

# **Design of a Tissue Sample Grinder for Preparation in Biochemical Analysis**

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**Abstract:** The tissue sample grinder processes surgically-removed tissue by freezing and grinding the sample into a powder (10  $\mu\text{m}$  diameter particles). Our design primarily focuses on force production and the grinding system components. Testing of the grinding mechanism demonstrated that the most effective process delivers approximately 80 pounds of force to the tissue sample with a hemispherical pulverizing head. To drive the motion, two solutions were considered: a motor-driven design and a pneumatic design. Between these two alternatives, the pneumatic design best fulfills the project requirements because of efficiency and cost effectiveness. The shape of the stainless steel grinding chamber effectively contains the sample and matches the curvature of the grinding head. In addition to grinding, the sample must be frozen and kept cold with liquid nitrogen. Our design features an insulated grinding chamber to prolong cooling effectiveness after the initial addition of liquid nitrogen. The polystyrene foam insulation holds a dry ice and alcohol bath to keep the sample frozen during grinding. A prototype is currently under construction. Future work will focus on liquid nitrogen delivery and prototype testing.

**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. Sample should be easily collected.

## 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 may be most effective for the patient. A more accurate and informative analysis of the tissue is often desired, and therefore, a tissue sample may be sent to a molecular biology laboratory for profiling of DNA, RNA, and protein. This information about the patient's tissue sample 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, which can then be analyzed. 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, thus 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, which could be sent to a laboratory for testing.

### **Design Requirements and Constraints**

The final design of this device must fulfill several requirements, as given by the client (Appendix A). The device should freeze a tissue sample less than a gram in mass (most likely with liquid nitrogen) and subsequently grind the sample to a powder the consistency of powdered sugar (10  $\mu\text{m}$  diameter). After analysis, the sample should be able to be collected efficiently (at least 38 mg of the sample) in a vial or other container that can be removed from the device for future biochemical analysis. Post-processing, the device should be easily cleaned to avoid cross sample contamination. This device should be able to prepare up to 40 tissue samples per day. It is preferred that the processing time for a single sample be comparable to or less than the time necessary for the current manual process (15 minutes). When not in use, the device itself could be kept cold in order to facilitate the freezing process when it is in use.

The constraints for the design of this device are relatively few. All materials that come in contact with the sample must be able to withstand the cold temperature of liquid nitrogen ( $-196^{\circ}\text{C}$ ). The device should fit on a laboratory bench. Also, the device should have removable components to aid in cleaning between sample processing. Our prototype is limited by the funds of the biomedical engineering department. If this product were manufactured for use in a pathology lab or clinical environment, the cost for them would be greater, selling for around \$20,000.

## Existing Grinding Products on the Market

### *BeadBeaterä*

Biospec Products, Inc. currently produces the BeadBeater™ (Figure 1), a bead milling homogenizer. In the past, this device has been used to disrupt small cells like yeast. This disruption occurs when the machine stirs and agitates the 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 tissue is not frozen and the device has no cooling unit. For grinding, the volume of beads should be greater or equal to the volume of the tissue sample. The beads are available in glass, stainless steel, zirconia, chrome steel, and tungsten. Tougher materials require beads with a higher density (Meyer, 2002).

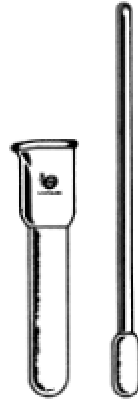


**Figure 1: The BeadBeaterä .** The top unit spins the metal or glass beads to create collisions with the tissue particles. The collision force breaks apart the tissue. Beads must be cleaned after each use (Meyer, 2002). Image from <<http://www.biospec.com>>.

