Design of a Cryogenic Tissue Pulverizer for Biochemical Analysis Preparation

Sara Alford – Team Leader
Christine Koranda – Communications
Carla Maas – BWIG
Ryan Roth – BSAC

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Advisor: Paul Thompson
Clients: Jeff Ross and Charles Tessier
Abstract:
The cryogenic tissue pulverizer processes surgically-removed tissue by freezing and grinding the sample into a powder (10 µm diameter particles). To accomplish this task, our design incorporates a pneumatic driven grinding head with a grinding chamber. After careful consideration between motor and pneumatic driven designs, a pneumatically grinding mechanism was chosen for the final design. The pneumatic cylinder, controlled by a timer circuit and solenoid, delivers the power needed to grind the sample. The sample is placed in a grinding chamber surrounded by a polystyrene insulated cooling chamber containing a dry ice and alcohol bath. This ensures that the tissue will remain frozen throughout the grinding process. A detachable grinding head attaches to the pneumatic cylinder and pulverizes the sample. The contour of the grinding head and the curvature of the grinding chamber were matched in order to maximize tissue contact. Our efforts this semester have been focused on building a working prototype. It was tested using liver tissue samples and its performance compared to the existing method using a mortar and pestle.

Design Problem:
To design a device that completes the preparation process done manually to prep a tissue sample for biochemical analysis. The device should freeze the tissue (with liquid nitrogen), and grind it to a powder. The sample should be easily collected.

Overview of Project Work:
Work on this project began in BME 301 during the spring semester of 2002. Initial design mechanism for the grinding method and background research was focused on during that semester. Three designs were initially considered. The next semester, fall 2002, focused on choosing a method to deliver the power needed to grind. A motor driven and pneumatic driven design were explored thoroughly before choosing a pneumatically powered grinding mechanism. Initial testing to determine the best mechanism of pounding and shape of the grinding head and bowl were undertaken. Near the end of the semester, the development of a prototype was started. This last semester continued work on the prototype, including the designing of a sample grinding chamber and cooling chamber. An electrical timing circuit was made to control the pounding rate of the grinding head. The prototype was working by mid-semester, and then tested with liver samples in the lab to rate its performance. The project was displayed at the
engineering EXPO, and was awarded an honorable mention as well as a K-12 Outreach Award. A patent proposal was submitted, but was not accepted by WARF.

**Background:**

*Biological and Clinical Rationale*

When a tumor is removed from a patient during a surgical resection, a pathologist analyzes it with microscopy. The information about the tumor cells obtained through this method is often insufficient to determine precisely what kind of treatment is most effective for the patient. A more accurate and informative analysis of the tissue is desired, and therefore the tissue sample is sent to a molecular biology laboratory for profiling of DNA, RNA, and protein. This information is important in determining a possible specific treatment that may inhibit or decrease tumor growth. However, before a profile can be completed, the tissue sample must be preserved and prepared for the molecular testing. The current preparation procedure involves freezing the sample with liquid nitrogen and using a mortar and pestle to grind up the sample to a fine powder. This process is tedious and time-consuming. A molecular biologist may spend several hours per day solely grinding samples. Our proposed device would replace the current manual preparation of tissue samples, allowing the researcher to spend his/her time on other tasks. Ultimately, this device would be placed in a clinical setting so that a physician or other health care staff could simply insert the fresh extracted sample and later remove the ground sample, to be sent to a laboratory for testing.

*Design Requirements and Constraints*

The final design must fulfill several client requirements (Appendix A). The device should freeze a tissue sample, less than a gram in mass, with liquid nitrogen and subsequently grind the sample to a powder the consistency of powdered sugar (10 µm diameter). After analysis, the sample should be efficiently collected. The device needs to have the capability to prepare 40 tissue samples per day, with each sample processing time less than or comparable to the manual processing time of 15 minutes. All parts in contact with the tissue sample should be removable or easily cleaned to avoid cross contamination of tissue samples. Lastly, the device should fit on a laboratory bench.
Cryogenic System Design Considerations:

Due to the use of liquid nitrogen, our group needs to be aware of its implications to the overall design. In general, a cryogenic storage device must be designed to withstand forces resulting from internal pressure, the weight of contents, and bending stresses. All materials that come in contact with the sample must withstand the cold temperature of liquid nitrogen (-196°C).

Most cryogenic devices are based on the concept of a dewar flask principle – a double walled container with the inner space being well insulated. In this design, the inner vessel is constructed of a material compatible with the cryogenic fluid, making material compatibility a major factor in designing a system (Flynn, 1997).

Properties and behavior of materials included must be considered at low temperatures since they often vary significantly from room temperature. These factors include thermal properties such as the ability to conduct heat as well as thermal expansivity, a material’s cyclic expansion and contraction due to a change in temperature from low to room temperature.

It also includes mechanical properties such as ductility and brittleness. Materials exhibiting low-temperature embrittlement should not be used in cryogenic systems. When a material is subjected to a force of high enough stress level, the elastic behavior of the material will no longer hold. The material becomes brittle, breaking without any more deformation, or becomes ductile, permanently deformed. Both results lead to system failure and inadequate performance. Material’s brittleness is related to its ability to dislocate under stress, which is related to structure. Metals, specifically face-centered cubic (fcc) metals and their alloys, are most often used in cryogenic equipment. These materials include metals such as aluminum, copper, and nickel. The body-centered cubic (bcc) and the hexagonal close packed metals are less desirable low-temperature devices because they are more apt to become brittle. Plastics and glass are less desirable materials because they tend to be very brittle and can shatter upon contact with a substance as cold as liquid nitrogen. When stress is applied to glass, atomic bonds in the structure rupture causing the propagation of a crack resulting in a fracture of the glass piece (Flynn, 1997).
Important considerations must also be taken into account concerning the use of liquid nitrogen in this device. Some general properties of liquid nitrogen are given in Table 1.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Molecular weight of N₂</td>
<td>28.01 g/mol</td>
</tr>
<tr>
<td>§ Boiling Point (at 1 atm)</td>
<td>-196°C</td>
</tr>
<tr>
<td>§ Density</td>
<td>8.07x10⁻⁵ g/m³</td>
</tr>
<tr>
<td>§ Heat of Vaporization</td>
<td>199 kJ/kg</td>
</tr>
<tr>
<td>§ Nitrogen gas evolved per volume liquid nitrogen</td>
<td>0.7 m³ vapor per 1 L (0.001 m³) liquid nitrogen</td>
</tr>
</tbody>
</table>

Table 1: Properties of Liquid Nitrogen (N₂). Information from the following sources *("Periodic Table", 2002), § (MacNeil, 2002).

In general, the container that holds liquid nitrogen must be a good insulator. A dewar is a specially made container designed to hold substances as cold as liquid nitrogen; unfortunately, most dewars are expensive. A thermos is an alternative container for liquid nitrogen; however, the liquid nitrogen will evaporate faster, and the outside of the thermos will become very cold to touch ("Physics Van", 2002). Evaporation of the liquid nitrogen will cause high pressure inside the thermos. Drilling a hole in the thermos allows nitrogen vapor to escape preventing explosions. Care must also be taken when handling liquid nitrogen to prevent serious frostbite burns resulting from direct contact with liquid nitrogen or contact with a surface that was cooled by liquid nitrogen. Safety glasses and waterproof welder’s gloves should be worn (K3PGP Experimenter’s Corner, 2002). Since the nitrogen gas that evolves from the liquid state can also be hazardous, liquid nitrogen should only be used in well-ventilated areas (MacNeil, 2002).

An insulated chamber containing a coolant can also be used to surround a desired area to prolong the time the chamber is cool. Dry ice or dry ice with alcohol can be used as a coolant. To contain the coolant, polystyrene foam insulation can be used. This is a non-deteriorating and vapor-proof material used commonly to ship dry ice around the country (ansciproducts.com, 2002).
Existing Grinding Products on the Market

A comprehensive search of existing products on the market solidified the need for the designing of a new device. Devices currently being sold do not meet all requirements set by our client. Although most products grind tissue, they are not compatible with liquid nitrogen. The following devices were considered in detail to aid design of our cryogenic tissue pulverizer:

BeadBeater™

Biospec Products, Inc. currently produces the BeadBeater™ (Figure 1), a bead milling homogenizer. This device is used to disrupt small cells like yeast. The machine operates by stirring and agitating glass beads, which collide with cells, resulting in crushing. Recently researchers have begun using the BeadBeater™ to grind plant and animal tissue with bead sizes of 1.0 to 2.5 mm. The beads are available in glass, stainless steel, zirconia, chrome steel, and tungsten. Tougher materials require beads with a higher density (Meyer, 2002).

Figure 1: The BeadBeater™. The top unit spins the metal or glass beads to create collisions with the tissue particles. The collision force breaks apart the tissue (Meyer, 2002). Image from <http://www.biospec.com>.

Pestle and tube homogenizer

Pestle and tube homogenizers (Figure 2) consist of a Teflon pestle precisely fitted to a tube. The distance between the tube and pestle is specialized for the particular cell type to be homogenized. The pestle spins and grinds the sample with a shearing force. The pestle is pushed downward and the sample can only pass the spinning pestle if it is
smaller than the clearance between the tube and pestle (Seetharam and Sharma, 1991). The tube is typically placed in a beaker of ice to keep the tissue cold. The main problem with homogenizers is preparation, as the tissue must be grinded prior to processing (Burgess, 2002).

**Figure 2: The Potter-Elvehjem Homogenizer (LabGlass, 2002).** The precision-fit tube is on the left, and the pestle that is attached to a motor is shown on the right. To homogenize a sample, the pestle is forced downward to the bottom of the tube. Image taken from <http://www.lab-glass.com>.

**Bio-Pulverizer™**

The Bio-Pulverizer™ consists of a stainless steel base and a piston that uses freeze fracturing to grind a tissue sample. The tissue is pre-frozen with liquid nitrogen and inserted into a pre-cooled base. The piston and spring loaded hammer are connected via a trigger loaded mechanism (Figure 3). The sample is then pounded once or twice to achieve desired consistency. It is then inverted to drop sample into appropriate collection vial (Meyer, 2002).

