrene box. The tension on the chain will, over the course of 30-50 runs, cause the polystyrene to warp towards the box interior. This deformation of the box will cause the conveyor to sink inward, allowing the chain to sag. This deformation, if large enough, will cause the teeth to not properly mesh with the sprocket assembly and can lead to conveyor failure. Maintaining proper gear connection is important to prevent problems during freezing.

When the conveyor is assembled, the user must ensure that the sprockets are horizontally aligned, otherwise the chain may become displaced from the sprockets. This can cause the straws to fall off the chain or for the chain to completely dislodge from the conveyor. Thermal expansion and contraction of the device can lead to loose connections between parts, which may also cause the chain to sag or fall. All parts must be firmly connected, and any parts that have worn or broken connections should be replaced.

Repeated exposure to cold nitrogen vapor and humid environments can also cause water vapor to condense within and on the motor, which will lead to the motor rusting if not addressed. The motor should be removed from the device and placed in a dry environment for storage after each use. Usage of dehumidifying agents, such as desiccants, can also help prevent wear on the motor from water vapor freezing.
VITA

Melissa Kaye Eskridge was born in Biloxi, Mississippi, the daughter of James and Gloria Eskridge. She attended high school in Ocean Springs, Mississippi where she became passionate about human health, genetics, and medical technology. Melissa attended Louisiana State University and received her Bachelor of Science in Biological Engineering in May of 2014. Shortly after, she began her Master of Science program in Biological and Agricultural Engineering. She anticipates that she will graduate with her Masters in May of 2017.