### *Pestle and tube homogenizer*

Pestle and tube homogenizers (Figure 2) consist of a Teflon pestle that precisely fits into 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

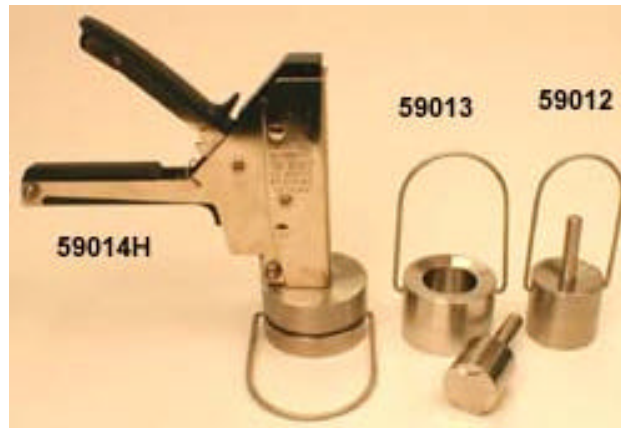
pushed downward and the sample can only pass the spinning pestle if it is smaller than the clearance between the tube and pestle (Seetharam and Sharma, 1991). The tube is typically placed in a beaker of ice to keep the tissue cold. The main problem with homogenizers is that the tissue must be prepared ahead of time by grinding or shearing with a scissors (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>.

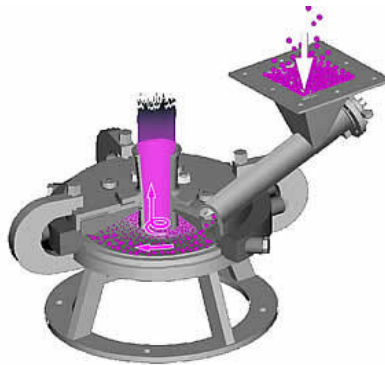
### *Jet Pulverizer*

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. The device can operate to precise specifications with little or no contamination or additional heat (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. The 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 just a few grams of a compound in the 1-in. jet mill,

the smallest of its kind. These mills are small, easy to disassemble and easily cleaned. Disassembly requires no hand tools (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>

## Literature Research

### *Cryogenic System Properties*

A cryogenic storage device must be designed to withstand forces resulting from internal pressure, the weight of contents, and bending stresses. 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 must be constructed of a material compatible with the cryogenic fluid, making material compatibility a major factor in designing a system. The container must be designed to withstand the forces resulting from the internal pressure, weight of the contents, and bending stresses. A vapor vent line is used as a source of venting and preventing over pressurization (Flynn, 1997).

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

temperature, and its mechanical properties such as ductility and brittleness. Lastly, the compatibility of materials with the cryogenic fluid being used must also be considered.

If a material exhibits low-temperature embrittlement, the material should not be used in a cryogenic system. 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 will become brittle, breaking without any more deformation, or it will become ductile, becoming permanently deformed. Both results will lead to a system failure and inadequate performance. A material's brittleness is related to its ability to dislocate under stress, which is related to its structure. Metals, specifically the face-centered cubic (fcc) metals and their alloys, are most often used in cryogenic equipment. These 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, the 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.

<b>Property</b>	<b>Value</b>
*Molecular weight of N <sub>2</sub>	28.01 g/mol
§ Boiling Point (at 1 atm)	-196°C
§ Density	8.07x10 <sup>5</sup> g/m <sup>3</sup>
*Heat of Vaporization	199 kJ/kg
§ Nitrogen gas evolved per volume liquid nitrogen	0.7 m <sup>3</sup> vapor per 1 L (0.001 m <sup>3</sup> ) liquid nitrogen

**Table 1: Properties of Liquid Nitrogen (N<sub>2</sub>).** Information from the following sources \*("Periodic Table", 2002), § (MacNeil, 2002).