**Figure 3: Images of the Bio-pulverizer.** The spring loaded bio-pulverizer is shown on the left. The mortar and piston are shown individually on the right. This device grinds by freeze fracturing. Image taken from <http://www.biospec.com/Brochures/cryog/BioPulv.html>.
Another design currently on the market is the Jet Pulverizer™. It is designed to grind any type of crystalline or friable materials, producing product in the size range of 0.25 to 15 microns. The biological design of the pulverizer is able to process samples smaller than 1 gram into a small centrifuge tube with 80% collection efficiency (Jet Pulverizer Company, 2002).

Pulverization occurs in the central chamber of the jet energy mill (Figure 4). The process material approaches sonic velocity around the perimeter of the chamber by multiple jets of air or steam. No grinding media is involved. High-velocity collisions between particles of the process material reduce the particle size. The interior of the chamber allows recirculation of over-sized particles, which enhances the incidence and the effect of collisions. As particles reduce in size and progressively lose mass, they naturally migrate toward the central discharge port. (Jet Pulverizer Company, 2002).

The process involves no moving parts and is suitable for many materials, including cryogenic ones. Laboratories can process small quantities in the 1-in. jet mill, the smallest version of the device. These mills are small, easy to disassemble and easily cleaned (Jet Pulverizer Company, 2002).

**Figure 4: Image of the Jet Pulverizer.** The material is placed into the jet energy mill where it is propelled by compressed gas at near sonic velocities. The material collides with other particles and breaks apart. When the particle is small enough, it is able to exit through the center port (Jet Pulverizer Company, 2002). Image from http://www.jetpul.com
Grinding Mechanism Design Alternatives:

To simplify and focus efforts, our group initially divided the project into different components: cooling/freezing, grinding, and sample collection. During the first semester of this project, the grinding method was deemed most important and central to the design of our device. Focus was directed towards possible mechanisms for grinding; assuming liquid nitrogen could be pumped or poured via an undetermined mechanism. Our preliminary design solutions included a plethora of mechanisms such as sliding plates, beads, pressurized air, pounding hammers, blenders, and funnels. From these designs, we chose to focus on three main ones to seriously consider and develop further. Based on test results grinding mechanism effectiveness, as well as other criteria, the grinding mechanism was chosen.

Grinding Mechanism # 1: The Blender

One design option explored was the blender (Figure 5). Operating like a kitchen blender, this design uses sharp blades to break frozen sample into fine particles. Blades are connected through a metal shaft to a motor, which supplies the energy needed to break the sample. By covering the blending area with a lid, sample loss is prevented. After the sample is broken, it is washed into the collection vial through the discharge port with small amounts of liquid nitrogen. The discharge port is selectively opened when the sample has finished blending.

![Blender Diagram]

**Figure 5: Blender.** The sample is cut into smaller pieces by the spinning blades of the blender. The liquid nitrogen spray will wash the sample through the discharge port and into the collection vial.
**Grinding Mechanism # 2: A Funnel Grinder**

This design uses a metal cone that rotating around its central axis to grind the sample. After a sample insertion, the motor rotates the cone creating a shear force against the sample. Only particles sizes of appropriate size are allowed through the discharge port into the vial (Figure 6).

![Funnel Grinder Diagram](image)

**Figure 6: Funnel Grinder.** The motor spins a cone shaped grinder to break apart the sample by shear forces. When the sample reaches an appropriate size it enters the collection vial through the discharge port.

**Grinding Mechanism # 3: The Impulse Sampler**

The impulse sampler design incorporates the same catabolic mechanism as the cryogenic pulverizer, but attempts to further the process using automation (see Figure 7). After the sample is then pulverized and consequently broken into smaller particles by impulses from the pulverizing head. A motor controls the impulses through a spiral gear, raising the head with each turn of the motor.
**Figure 7: Impulse Sampler.** The impulse-sampler freezes tissue samples with liquid nitrogen and processes them with impulses delivered by the motor.

**Evaluating of Early Grinding Designs:**

**Initial Testing:**

To better evaluate our designs, our group decided to conduct initial testing. We tested the following methods: two plates sliding past each other, a shaker with ball bearings, a rotating pestle and a hammer repetitively pounding (Pictures in Appendix B). Two flat surfaces, providing shear forces to the frozen tissue sample, did not adequately grind the sample. This process only shredded small layers of cells. Next, we looked at metal beads in a confined container. The sample stuck to the beads and was hard to isolate. The hammering test revealed the need for a sample containment mechanism. Otherwise, sample tended to project bits outward. With an enclosed container, it was broken into smaller pieces, not a fine powder. We then decided to try the rotating pestle motion. This motion did not initially break the sample appropriately, but was the best method for achieving a fine powder once the sample was preprocessed into smaller pieces. Based on these results, a hybrid design incorporating a pounding hammer motion first, switching to a rotating pestle motion later would provide optimal grinding of frozen tissue.
Another observation helped realize the importance of cooling. The tissue remained frozen for around 3 – 5 minutes after being exposed to room temperature directly after immersion in liquid nitrogen. This led to trouble maintaining frozen tissue samples. Thawing and refreezing led to tissue freezing hard onto surfaces.

**Evaluation Process:**

Each of these grinding designs had its advantages as well as disadvantages. As shown in the design matrix (table 2), we used appropriate criteria to judge each design. Grinding efficiency, due to its importance, was weighted by a factor of 4.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Blender</th>
<th>Funnel Grinder</th>
<th>Impulse Sampler</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grinding Efficiency (4x)</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Simplicity</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Sample Collection Efficiency</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Durability</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Noise Acceptability</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Cleaning</td>
<td>-</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Processing Time</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pressure Release</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total +</td>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Total 0</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total -</td>
<td>-6</td>
<td>-4</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total Score</strong></td>
<td><strong>-4</strong></td>
<td><strong>2</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>

**Table 2: Design Matrix.** Eight criteria were used to evaluate the three grinding designs, with grinding efficiency weighted by a factor of 4. A plus (+) was given when the design met the criteria. A zero (0) was given when the design neither met the criteria nor failed. A minus (-) was given to the design if it failed the criteria. The total score was obtained by adding all criteria with pluses equal to 1, zeros equal zero, and minuses equal to –1.

Based on this initial evaluation of the grinding mechanism, the impulse sampler was chosen for our final design. The exact grinding sequence incorporates several hard impact pounds to break up the sample followed by rotation of the head around its central axis, providing shear force. This combination effectively produces a homogenous fine powder.

**Grinding Head Shape and Chamber Design:**

Next, further testing was conducted to determine the most effective grinding head shape and grinding container. The grinding effectiveness of three different objects was
tested to help determine the optimal grinding chamber design and head shape. The three sets of stainless steel grinding tools included round bowls with slanted sides, a flat bottomed (7.6 cm) soup ladle with completely rounded surfaces (4.1 cm) and common kitchen spoons with completely rounded surfaces. Comparisons of the bowls to the ladles test for shape, while, comparisons of the ladles to the spoons test for size. Each testing set was composed of two duplicate items (i.e. two identical bowls) to achieve a close fit during grinding of shelled peanuts.

We performed preliminary testing to identify the best method for testing. The same person pounded to avoid inconsistencies in pounding force. A board was used to distribute the force over the grinding object. Approximately 15 impulses were applied to the bowls and ladles, while only 1 pound was applied to the spoons.

We observed a heterogeneous mixture resulting from the slanted bowl. Smaller peanut particles were present around the periphery of the sample. We attributed this result to the edges grinding more effectively than the flat surface. Overall the soup ladles ground the sample to a much smaller homogenous consistency with the same amount of pounding, suggesting that the rounded surfaces are more effective at grinding. The spoons pulverized the peanuts to a small powder with only 1 pound. This suggests that a smaller area concentrates the force to grind the material better. This is probably due to the increased pressure on the peanuts since the spoon has a smaller surface area than the ladle. Since pressure is the force per unit of area, smaller areas with the same force will have higher levels of pressure. This is also intuitive since the same force was applied to many more peanuts in the ladle (approximately 15) than in the spoons (2 peanuts).

Future testing could be more quantitative. The average size of the larger peanut particles could be recorded. Since our client wants a fine powder, we found qualitative observation an acceptable method of analysis, especially since each tool set had clear differences in resulting particle size. Another improvement to this experiment would be the use of frozen materials to more accurately represent the frozen tissue. Due to the limitations of our room temperature setting (no cooling materials such as dry ice or liquid nitrogen), we did not test frozen materials such as chicken breast.
Grinding System Design Alternatives:

Based on initial testing data, the group tackled the overall grinding system design. Two plausible methods of powering the grinder were designed: motor driven or pneumatically driven.

Grinding System Design #1: A Motor Driven Grinder

The motorized design operates with motor to provide impulses driven by a spiral gear (Figure 8). A spring lifts the pulverizing head to reset the pulverizing head after each impulse. A second motor rotates the pulverizing head to distribute the sample. These motors are connected through a gear, allowing simultaneous vertical and rotational movement. All motors are controlled by a microprocessor. The design is also somewhat simple with only a gear as the most complicated component. The motors are also isolated from the cold chamber preventing generated heat from interfering with the cooling area.

![Figure 8: Motorized Design](image)

The motorized design uses three motors to spin, pulverize, and collect the sample. The dashed line represents the motor going into the page.

Grinding System Design #2: Pneumatically Driven System

This design uses a lab air supply to drive the overall grinding system (Figure 9). An electrical timing circuit connected to the solenoid valve controls the pounding pattern.
After filtration in the regulator, air flows into the pneumatic cylinder to provide linear force to break-up the sample. A pivot mount will support the cylinder.

![Diagram of Pneumatic Driven System](image)

**Figure 9: Pneumatic Driven System.** The electrical timing circuit controls the solenoid valve which in turn directs the air supply.

**Evaluation of Grinding System Designs:**

There are several advantages and disadvantages to each system design which influenced our decision when compared in a design matrix (Table 3).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Motor</th>
<th>Pneumatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grinding Efficiency (4x)</td>
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</tr>
<tr>
<td>Cost (2x)</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Power Acceptability (2x)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Size/Weight</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Simplicity</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Flexibility to Design</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total +</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Total 0</td>
<td>-3</td>
<td>0</td>
</tr>
<tr>
<td>Total -</td>
<td>-3</td>
<td>7</td>
</tr>
<tr>
<td>Total Score</td>
<td>-3</td>
<td>7</td>
</tr>
</tbody>
</table>

**Table 3: Design Matrix.** Five criteria were used to evaluate the three grinding designs, with grinding efficiency weighted by a factor of 4 and power acceptability weighted by a factor of 2. A plus (+) was given when the design met the criteria. A zero (0) was given when the design neither met the criteria nor failed. A minus (-) was given to the design if it failed the criteria. The total score was obtained by adding all criteria with pluses equal to 1, zeros equal zero, and minuses equal to –1.

Pneumatic cylinders tend to be lighter and less complicated than electrical actuators since there are no mechanical parts such as gears, shafts, or cams. The cylinder is controlled by simply fluctuating the electrical signal to the solenoid. Changing the bore size of the pneumatic cylinder proportionally changes the forces created by the
cylinder. Pneumatics allow for continuous application of force, facilitating sample pulverization. The pneumatic grinder also has several disadvantages. It is difficult to stop the piston during a stroke. This may be a safety issue. Maintenance and repair will require both electrical and pneumatic experts, increasing maintenance cost.

The motorized design is also expected to be more cost effective and smaller than the pneumatic design. The smaller size is better for laboratories where lab bench space is often limited. Also, the lab does not need access to compressed air. The motorized design is not without disadvantages. The motorized design is not able to provide continuous force but instead delivers impulses, which are a less effective means of grinding. Motor electronics will less likely experience resistivity changes due to the cold.

Our final decision to use pneumatic power was further supported by professor advice. Initially, in conversations with Professor Roderic Lakes, we learned that using an electrical motor is an indirect way to apply force since it changes electricity to circular motion to linear motion. For an electric grinder, he suggested seeking out other options. Upon hearing the requirements of our design Professor Frank Fronczak highly recommended pneumatic power for our final design. Taking their advice, pneumatic power was chosen for our final design and warranted a thorough background search on pneumatic systems.

**Background on Pneumatic Systems:**

Pneumatics is the study of the behavior of gases. Compressed air performs work by either expansion or direct forces (Morden, 1966). Although air is not a perfect gas, the various gas laws (Figure 10), are useful for reasonable estimations in pneumatic situations.

\[
\begin{align*}
PV &= \text{constant} \\
V/T &= \text{constant} \\
\frac{P_1V_1}{T_1} &= \frac{P_2V_2}{T_2} \\
PV &= mRT
\end{align*}
\]

*Figure 10: Ideal Gas Laws.* These equations are used with providing values in different pneumatic situations.
Pneumatic tools operate on compressed air. Hospitals have many uses for compressed air including pneumatic drills, saws and ventilators. (Jacob and Kumaresh, 2001). Other general purpose pneumatic tools drill, rivet, chip, and caulk. These tools fall into two main categories: percussive and rotary. Riveting hammers, which fall under the percussive category, provide between 700 to 3,000 impacts per minute (British Compressed Air Society, 1955).

Pneumatic tools offer many advantages over mechanical tools. They provide linear motion without complicated mechanical parts such as gears, cams or shafts. By varying the air pressure, the applied force can be controlled. This applied force can also be easily maintained for durations of time. Speed can be controlled by changing valve and pipe sizes (Morden, 1966). Pneumatic circuits can be developed with many parallels to electric circuits, allowing for designs with precise control of piston action. An explanation of valves common to these circuits can be found in Appendix C.

The primary device for changing compressed air into operation power for tools is the cylinder. The standard components of a cylinder are the barrel, end covers, piston, and piston rod. The force generated by the piston is given by the following equation: 
\[ F = pA \]  
(F = force, \( p \)=pressure; A = area of piston).

The simplest pneumatic cylinder is the single-acting type (Figure 11). The cylinder is fed compressed air from a compressor. Assuming the piston starts in the upright position, the air expands inside the chamber above the piston forcing the piston down. The lower chamber of the piston is connected to a vent that allows the air underneath the piston to exit, decreasing resistance to the moving piston. The piston is returned by a spring (Pinches and Callear, 1996).
**Delivery of Liquid Nitrogen Design Alternatives:**

Switching over to the cooling aspects of the design, a mechanism to cool the sample was first investigated. Cooling needed to be investigated on a number of different levels. Besides keeping the tissue frozen, the group researched methods of automating liquid nitrogen dispersal, attempting to eliminate the need for a lab technician to manually pour liquid nitrogen on the sample.

There are a few different mechanisms for freezing such as immersion in a tank of liquid nitrogen, spraying liquid nitrogen from above, or flooding the grinding chamber with liquid nitrogen. Immersing an unfrozen item will create a thin crust on the item by freezing the outer layers of the material. The thin crust formed by immersion is beneficial since it prevents the material from sticking to the sides of the container (Agnelli and Mascheroni, 2000). This mechanism needs to include a complex method of lowering and raising the sample plate. Liquid nitrogen spray can be created with liquid nitrogen and helium gas. The helium is pushed into the gas chamber of an injector at a higher pressure than the liquid nitrogen is stored at. This forces the liquid nitrogen through a nozzle where tiny liquid nitrogen droplets spray down onto a sample (Felizsaz *et al.*, 2000).

Flooding the chamber with liquid nitrogen would be the easiest of the three and is the mechanism tentatively chosen for our design. A valve controls flow into the chamber. When the tissue sample is first placed in, the valve opens and allows a certain volume of liquid nitrogen to flow into the sample container. Ideally, it would be coupled with a thermostat to provide feedback. This feedback will trigger the valve to open once the...
temperature reached a certain warmth threshold to re-cool the chamber. Currently, only two alternatives for liquid nitrogen flow are available in the cryogenics industry: an expensive, high-flow-rate pump and a pressurized sealed tank ("Cryocare Report", 1996).

**Liquid Nitrogen Delivery System #1: Pressurized Valve System**

The pressurized valve design utilizes the normal evaporation pressure which builds up when a container with liquid nitrogen is sealed (Figure 12). A liquid discharging device, a valve and tubing, dispenses liquid nitrogen (MVE Form 2195, 2002). The valve used in this design can be coupled to a temperature feedback loop from the grinding chamber, allowing for regulation of the flow of liquid nitrogen to maintain appropriate coldness. The amount of fluid dispensed with this method will vary slightly; however, the overall effect of freezing the tissue sample sufficiently is more important than the exact quantity delivered.

![Figure 12: Pressurized System.](image)

The pressure developed inside the tank pushes the liquid nitrogen out. The tube provides a valve that can be closed or opened, allowing pressure to build and then liquid nitrogen to be dispensed.
Liquid Nitrogen Delivery System #2: Un-pressurized Pump System

Using a pump to deliver liquid nitrogen to the sample chamber is the second alternative to consider for this device. A cryogenic pump is defined as a pump that cools a surface to approximately –255°C in order to produce a very low vacuum with pressures of about $10^{-8}$ mm Hg (“Online Dictionary”, 2002). Pumps currently on the market include both manual and automated pumps. The manual pumps are simple in design and affix directly to storage dewars. If a manual pump, such as the one shown in Figure 13, were incorporated, we would modify the device to automate operation of the pump.

![Figure 13: A Manual Pump](http://www.brymill.com/catalog_4_nitro.htm)

The automated pumps have a hermetic design, which involves both a motor and a pump. Devices such as the one displayed in Figure 14 minimize the transfer of ambient heat from the motor to the cryogen using a vacuum system.
Figure 14: Hermetic Pump. The SH-100 Single Stage Hermetic Scroll Pump manufactured by Varian, Inc. is one of many automated pumps currently available on the market. (http://www.varianinc.com)

Other devices effectively minimize heat transfer by separating the motor from the cryogen with a long shaft (Barber-Nichols, Inc, 2002). An automated pump specially designed to handle cryogenics provides a highly efficient method of delivering liquid nitrogen. The major drawback of automated pumps is the high cost (several thousand dollars) whereas the manual pumps cost in the range of $100-$200.

Advantages and Disadvantage of Cooling Delivery Systems:

Pumps are advantageous because they require few or no modifications to be incorporated into the overall design. The pump connects to a dewar to obtain liquid nitrogen which could then be pumped through tubing into the sample chamber. This option allows the liquid nitrogen to be stored separately from the motor of our device, which increases the chances that a motor functions properly at reasonable temperatures. Incorporation of a manual requires additional modifications to completely automate the process. In addition, a pump is a commercially available, reliable way to transfer liquid nitrogen.

A pressurized valve system is a simple, comparatively cheaper delivery method. It is currently not commercially available; therefore, we would need to further design this component to be automatic. A valve functional at the cold temperatures associated with liquid nitrogen (-196 degrees Fahrenheit) would need to be found. A quick browsing of the Cole-Palmer lab catalog (1999-2000) did not yield any valves for low temperature
cryogenic systems; therefore a more rigorous search would need to be conducted. Also, a temperature gauge could easily be integrated, thereby keeping the temperature in the sample-grinding chamber below a temperature threshold during the entire preparation process. Based on these advantages, we would like to incorporate one into the design, but based on cost limitations of this project, it is not feasible. Therefore, manual cooling of the sample was decided to be the best means possible with the resources available.

**Sample Chamber Design:**

Next, the focus turned to designing a sample chamber that would meet the temperature requirements. Before the tissue grinding process can begin, all materials that contact the tissue must be cooled. Pre-cooling and cold storage of the device is accomplished by either periodic liquid nitrogen application or an additional cooling system. Adding liquid nitrogen periodically is ideal, therefore different cooling systems were considered. Two options currently exist: a dependent storage of the device in a freezer or an independent cooling system. The necessity of a freezer would be a major drawback; therefore an independent cooling system was incorporated in the sample chamber design.