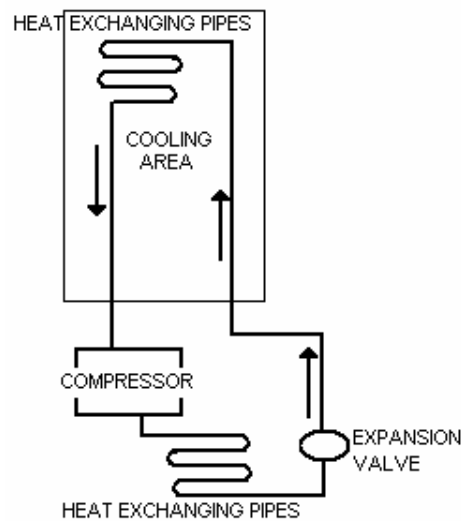
In general, the container that holds liquid nitrogen must be a good insulator. A dewar is a specially made container designed to hold substances as cold as liquid nitrogen; unfortunately, most dewars are expensive. A thermos is an alternative container for liquid nitrogen; however, the liquid nitrogen 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 a high pressure to build up inside the thermos. Drilling a hole in the thermos can allow the nitrogen vapor to escape; otherwise, it may explode. Care must also be taken when handling liquid nitrogen to prevent serious frostbite burns that may result from direct contact with liquid nitrogen or contact with a surface that was cooled by liquid nitrogen. 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). Safety glasses and waterproof welder's gloves should be worn (K3PGP Experimenter's Corner, 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).

### *Refrigerators*

As seen in Figure 5, refrigerators have five basic components including a compressor, internal heat exchanging pipes, an expansion valve, external heat exchanging pipes, and refrigerant. The refrigerant cycles throughout the entire system absorbing or dissipating heat. The cycle begins with the compressor raising the pressure and temperature of the refrigerant. The refrigerant travels to the external heat exchanging coils, and the excess heat is dissipated.

The refrigerant moves through the expansion valve and evaporates after entering a low- pressure area. Absorbing heat while in the internal heat exchanging pipes, the refrigerant cools the internal unit (where food or laboratory samples are typically stored). The hot refrigerant then enters the condenser and the cycle repeats (Brain, 2002). Laboratory quality refrigeration and freezer units are expensive. A 0.12 m<sup>3</sup> (3.8 ft<sup>3</sup>), -30° C Biopac laboratory freezer costs \$1,160.00 (BioCold Scientific, 2002).



**Figure 5: A schematic of a refrigeration system.** The arrows indicate the direction of the refrigerant flow. The refrigerant is recycled in a continuous loop either absorbing heat from the cooling area or dissipating heat in the external heat exchanging pipes.

#### *Pneumatic Systems:*

Pneumatics is the study of the behavior of gases. Compressed air can perform work by either expansion or direct forces (Morden, 1966). Even though air is not a perfect gas, the various gas laws, shown below in figure 6, can be used to reasonably estimate values in pneumatic situations.

$PV = \text{constant}$ $V/T = \text{constant}$  $\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$	$PV = mRT$  P = pressure V = volume T = temperature M = mass of gas
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**Figure 6: Ideal Gas Laws .** These equations are used with providing values in different pneumatic situations.

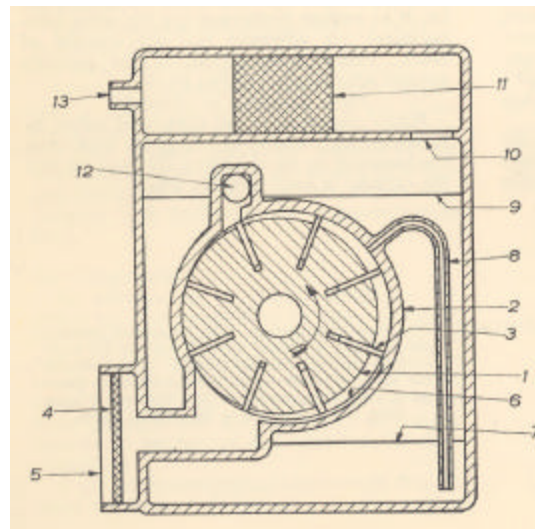
Air pressure is frequently measured in bars where 1 bar =  $10^5$  N/m<sup>2</sup>. Another common unit in pneumatics is the f.a.d, which stands for free air delivered. One f.a.d. is the quantity of air flowing at atmospheric pressure and 20°C (Pinches and Callear, 1996). It could also be thought of as volume per time. Compressors frequently rate their output in units of f.a.d.