When designing our sample chamber, two other requirements besides the temperature concerns governed our design. The chamber needs to be rounded in shape to match the grinding head. Also, the chamber should provide a means for sheltering the sample to minimize the amount of sample spray during grinding. Secondary to these is the ease of sample collection. Early in the project, two options were considered. One option uses a hole at the base of the sample chamber, which can be selectively opened to allow the sample to pass through. The other option inverts the sample chamber, dropping ground sample into a funnel. When building the prototype, our group did not consider this design constraint as vital, and was not pursued further. The lab technician needs to manually place the sample in a test tube, taking them about one minute.

**Liquid Nitrogen Volume and Time Testing:**

To better understand the temperature limitations as well as amount of pressure building up in the sample chamber; a testing protocol was created to determine the
required volume of liquid nitrogen needed to completely freeze a tissue sample and time until sample thawing. Each mouse liver tissue sample, 1 cm³ in size, was placed in a mortar, and a specific volume of liquid nitrogen was poured over the sample. Liquid nitrogen volumes ranged from 6-20 mL. The time required for the liquid nitrogen to evaporate, as well as the first sign of sample thawing was recorded. Thawing was determined by a change in the color of the sample. It was observed that at least 15 mL of liquid nitrogen was required to completely freeze a sample and the tissue began to thaw after 5 minutes. Further testing of three repetitions per sample for 15 mL and 20 mL of liquid nitrogen were completed. Thawing times were averaged. Data and calculations can be found in Appendix D. A one-tailed t-test assuming independent samples was performed, resulting in a p-value of 0.09, meaning that for a 95% confidence level, the addition of 15 mL and 20 mL of liquid nitrogen was not statistically significantly changing thawing time. Therefore, the volume of liquid nitrogen used to freeze a sample can be in the range of 15-20 mL. These values were then used to do pressure calculations found in Appendix E.

Chamber Insulation Design Alternatives:

Based on the testing data, a tissue sample stored in the device for any extended amount of time longer than 5 minutes will begin to thaw. To ensure that the sample remains frozen during the entire grinding process, the device should have an additional system to keep the tissue frozen. To effectively keep the sample chamber cold, two insulation designs, an insulated chamber and an additional dry ice insulated chamber were developed.

Chamber Insulation Design #1: Insulated Chamber

In this simple design, the grinding sample chamber is surrounded by a thick layer of insulating material (Figure 15). When liquid nitrogen is placed in the sample chamber, the chamber is cooled, freezing the sample. Because of the insulation, the amount of time needed for the sample chamber to warm up is lengthened. Depending on the thickness and properties of the material chosen, the exact time it takes before the sample thaws can be manipulated.
Figure 15: Insulated Grinding Chamber. Insulation is wrapped around the grinding chamber to keep the chamber from warming up as fast.

Chamber Insulation Design #2: Additional Dry Ice Insulated Chamber

In this design, another chamber underneath the grinding chamber is included. This insulated container holds a dry ice and alcohol mixture which simulates an -80 °C freezer, significantly increasing the time the sample chamber is cold enough to keep the sample frozen.

Evaluation of Insulation Designs:

By simply surrounding the device with insulation, the increased time that the sample remains cold will not be significant enough. The dry ice and alcohol bath would drastically improve the time the sample remains frozen. The dry ice system is a more complex system than the insulation, but not unreasonable. Dry ice and alcohol are
common place in many medical and biochemical laboratories. Based on this, the dry ice and alcohol chamber was chosen for our final design.

Final Design: Cryogenic Tissue Pulverizer

For our final design we chose a pneumatic driven grinding mechanism with a sample chamber complete with a dry ice and alcohol insulated bath underneath (Figure 15). This semester, our group focused on building the electrical control to the solenoid and designing the grinding head, grinding chamber, and cooling chamber. Prototype development continued with funds from the University of Wisconsin Biomedical Engineering Department and Engineering EXPO. A detailed Ironcad drawing with dimensions is included in Appendix F.

Figure 15: Drawing of the Pneumatic Driven Final Design. A pneumatic cylinder will pound into a grinding chamber. The grinder chamber is surrounded by another chamber which will be filled with a dry ice and alcohol coolant mixture to ensure that the sample chamber remains cold.
Structural Components

Sample Chamber and Metal Insulating Chamber

Design development of the sample chamber over the past two semesters has focused on keeping the tissue sample cold during the grinding process while addressing other client requirements. Initial designs for the sample chamber were minor variations of the final design. The general design needs to have both a rounded bottom to mimic the shape of the mortar it is replacing and sides that prevent any sample from escaping from the chamber during grinding. The chamber itself needs to be removable to facilitate ease in cleaning between samples. While in use, the sample chamber needs to be securely attached to prevent its movement during grinding.

Constructed from stainless steel, the final design of the sample chamber features a thin, wide rim connected to a hollow cylinder and a pipe cap. The wide rim has two holes that allow the sample chamber to be placed over two screws of the metal insulating chamber and tightened in place with wing nuts. The height of the hollow cylinder (approximately three inches) adequately prevents the sample from exiting the chamber during grinding. The pipe cap is nearly hemispherical and forms the rounded bottom of the sample chamber.

The sample chamber fits inside the metal insulating chamber, and the wide rim rests on top of the insulating chamber. The space between the walls of the metal insulating chamber and the sample chamber provides room for a dry ice and alcohol mixture that further insulates the sample during the entire grinding process in addition to cooling from the liquid nitrogen (Figure 16). The metal insulating chamber is formed from a larger hollow cylinder and two thin rectangles that allow the chamber to be attached to the main structure of the device. Mounted on each thin rectangle is a screw to provide a point of attachment for the sample chamber. Each rectangle also has a lateral hole to allow for a second set of screw attachments to the L-brackets that support the insulating chamber. The bottom of the metal insulating chamber features a smaller hemispherical dome that mirrors the bottom of the sample chamber in order to allow the dry ice and alcohol mixture to fully surround and insulate the bottom of the sample chamber. AutoCAD drawings of the sample chamber can be found in Appendix G.
Figure 16: Ironcad Drawings of Sample and Cooling Chamber. A cooling chamber surrounds the inner grinding chamber. The cooling chamber contains a dry ice and alcohol bath.

**Grinding head**

The grinding head was machined from stainless steel and its shape was determined to most closely match the contour of the inside curvature of the pipe cap that forms bottom of the sample chamber. The radius of curvature for the grinding head was chosen to be the same as that of the pipe cap to maximize the contact surface area during grinding. To firmly attach the grinding head to the pneumatic cylinder, the grinding head features a threaded hole the same size as the screw at the end of the pneumatic cylinder. The grinding head can be easily cleaned due to the incorporation of a threaded cylinder attachment. AutoCAD drawing of the grinding head are in Appendix G.

**Stand**

The design of the stand for the device was implemented with three basic objectives: withstand linear force exerted by cylinder, hold cylinder upright, and incorporate simple component attachment. The stand must withstand the linear force of the cylinder. The design is rectangular, incorporating four components: top, bottom, and two sides. The top and bottom of the stand withstands three-point bending, while the two sides withstand tensile force. The amount of force exerted by the cylinder is determined by the amount of air pressure applied. The average expected lab air pressure produced by a building’s HVAC system is 100 psi (Fronczak, 2002). Estimating a bore diameter of one square inch, to simplify calculations, the stand is able to respond with a 100 pounds force.

The other two main concerns are easily addressed by specializing design for linear force. To hold the cylinder upright, the base was chosen as a plane. The plane provides enough material on all sides to prevent the cylinder from falling over. Simple attachment of components is achieved by plastic ties wrapped through holes in the side supports.
The local hardware store was the best place for materials. Considerations for part selection include cleaning efficacy, durability, and strength.

A steel rod acts as the top of the stand. The rod simplifies attachment of the cylinder mount provides adequate strength against the three-point bending. The pivot mount, a Bimba bracket kit, was chosen to minimize buckling. The permanent pivot mount is carefully aligned over the entrance to the grinding chamber to minimize buckling in the direction the pivot did not swing. Since a pivot mount reduced buckling in only one plane, a ball and socket joint would have been preferable.

A plane of wood serves as the stand base. It is relatively lightweight, easy to attach bolts to; however, wood is not easily cleaned, especially the combination of unfinished wood and animal tissues. For this reason, a piece of sheet metal was chosen to cover the plane of wood. The wood also holds the rest of the stand upright. The sides of the stand are two steel posts with holes down the side. They are strong enough to withstand force produced by the cylinder, easily cleaned, and allow easy attachment of other components. All components are easily bolted together to provide a secure structure for the cylinder, sample chamber, cooling chamber, solenoid, and upright regulator. AutoCAD drawings of the stand are in Appendix G.

**Stand Strength: Three-Point Bending Analysis**

The cylinder is mounted by a pivot joint at one end. This pivot joint is connected to rod and support bars. For this situation, three-point bending is a concern. The cylinder will deliver 100 pounds of force to the tissue sample and to the mounting hardware. The rod must withstand bending, and the support bars must withstand the tension applied. The equations for three-point bending are shown below (see Equations 1.1).

(Equation 1.1) \( \sigma = \frac{My}{I} \)

where \( M \) = moment; \( y \) = distance from neutral axis; \( I \) = inertia; and \( \sigma \) = maximum stress.

(Equation 1.2) \( \sigma = E\varepsilon \)

where \( \sigma \) = maximum stress; \( E \) = elastic modulus; and \( \varepsilon \) = strain.

(Equation 1.3) \( I = \left( \frac{\pi}{4} \right) * r^4 \)
where $I = \text{inertia}; \ r = \text{radius}.$

The maximum stress for a particular material is often known from previous stress-strain experiments involving graphs of equation 1.2. Using this maximum stress of the material and comparing the value to the maximum stress generated from bending (see Equation 1.3), we determine if the material is suitable or if the dimensions for the material are suitable. A new material may offer a stronger alternative; however, if more strength is needed from the same material, altering the dimensions of the material to increase the distance from the neutral axis will also increase maximum stress allowed prior to failure.

**Pneumatic Components of Final Design:**

*Pneumatic Cylinder:*

According to the Bimba cylinder catalog, a 1-1/16” bore air cylinder will provide a force of approximately 0.9 times the air line pressure. With lab pressure varying between 80 – 120 psi, the cylinder provides 72 – 108 lbs of force. This value will be stabilized with a regulator.

After the bore size is selected, there are many Bimba cylinders to choose from. Following the advice of Professor Frank Fronczak, we chose a single acting disposable cylinder (Figure 17). The spring operated return stroke allows the cylinder to retract without the use of the air supply. Disposable cylinders are inexpensive, light duty cylinders that cannot be serviced if seals deteriorate.

*Figure 17: Bimba Single Acting Pneumatic Cylinder.* Shown with screw-in brass fitting. Digital camera image of actual purchased 1-1/16” bore cylinder. (11” in length).
We chose a 4” stroke length for the cylinder. Only the last 1” of this stroke length will be used since the cylinder needs only to clear the maximum sample height. Also, with the appropriate valve cycling pattern, the cylinder will not fully retract, preventing sample loss, since the grinding head will remain deep within the grinding chamber edges.

A circular rod shape was chosen for flexibility in future modifications. The circular rod shape leaves open the possibility for the future design of a twisting motion in the rod to facilitate shear forces to grind the sample.

The additional option “N” (low temperature seals and lubrication), was added to our cylinder. At cold temperatures the cylinder’s life is shortened due to the changing consistency of lubricants, overstressed metal, and damaged and brittle seals (Korane, 2002). The low temperature seals extend the operating range of our cylinder to encompass -40° to 200°F for only $0.80.

We discarded the option of ordering an attached solenoid valve to reduce cost and allow more flexibility in our design. Our final cylinder order consists of a 094-PN cylinder special ordered, pivot brackets (mounting), and piston rod clevis (mounting) from Price Engineering, a Bimba Distributor.

Regulator/Filter:

To implement the pneumatic system and ensure proper maintenance of moving parts, a filter/regulator is incorporated (Figure 18). The air pressure from the HVAC system installed within a building is expected to be near 120 psi. The pressure will fluctuate due to the number of users on the HVAC system. A regulator is necessary to control these pressure fluctuations and prevent the pressure from exceeding the chosen level set by the user. The regulated pressure drives the cylinder in regular intervals. For our design, we selected a filter coupled with a regulator.

The other component of the combination, the filter, removes debris and liquid from the pressurized air. Unfiltered debris and liquid results in excess wear on the cylinder, limiting its lifetime. Vertical placement is essential for proper filter function.
Figure 18: The Filter/Regulator. This filters debris and liquid while regulating air flow into the pneumatic cylinder. Digital camera image of Parker regulator. (9” in height)

_Solenoid Valve:_

Cylinder control is established through controlling the air line. This is performed with a simple 3-way, 2-position inline valve (Figure 19). The “3 way” describes the 3 ports for the valve including: inlet, vent, and out (to cylinder). Only 2 ports are connected to the air line system, since the cylinder has a spring operated return. The third port is left open as a vent to release air on the cylinder's return stroke. The solenoid allows the design to be electrically controlled, turning electrical energy into mechanical movement of the valve. Energizing the solenoid causes the valve to open, allowing air flow to the cylinder and extending the rod to pound. Upon de-energizing, the valve opens to vent air to the environment, allowing the spring retraction of the cylinder’s rod to reset the stroke.

Figure 19: Solenoid Valve. The solenoid valve controls the cylinder motion during grinding. Digital camera image of purchased Humphrey solenoid valve. (3” in height).

On the advice of an engineer at Price Engineering, we selected the 31-E1-120-VAC solenoid valve. The 1/8” port allows attachment to our cylinder’s air tubing without size adaptors. The wires extending from the solenoid valve are connected to an electrical wall socket to provide the electrical source. A timing circuit will control this electrical source (see Electrical section).
Additional Accessories:

From Price Engineering we ordered 5 push-in fittings and 10 feet of air tubing to connect all of the pneumatic parts together. The fittings have male pipe threads that fit directly into the ports of the cylinder, solenoid valve, and regulator. The air tubing is pushed into the fitting and instantly locks into the fitting, providing a superior air seal. Teflon tape was used to seal the threading on the fittings to ensure minimal air loss.

Electronic Control of Final Design:

Main Timing Circuit

The main timing circuit is the original creator of the desired ON/OFF signal. Our circuit, based from designs by W.D. Phillips, is composed of the following components:

- 555 timer: 8 pin LM555
- 2 capacitors: 47 uF and 1 uF
- 2 resistors: 1 kΩ and 680 Ω
- potentiometer: 5 MΩ
- light-emitting diode (LED)
- voltage source 9 V

The circuit was built for initial testing on a protoboard (Figure 20). The potentiometer, a variable resistor, was added to allow the most variability. This ON time eventually corresponds to the time solenoid valve is open. Resistor and capacitor values were chosen to provide approximately a frequency of 1 Hz, translated to the pneumatic system to 1 pound/second.

The following equations (Phillips, 2003) show the controlling variables:

\[
\begin{align*}
  f &= \frac{1.44}{(R_1 + 2R_2) \cdot C} \\
  ONtime &= 0.69 \cdot (R_1 + R_2) \cdot C \\
  OFFtime &= 0.69 \cdot (R_2) \cdot C \\
  DutyCycle &= \frac{ONtime}{OFFtime}
\end{align*}
\]
In our particular circuit $R_1 = 1\, \text{k}\Omega$, $R_2 = \text{potentiometer}$, and $C = 1\, \text{uF}$. We adjusted the potentiometer to roughly obtain a frequency of 1 Hz by watching the LED flashing.

After satisfactory results were obtained from the circuit, we soldered the same components to a smaller protoboard to minimize size and loose wires. After several small kinks of open circuits and misalignment of the LED were resolved, our circuit remained stable (earlier it would stop functioning when shaken).

The timing circuit connects to an AC to DC converter labeled to provide 9V, freeing our device from batteries. Digital multimeter readings revealed the product to provide 14.3V. Before connecting our circuit to the AC to DC converter, the circuit was gradually tested with an increasing voltage from the lab power supply. When no problems resulted in testing up to 16 V, the timing circuits was powered directly by the AC to DC converter.

![Image](image.png)

**Figure 20: Main Timing Circuit.** The potentiometer is blue at the top of the circuit. The 555 timer is the black 8 pinned integrated circuit. The output voltage is taken across the LED, or through the black and green wires on the right side of the circuit. (1” in width).

**Logic Relay**

To connect the action of the main timing circuit to the solenoid’s power supply, a logic relay (Figure 21) was recommended and provided by Burke O’Neal, instrument innovator. The relay increased flexibility over simpler DPDT relays since it accepts input from a larger range of voltages. Therefore no voltage divider is necessary to cut down the output voltage of the main timing circuit to the exact voltage necessary for the DPDT relay. This relay is of the “normally closed” type, meaning that the electrical line is kept open-circuited until a signal is received at the input ends of the logic relay. The top terminals are connected, regardless of polarity, into the power source line to the solenoid.
The lower terminals must be connected to the output of the main timing circuit with careful regard to polarity.

The signal from the main timing circuit provides the stimulus to the logic relay, turning on an internal electromagnet. The magnet moves an armature, which acts as a switch, to close the circuit, allowing the current to the solenoid to pass. When the stimulus is no longer provided by the main timing circuit (OFF time of main timing circuit), the magnet turns off, the armature returns, and the switch is opened, ceasing current to the solenoid (HowStuffWorks, 2003).

Figure 21: Logic Relay: Digital camera image. Upper terminals connect to 120 V AC line. Lower terminals connect, with correct polarity, to output of the main timing circuit. (2” width).

Integration into Device

The main timing circuit and logic relay were soldered together to form one circuit connected to the 120 V AC power wires to the solenoid valve (Figure 22). Heat shrink wrap was attached to all bare connecting wires. A plastic Gladware™ container isolates the circuit for safety, stability, and organization of wires. The container prevents any spilled liquids from short circuiting the electronics or damaging components. It also isolates the user from the 120 V AC wires.

Figure 22: PSpice diagram of timing circuitry.
**Working Prototype:**

Work on the prototype began in the fall of 2002 with the development of pneumatic components. Design and machining of the grinding chamber, cooling chamber, and grinding head were done at the beginning of the spring semester of 2003. Insulation was then incorporated into the design by mid-semester. Electronic control was simultaneously being developed via other group members. A circuit was built and added to the prototype. The entire device was functional in March 2003 (Figure 23). Pictures of the prototype are included in Appendix H. A budget is included in Appendix I.

![Figure 23: Working Prototype.](approximately 22" in height, and 18" in width).

**Prototype Testing**

Testing of the prototype occurred at the end of the semester. We had four main concerns: grinding to desired particle size, appropriate processing time, minimal sample loss, and adequate cooling during entire process. Initially, just before we began testing, we noticed our client’s lab air pressure was only 30 psi, less than half of our anticipated
80 – 100 psi. This variable may have affected our results. Despite this altercation, we continued testing the device on mouse liver tissue.

Testing continued with the supplies (tissue, dry ice, alcohol, liquid nitrogen, safety gloves, safety glasses, and a scraper) provided by our client. As were limited to 2 mouse liver tissue samples, we decided to discard plans to compare grinding results between manual and automated grinding. There was also disagreement between our clients on whether a protein assay was appropriate for quantization of grinding. Dr. Jeff Ross suggested homogenizing the samples and then performing the protein assay to determine how much protein was released from the cells. Charles Tessier, the graduate student who works most often with the tissue grinding, opposed the test. He argued that the homogenization would equal out the grinding results and did not regard a protein assay as an effective judge of particle size. With only 2 samples, no statistical comparisons were possible since we would be unable to estimate standard deviations or means with only 1 sample per treatment. The group collectively decided that submitting the 2 tissue samples to automated grinding would be more worthwhile.