The gas laws show that decreasing volume will result in either an increased temperature or pressure. Compressors decrease the volume of a gas, thus increasing temperature and pressure. A compressor is defined as “a machine that aspires, or draws in air or gas, at any pressure—usually atmospheric—compresses it and delivers it at high pressure” (British Compressed Air Society, 1955). Two common types of compressors are piston and rotary.

Reciprocating compressors compress the air within cylinders removing heat through compressor walls. When the piston rises, air is aspired into the cylinder. When the piston lowers, the air is compressed and delivered from the cylinder (British Compressed Air Society, 1955).

Rotary compressors operate as cylinders without valves (Figure 7). Flooding the cylinder with oil creates the seals, which substitute for valves. This oil helps dissipate the heat created by the volume decrease. A spinning rotor delivers compressed air as the space between the wall of the rotor and the rotor decreases after 90° of rotation. Next the oil is injected into the chamber to

absorb heat and further compress the air. The oil is filtered from the air in a compartment outside of the rotor area (Morden, 1966).



**Figure 7: A rotary compressor.** Components are as listed: 1. Rotor; 2. Drum; 3. Vane; 4. Inlet filter; 5. Inlet 6; Inlet groove; 7. Oil level; 8. Oil injection pipe; 9. Baffle for oil separation; 10. Air passage to filter; 11. Oil filter; 12. Outlet to pressure chamber; 13. Outlet. Image from Principles of Pneumatics (Morden, 1966)

Pneumatic tools operate on compressed air. Commonly found in hospitals, compressors are used to power surgical instruments such as pneumatic drills and saws and also ventilators (Jacob and Kumaresh, 2001). Other common pneumatic tools can drill, rivet, chip, and caulk. These tools fall into two main categories: percussive and rotary. Riveting hammers, which fall under the percussive category, can 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. Speed can be controlled by changing valve and pipe sizes (Morden, 1966). Pneumatic circuits can be developed with many parallels to electric circuits. This allows for designs with precise control of the piston action. An explanation of valves common to these circuits can be found in Appendix B.

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:

$$F = pA \quad F = \text{force, } p = \text{pressure; } A = \text{area of piston}$$

The simplest pneumatic cylinder is the single-acting type. 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 and decrease resistance to the moving piston. The piston is returned by a spring (Pinches and Callear, 1996).

A slightly more complicated version of the pneumatic cylinder is the double-acting cylinder. Assuming the piston starts in the upright position, the valve to the upper chamber is blocked. The compressed air travels through the open valve down into the lower chamber of the cylinder. The air expands thereby lifting the piston. The air above the piston is forced up and the valve to the upper chamber is triggered to open. The valve to the lower chamber closes. When this valve opens, air rushes in, expands, and pushes the piston down. Double-acting cylinders also have exhaust valves so that the chamber that the piston is moving into is able to vent out and decrease resistance to the piston (Pinches and Callear, 1996).

## Design Alternatives

To simplify our task of designing the tissue grinder, we divided the process into three components: cooling/freezing, grinding, and collection. Our first semester, we focused mainly on the type of grinding mechanism optimal for the sample and system. Based on initial test results, we found that a series of impulses in conjunction with a twisting shearing motion was the ideal grinding motion. Next our group considered the different methods of providing the desired

grinding mechanism. Two main sources of power, motor and pneumatic, were researched and seriously considered. During this time, part of the group also focused on methods to delivery the liquid nitrogen. Afterward, the insulation and grinding chamber were designed. To help with both decisions, our group underwent two more experiments to better define our constraints and systems. Our group agreed that the collection mechanism was not an important aspect of the function of the device, and would be designed as an added feature later on if time permitted.