Any similar future testing of particle size would be most effectively determined with either a microscope or fine mesh in a freezer room or outside on a day below freezing to prevent sample melting. Previous testing revealed tissue to melt very quickly at room temperature, making such tests difficult.

After we began grinding, we noticed the time required for the whole process was much shorter than manual preparation. Judging by the particle size and rate of change from large to small particles per unit time, we concluded a grinding period of 1 minute was sufficient. A second sample was tested to check for consistent and repeatable grinding.

The particle sizes produced by our device had a greater range than manual preparation. The sizes of the particles ranged from one millimeter to approximately ten microns. Professional observation by Charles Tessier, who has processed many samples manually, rated the grinding efficiency as 80% acceptable. This rating shows room for improvement. To better grind the sample, a shaking plate may be implemented into the device to redistribute and center the sample directly underneath the grinding head. A
turning grinding head would contribute shearing forces to grind the remaining larger tissue pieces.

Complications during testing lead us to brainstorm a creative solution that should be implemented. The first complication we encountered was cooling the grinding head. When removed and placed in dry ice to cool, the grinding head’s threaded hole diameter decreases (the material contracts as it cools) making attachment to the piston rod difficult. However, when left attached, the grinding head was difficult to cool, leading to melting tissue problems (Figure 24).

![Figure 24: Difference between properly cooled grinding head and room temperature grinding head. The picture on the left shows the tissue quickly melting on the room temperature grinding head, while the picture on the left shows the powdery tissue remaining frozen on the grinding head.]

Testing our prototype affirmed its strengths and weaknesses. The main goal was to determine whether it worked properly. This goal was achieved, albeit room for improvement. More pictures from testing as well as a procedure for device use are included in Appendix J and K respectively.

**Ethical Concerns:**

The main ethical issue concerning this device is safety for the user. Before this device could be marketable, it would have to be thoroughly tested to ensure no harm will result to the user. The outside of the device must be a reasonable temperature, one that does not cause discomfort or frostbite burns. If the exterior is found to be too cold to touch, the manufacturer will need to require cryogenic gloves to be worn whenever the user might touch the device. It will have to be determined if wearing gloves is also necessary when collecting the ground sample. During our testing of the device, we found
touching of the exterior metal to not result in any user discomfort. Warning labels for the extremely cold temperatures inside the chamber may be required.

In addition to safety in handling the device, another concern is how often the user will need to handle liquid nitrogen directly during the entire process. The amount of handling or transfer of liquid nitrogen should be minimized in conjunction with this device in order to prevent possible burns from the cryogen. With the current prototype, the user must transfer liquid nitrogen to the device’s sample chamber for each sample to be processed.

While safety is the major ethical issue with this device, the cleaning and sterilization of the device is also a concern. The method chosen for disinfecting the sample chamber between successive samples processed must be reliable and efficient in order to prevent contamination of the next sample inserted into the device. The sample chamber is made of stainless steel, so it can be cleaned thoroughly with an autoclave. Also, this procedure should be well documented so any user would be able to follow the instructions and disinfect the sample grinding area between samples.

Another possible ethics issue with the device may occur if the device falls into the wrong hands. Certain individuals may want to sample tissues in order to design destructive carcinogens or proliferate disease. This is not the scope of the tissue sampler demographic. We encourage the use of the tissue preparation device to provide a resource for researchers trying to eliminate cancer. Any use of the device for destructive means is discouraged.

Our project does not directly involve animal care, although we do use mouse tissue samples provided by our client. If our client’s research advances to human subjects, different protocol will need to be followed. Although we are not personally in charge of ethical animal care, as members of a design team, we must still assure proper treatment and protocol is followed in sacrificing the mice. Verifying that our client has consulted the Research Animal Resources Center (RARC) is an important part of ethical testing, since tissue is collected from the mice liver. Since mice tissue could be a biohazard, we carefully consulted with our client before safely labeling and disposing of the ground mouse tissue.
Regulation Concerns:

The Center for Biologics Evaluation and Research (CBER) regulates medical devices that collect, process, test, and manufacture blood, blood components, and cellular products. The CBER has developed The Device Action Plan of 1997 and implemented this plan on April 26, 1999. This plan helps to ensure that the policies of the CBER are consistent with those of the Food and Drug Administration’s (FDA) Center for Devices and Radiological Health (CDRH), the Office of Regulatory Affairs (ORA) and the Office of Chief Council (OCC). The CBER website has many links to the FDA (CBER, 2002).

Currently the FDA has three classes of regulation for medical devices. Information our group found relevant is included in Appendix L. Class III are the most regulated and are typically devices, which either support or sustain human life or have an unreasonable high risk of illness or injury associated with them. These devices all require pre-market approval. Class II devices are subject to certain FDA standards. Class II devices are subject to only general controls (FDA, 2002).

New devices that cannot be compared to an equivalent existing device of either Class I or II will be classified as Class III. This classification will remain until a Premarket Approval (PMA), Product Development Protocol (PDP), or a petition to the FDA to reclassify the device as Class I or II is submitted. This means that we will need to initially submit a PMA on our device and wait to see if the FDA determines “substantial equivalence” with another preexisting device. If they categorize our device as Class I or II, then we do not need to apply for approval. If they categorize our device as a Class III (“new” device) we will need to either petition the FDA to reclassify it, or wait for further classification (FDA, 2002).

Our device seems to fit between two categories: “Clinical Chemistry and Clinical Toxicology-General purpose laboratory equipment labeled or promoted for a specific medical use” and “Hematology and Pathology Devices – Tissue processing equipment.” The chemistry categorization is Class I, and the tissue-processing category is exempt from PMA forms (FDA, 2002). This suggests we will not have difficulty obtaining FDA approval for our device.

Future Concerns and Direction of the Project:
As all four team members are graduating May 2003, our work on the device will cease. Testing revealed several key developments that will greatly improve our device. Due to time constraints we are not able to add them. Any future student group interested in prototype development should consider the following concerns and suggestions for future development.

Grinding:

After testing our system at 30 psi, our sample was rated 80% acceptable. Several variables can be changed to improve grinding to a higher acceptance rate. Running the device with air pressure at 80 psi will more than double the force applied to the sample. If higher air pressure is not available, several alternatives exist. A larger cylinder bore size will increase force applied to sample. A modification to rotate the grinding head will add shearing forces to the grinding, proven by earlier testing to create finer granules. Vibrations added to the sample chamber will help distribute the larger tissue pieces for better grinding.

Cooling:

As mentioned in testing, the current procedure for cooling the grinding head is insufficient. To remedy this problem, a switch bypass should be added to the timer circuit to enable the user to flip a switch to submerge the grinding head into the sample chamber for a prolonged period. The switch directly connects the solenoid to the AC power line, avoiding the timing pattern and instead providing constant current. This current holds the solenoid valve open, allowing constant airflow to the pneumatic cylinder. With the pneumatic cylinder piston fully extended, the grinding head is deep within the sample chamber, ideal for immersion of the grinding head in liquid nitrogen. This method effectively cools the head without complications associated with reattaching a pre-cooled grinding head.

Closer fitting polystyrene will improve insulation. While we were limited to removable insulation for ease of cleaning the device, future development should include finding a tighter fitting insulation. Other possibilities include quick drying liquid
polystyrene that can be painted on. If this option is pursued, the design must ensure that the metal can be autoclaved for proper sanitation.

Additionally a simple circuit with a thermocouple, comparator and sound alarm should be installed to alert the lab technician if the device temperature rises above freezing. The thermocouple could be either submerged in the dry ice bath or integrated into the metal. This addition is an important and inexpensive safety measure.

**Ergonomics:**

Several minor modifications should be made to the grinding and cooling chambers to simplify assembly and cleaning. The lateral holes for the wing-nuts are placed extremely close to the attachment creating too tight of a sample chamber fit. This attachment site needs to be moved out laterally. A similar wing-nut attachment site should be adapted to the cooling chamber as presently a wrench is required for loosening the chamber. The current design does not allow easy emptying of the dry ice and alcohol bath. Wing-nuts at the site allow removal and assembly to occur without tools.

**Conclusions:**

After 3 semesters of work we have achieved an 80% grinding efficiency under limited conditions of 30 psi. Although the automation is not currently as effective as manual grinding, the process cuts grinding time remarkably to 1 minute. At the current stage, our device at least provides the initial preparation which will lead to proper consistency with a small additional amount of manual work. Our device provides a much more stable grinder than the fragile ceramic mortar and pestle of our client.

Pneumatic power and baths of liquid nitrogen and dry ice/alcohol do appear to be effective design choices. All recommended changes to the design are merely additions and not changes to the basic principles we have devised. The stand and machined chambers remain solid through the pounds of the cylinder and harsh cryogenic conditions. Our circuit is reliable, isolated, and safe. Provided with the procedure for operation, our client should be readily able to grind and freeze his tissue samples in our device.
References and Resources


Burgess, A. Biocore Lab Instructor, Personal Communication 2/21/02.


www.bimba.com/techctr/tb052199b.htm


MacNeil, K. “Risk Assessment: Handling, Transportation and storage of liquid nitrogen and other cryogenic material.” Bristol University, School of Chemistry. 8 Apr 2002.  
http://www.chm.bris.ac.uk/safety/lnitcry.htm


Minnesota Valley Engineering (MVE) Cyrogenics. Form 2195: Liquid Discharging Device Installing and Operating Instructions. Received April 30, 2002.


http://www.harcourt.com/dictionary/def/2/6/1/0/2610600.html

“Periodic Table of Elements: Nitrogen.” Environmental, Chemistry and Hazardous Materials Information and Resources. 8 Apr 2002.  
http://environmentalchemistry.com/yogi/periodic/N.html

"Physics Van Outreach Program." University of Illinois Physics Department. 2 Feb 2002. 


University of Illinois Physics Department. Physics Van Outreach Program. 2 Feb 2002.  

**Acknowledgements**

Dr. Jeff Ross – client, testing advice, testing supplies  
Charles Tessier – client, testing advice, testing supplies  
Paul Thompson – general advising  
Burke O’Neal – logic relay assistance  
Bill Hagquist – machine shop and AutoCAD expertise  
Prof. Frank Fronczak – pneumatic expertise
Appendix A - PDS
Tissue Sample Preparation Device for Biochemical Analysis
Version 7 – May 5, 2003

Team Members: Sara Alford
Christine Koranda
Carla Maas
Ryan Roth

Client: Jeff Ross and Charles Tessier
UW – Medical School
Department of Oncology

Advisor: Paul Thompson

Function: The device will grind a frozen tissue sample into a fine powder, replacing the manual mortar and pestle grinding technique currently used. It will also include a cooling method using liquid nitrogen keep the tissue frozen. The sample should be easily collected after grinding.

Client Requirements:
- Sample and tools used must be kept cold at all times during the process
- Must salvage as much of tissue sample as possible
- Processing time should not exceed that for manual preparation (approximately – 15 minutes)
- Device to be used 30 – 40 sample/day
- Grind tissue to the consistency of powdered sugar (10µm diameter granules)
- No cross sample contamination

Design requirement:

1. Physical and Operational Characteristics
   a. Performance requirements: Tissue should be easily inserted into the device. After grinding completion, tissue sample should be removed and grinding area cleaned before the next sample. Device will be in operation 3 – 5 hours per day. On average the device may have to be turned on 1 -3 times per day. It should be able to grind a soft tissue sample of a maximum sample size of 8 cm³ (2 x 2 x 2 cm). The tissue should remain frozen completely through the grinding experiment.
   b. Safety: Liquid nitrogen should be contained to avoid possible contact with skin and clothing. Instrument should be insulated to prevent skin burns. Cryogenic gloves and safety glasses may be required for the use of the device. A means to vent pressure should be included to avoid build up and possible explosion.
c. **Accuracy and Reliability:** Technique should grind sample to a powdered sugar (10 µm diameter granules) consistency. The device should provide an optimal means of sample collection.

d. **Life in Service:** The device should last 5-10 years with proper handling and maintenance. Around 30-40 samples could be analyzed daily.

e. **Operating Environment:** Device would function in a normal room temperature (approximately 20°C) biochemistry laboratory. An alternative to laboratory bench storage may be storing the entire device or certain components in a -20°C or -80°C freezer. The interior will be exposed to extremely cold (-196°C) temperatures. The exterior may be exposed to freezer condition (-20° or -80°C). The device will be handled by a laboratory technician.

f. **Ergonomics:** The sample should be easily inserted. The user’s hand should not be subjected to a cold temperature during sample insertion. There should be a method for easy refill of the desired coolant (dry ice and alcohol).

g. **Size:** The device should fit on a laboratory bench with a maximum volume of 0.9x0.61x0.61 m (3x2x2 ft).

h. **Materials:** Only materials that can withstand cold temperatures can be used (fcc metals such as Nickel, Aluminum, Stainless steel 18-8, Copper). Regular glass and plastics should not be used.

i. **Cleaning:** Any components of the device that come in contact with the sample should be removable for ease in cleaning and disinfecting (soap and water).

2. **Production Characteristics:**

   a. **Quantity:** One prototype will be constructed.

   b. **Target Product Cost:** If a pathology lab were to purchase the device, a reasonable range would be $5000-20,000. For our client’s biochemistry laboratory, a reasonable cost for the prototype is $1,000 – 1,500.

3. **Miscellaneous**

   a. **Standards and Specifications:** Premarket approval by the FDA will be required.

   b. **Competition:**

      i. Polytron system – a homogenizer that operates at room temperature to grind up sample.

      ii. Biospec BeadBeater – uses glass or stainless-steel balls to break apart sample, operates at room temperature.

      iii. Biospec Cryogenic Pulverizer – uses hammer to crush pre-frozen sample in mortar.

      iv. Jet Pulverizer – use air pressure to pulverize sample.
Figure 1B: From upper left clockwise. (1) Pouring liquid nitrogen, (2) grinding with funnel, (3) Beads, (4) Tight fitting container, (5) Ground tissue in mortar, (6) Shearing force with spatula.
Appendix C – Pneumatic Valves

The following is information found on valves used in pneumatic systems and cylinders.

Distribution valve

Example: plate valve that changes positions (“upper” and “bottom” seats) based on pressure.

How it works: When valve is in the “bottom” seat, air is delivered underneath the piston which is in the down position. This air forces the piston upward. Near the end of the piston’s stroke, air is compressed in the upper chamber by the decreasing volume of this chamber. This results in the valve switching to the “upper” seat. Air in the bottom chamber is now allowed to exit via an exhaust valve. Air from the compressor is now routed to the top of the cylinder since the valve is in the “upper” seat. This forces the piston down. Air is exhausted from the bottom chamber and the valve switches back to its “bottom” seat. The cycle then repeats. Depending on the design of the valve, the cycle frequency varies between 1000 –1500 rev/min (Morden, 1966).

Directional control valve

Example: 3-way valve

![Diagram](image)

Figure C1: A 3-way valve is the simplest valve for cylinder control.

How it works: Connects the cylinder to alternately the compressor or the exhaust without allowing the compressed air source to be released into the atmosphere. Control valves are also common with more ports. As the number of ports increases so does the switching capacity of the valve. 4-way valves can be substituted for 2 3-way valves in double-acting cylinders. 4-way valves have the disadvantage of needing a pipe to at least one chamber; this pipe can affect the speed of the response (Morden, 1966).

Example: poppet valve
**Description:** Seated valves use metal to metal seats or metal to elastomer seats for a better seal. Poppet valves provide low resistance when fully opened and a good seal when fully closed. These valves use a plunger to open/close the valve. Poppet valves are a more complicated design of control valves (Morden, 1966).

**Example:** slide valve

![Diagram of a slide valve](image)

**Description:** Slide valves are limited to control valves of 4 or less ports. The slider connects the desired side of the cylinder to the exhaust, while the other valve is connected to the air supply (Morden, 1966).

**Example:** Quick exhaust valve

**Description:** Allows air in rod end of cylinder to escape more quickly thus decreasing resistance to the stroke. A moving diaphragm seals the exhaust port when pressure is applied (when the chamber is filling). This diaphragm opens the exhaust port when the air flow changes (Morden, 1966).
Appendix D – Liquid Nitrogen Testing Analysis of Results:

<table>
<thead>
<tr>
<th>Sample</th>
<th>LN$_2$ Volume (ml)</th>
<th>Time for LN$_2$ Evaporation (min)</th>
<th>Time to Thaw (min)</th>
<th>Comments about Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>center not frozen</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>1.58</td>
<td>2.67</td>
<td>froze to mortar, center not frozen well</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>1.08</td>
<td>4.83</td>
<td>completely frozen</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>1.33</td>
<td>5.50</td>
<td>completely frozen</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>0.58</td>
<td>6.00</td>
<td>completely frozen</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>1.12</td>
<td>5.67</td>
<td>completely frozen</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0.67</td>
<td>6.33</td>
<td>completely frozen</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0.70</td>
<td>6.25</td>
<td>completely frozen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LN$_2$ Volume (ml)</th>
<th>Average Evaporation Time (min)</th>
<th>Average Evaporation Time (sec)</th>
<th>Average Thaw Time (min)</th>
<th>Average Thaw Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1.0</td>
<td>60</td>
<td>5.4</td>
<td>330</td>
</tr>
<tr>
<td>20</td>
<td>0.83</td>
<td>50</td>
<td>6.1</td>
<td>360</td>
</tr>
<tr>
<td>Difference</td>
<td>0.6</td>
<td>10</td>
<td>0.7</td>
<td>30</td>
</tr>
</tbody>
</table>

T-test for 15ml and 20 ml LN$_2$ volume

<table>
<thead>
<tr>
<th></th>
<th>15 ml Volume</th>
<th>20 ml Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.44</td>
<td>6.08</td>
</tr>
<tr>
<td>Variance</td>
<td>0.34</td>
<td>0.13</td>
</tr>
<tr>
<td>Observations</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pooled Variance</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Hypothesized Mean Difference</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>df</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>t Stat</td>
<td>-1.61</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td><strong>0.09</strong></td>
<td></td>
</tr>
<tr>
<td>t Critical one-tail</td>
<td>2.13</td>
<td></td>
</tr>
<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>t Critical two-tail</td>
<td>2.78</td>
<td></td>
</tr>
</tbody>
</table>

**Since the p-value (0.09) is greater than 0.05 (for a 95% confidence level), the difference in the thawing time, for the addition of 15 mL and 20 mL of liquid nitrogen is not statistically significant. Therefore, the volume of liquid nitrogen added can be in the range of 15-20 mL.**
Appendix E – Pressure Calculations

Liquid Nitrogen Properties and Pressure Calculations In Sample Chamber

<table>
<thead>
<tr>
<th>Atomic Mass N (g/mol)</th>
<th>14.00674</th>
<th>Atomic Mass N₂ (g/mol)</th>
<th>28.0134</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density of LN₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/cm³</td>
<td>0.807</td>
<td>m³</td>
<td>0.7</td>
</tr>
<tr>
<td>kg/m³</td>
<td>804</td>
<td>L</td>
<td>700</td>
</tr>
<tr>
<td>Heat of Fusion of N₂</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J/g</td>
<td>2.57E+0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J/mol</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J/kg</td>
<td>1.99E+0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J/mol</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J/mol</td>
<td>2.79E+0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas Constant R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J/(mol*K)</td>
<td>8.3143</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN₂ Melting Point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>°F</td>
<td>-345.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>°C</td>
<td>-209.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>63.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN₂ Boiling Point</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>°C</td>
<td>-195.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>77.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific Heat of Nitrogen (at 77.35 K, liquid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J/(kg K)</td>
<td>2042</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific Heat of Nitrogen (at 300 K, gas)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J/(kg K)</td>
<td>1042</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volume LN₂ to freeze sample once</th>
<th>Corresponding Vapor N₂ gas evolved LN₂</th>
<th>Mass N₂</th>
<th>n Moles N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02 L</td>
<td>0.014 m³</td>
<td>0.01614 kg</td>
<td>0.5762</td>
</tr>
<tr>
<td>20 mL</td>
<td>14 L</td>
<td>16.14 g</td>
<td></td>
</tr>
</tbody>
</table>

°C

\[
\text{assume } T = -78.5 = 194.65 \text{ K (dry ice temperature)}
\]

let V=volume of space in the sample chamber

actual volume of chamber will be V+1 cm³ (vol. of sample)

\[
P = \frac{F}{A} \quad PV = nRT
\]

\[
nRT = (0.57615 \text{ mol}) \times (8.3143) \quad J/(\text{mol*K}) \times (194.65 \text{ K})
\]

\[
nRT = 932.4307051 \quad J = N \times m = \text{kg} \times \text{m}^2/\text{s}^2
\]

Assume chamber shape is hemisphere and cylinder

\[
\text{Volume chamber} = (\pi) \times (\text{radius})^2 \times \text{cylinder height} + (0.5)(4/3)(\pi)(\text{radius})^3
\]

\[
\pi = 3.14159 \quad 0.000001 \quad \text{m}^3 = 1 \quad \text{cm}^3
\]
The above graph shows how pressure inside a sealed sample chamber will increase as temperature increases. The temperature inside the sample chamber begins at -196°C, the temperature of the liquid nitrogen added to the chamber. It is assumed that a dry ice bath surrounds the sample chamber. The high values of pressure that result, even when the chamber is at the temperature of dry ice, indicate the need for a vent hole to atmospheric pressure so that the pressure build-up does not cause structural damage to the stainless steel sample chamber.
Appendix F: Ironcad Drawing of Final Design with Dimensions
Appendix G – AutoCAD Drawings of Grinding Head, Sample Chamber, Cooling Chamber and Stand.
Appendix H – Prototype Pictures

Outer Cooling Chamber Top View:

Outer Cooling Chamber Side View Attached to Frame:
Bimba Cylinder Attached by Pivot Mount to Frame:

Grinding Chamber Side View:
## Appendix I – Budget for Semester

<table>
<thead>
<tr>
<th>Part</th>
<th>Quantity</th>
<th>Price</th>
<th>Purchaser</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pneumatic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bimba cylinder – 094-PN</td>
<td>1</td>
<td>$25.85</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Bimba pivot brackets – D-167</td>
<td>1</td>
<td>$2.70</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Bimba grinding head mount - D-166-1</td>
<td>1</td>
<td>$3.10</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Parker regulator/filter - 05E-02A18AA</td>
<td>1</td>
<td>$35.49</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Humphrey solenoid valve - 31-E1-120VAC</td>
<td>1</td>
<td>$45.40</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Fittings (1/8&quot;) - KQ2-H07-345</td>
<td>5</td>
<td>$7.50</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Tubing (10ft section) - AP-44-N</td>
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<td>$1.20</td>
<td>BME dept.</td>
</tr>
<tr>
<td><strong>Electrical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall outlet plug</td>
<td>1</td>
<td>$3.30</td>
<td>Carla</td>
</tr>
<tr>
<td>Plug-in flasher</td>
<td>1</td>
<td>$2.63</td>
<td>Carla</td>
</tr>
<tr>
<td>Electrical tape</td>
<td>1</td>
<td>$1.33</td>
<td>Carla</td>
</tr>
<tr>
<td>Switch pushbutton</td>
<td>1</td>
<td>$3.04</td>
<td>Carla</td>
</tr>
<tr>
<td>Gladware Container for Circuit Protection</td>
<td>1</td>
<td>$0.55</td>
<td>Carla</td>
</tr>
<tr>
<td><strong>Sample Chamber</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphere (unthreaded stainless steel butt-welded fitting cap)</td>
<td>1</td>
<td>$20.00</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Chamber pipe (stainless steel, 1.5&quot; diameter)</td>
<td>1</td>
<td>$10.00</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Chamber labor (UW-Machine shop)</td>
<td>--</td>
<td>$50.00</td>
<td>BME dept.</td>
</tr>
<tr>
<td>Cooling Chamber labor (UW-Machine shop)</td>
<td>1</td>
<td>Pending</td>
<td>EXPO</td>
</tr>
<tr>
<td><strong>Grinding head</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grinding head (nickel-plated cabinet knob, 1.25&quot; diameter)</td>
<td>1</td>
<td>$2.31</td>
<td>Christine</td>
</tr>
<tr>
<td>Grinding head (UW-Machine shop)</td>
<td>1</td>
<td>Pending</td>
<td>EXPO</td>
</tr>
<tr>
<td><strong>Support Stand</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheet metal</td>
<td>1</td>
<td>$5.65</td>
<td>Ryan</td>
</tr>
<tr>
<td>Wood for base (2&quot;x12&quot;x8&quot;)</td>
<td>1</td>
<td>$8.23</td>
<td>Ryan</td>
</tr>
<tr>
<td>Post for support beams</td>
<td>1</td>
<td>$9.00</td>
<td>Ryan</td>
</tr>
<tr>
<td>Threaded rod for cross-beam (5/8&quot;)</td>
<td>1</td>
<td>$2.29</td>
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<tr>
<td>U-bolts</td>
<td>2</td>
<td>$1.58</td>
<td>Ryan</td>
</tr>
<tr>
<td>Washers, nuts, and bolts</td>
<td>43</td>
<td>$6.23</td>
<td>Ryan</td>
</tr>
<tr>
<td>L brackets</td>
<td>2</td>
<td>$3.74</td>
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</tr>
<tr>
<td>Drill bit</td>
<td>1</td>
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</tr>
<tr>
<td>Tax</td>
<td>--</td>
<td>$3.23</td>
<td>Ryan</td>
</tr>
<tr>
<td>Washers, nuts, and bolts</td>
<td></td>
<td>$2.00</td>
<td>Carla</td>
</tr>
<tr>
<td><strong>Cooling Chamber</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cauk gun</td>
<td>1</td>
<td>$2.12</td>
<td>Sara</td>
</tr>
<tr>
<td>Foam board</td>
<td>1</td>
<td>$4.78</td>
<td>Sara</td>
</tr>
<tr>
<td>Foam board cauk</td>
<td>1</td>
<td>$1.98</td>
<td>Sara</td>
</tr>
<tr>
<td><strong>Miscellaneous Parts</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Item</td>
<td>Quantity</td>
<td>Price</td>
<td>Supplier</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>cabinet handles</td>
<td>2</td>
<td>$2.78</td>
<td>Sara</td>
</tr>
<tr>
<td>soup ladles</td>
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<tr>
<td>Pneumatic Total</td>
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<tr>
<td>Electrical Parts</td>
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<td>Sample Chamber Total</td>
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<tr>
<td>Support Stand Total</td>
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<td>Grinding Head Total</td>
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<tr>
<td>Cooling Chamber Total</td>
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<tr>
<td>Miscellaneous Total</td>
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<td>combo</td>
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<tr>
<td>Machining Pending</td>
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<td>EXPO</td>
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<td>Total</td>
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<tr>
<td>BME dept paid</td>
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<tr>
<td>team members paid</td>
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<td>EXPO Pending</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td>$297.04</td>
<td></td>
</tr>
</tbody>
</table>
Appendix J – Testing Prototype Pictures

Figure J1: Dry Ice and Alcohol Bath.

Figure J2: Sample after 30 impulses.
Figure J3: Cooling Grinding Head in Dry Ice.

Figure J4: Grinding Tissue Sample. Lab PSI was only 30 psi as indicated by the regulator.
Appendix K - Procedure for Use

The following includes a step-by-step procedure for using the Cryopneumatic Tissue Pulverizer.

Setup:

1. Affirm metal grinding chamber and head have been autoclaved
2. Immerse grinding head in liquid nitrogen*
3. Remove grinding chamber
4. Add dry ice and alcohol bath, stacking dry ice pellets around edge
5. Place grinding chamber in cooling chamber and tighten wing-nuts to secure grinding chamber
6. Pour approximately 5 mL liquid nitrogen in grinding chamber
7. Insert tissue sample
8. Allow sample to cool (approximately 30 seconds)
9. Align grinding head over grinding chamber entrance
10. Plug in both electrical cords
11. Attach air hose to air supply securely with ring clamp
12. Turn switch ON
13. Allow pulverization for approximately 1 minute
14. Turn switch OFF
15. Remove wing-nuts to remove grinding chamber and empty sample into vial.
16. Autoclave grinding components for next use

*Step 2 will vary depending on whether a circuit by-pass is added to allow the grinding head to be submerged in the liquid nitrogen while still attached.
Appendix L – FDA
(http://www.fda.gov/cdrh/devadvice/312.html)

A device is an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part, or accessory which is:

- Recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them.
- Intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals.
- Intended to affect the structure or any function of the body of man or other animals, and which does not achieve any of its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes.

Fact Sheet
(http://www.fda.gov/cdrh/devadvice/3131.html)

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TITLE 21--FOOD AND DRUGS
CHAPTER I--FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEALTH AND HUMAN SERVICES--(Continued)
PART 862--CLINICAL CHEMISTRY AND CLINICAL TOXICOLOGY DEVICES—

Subpart C--Clinical Laboratory Instruments
Sec. 862.2050: General purpose laboratory equipment labeled or promoted for a specific medical use.

(a) Identification. General purpose laboratory equipment labeled or promoted for a specific medical use is a device that is intended to prepare or examine specimens from the human body and that is labeled or promoted for a specific medical use.

(b) Classification. Class I. The device identified in paragraph (a) of this section is exempt from the premarket notification procedures in subpart E of part 807 and is exempt from the current good manufacturing practice regulations in part 820, with the exception of Sec. 820.180, with respect to general requirements concerning records, and Sec. 820.198, with respect to complaint files.