### **Initial Experimental Results:**

#### *Grinding Method:*

To better evaluate our designs, our group decided that it was necessary to conduct some initial testing. We tested the following methods: two plates sliding past each other, a shaker with metal beads, a rotating pestle and a pounding hammer. First, we tested the two flat surfaces that provided a shearing force to the frozen tissue sample. The sample was not being adequately ground up, merely just shedding a small layer of cells each time. This was not an efficient method of grinding nor did it work that well. Next, we looked at metal beads in a confined container. This also was not an ideal method since the sample would stick to the beads and was hard to isolate. Next we tried the hammering method. This test led to the conclusion that the sample needed to be in a tightly fitting container and hammer set. If not, since the sample was so hard – it tended to project bits outward and was not easily contained. With an enclosed container, it was broken up into smaller pieces, but not to a fine powder. Next, we decided to try the rotating pestle motion. When tested, it did not do as well with the initial breaking of the sample, but was the best method once the sample was in smaller pieces. Therefore, based on these results, a hybrid design incorporating a pounding hammer motion first, and then switching

into a rotating pestle motion later would be the best grinding method to use for the preparation of the frozen tissue.

Another point to mention about testing deals with the coolness of the sample. During testing, the group experiences trouble keeping the sample adequately cold. This led to thawing and refreezing of the sample, which then led to the sample sticking to the surrounding surfaces. The tissue remained frozen for around 3 – 5 minutes after being exposed to room temperature directly after immersion in liquid nitrogen. This will need to be further tested to better quantify the time until thawing. Next, the group conducted further testing to determine the most effective grinding head shape and grinding container.

#### *Grinding Head Shape and Chamber Design:*

The grinding effectiveness of three different objects was tested to help determine the optimal grinding chamber design as well as head shape for our device. The three sets of stainless steel grinding tools included rounded bowls with slanted sides and a flat bottoms (7.62 cm) soup ladle with completely rounded surfaces (4.14 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) so that a close fit for grinding could be achieved. Shelled peanuts were used as the grinding material.

We performed preliminary testing to identify the best method for applying force to the objects. We decided one person should use a hammer to pound a board placed on top of the grinding item. The same person consistently pounded to avoid inconsistencies in pounding force. The board was used to distribute the force over the grinding object. Approximately 15 impulses were applied to the bowls and ladles. Only one 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. This suggests that rounded surfaces are more effective at breaking apart a material such as peanuts. 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).

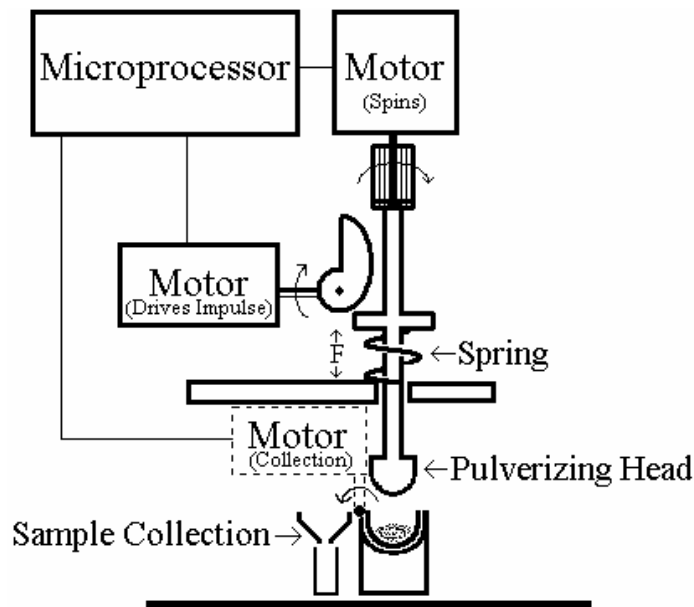
Future testing could be more quantitative. The average size of the larger peanut particles could be recorded. Since our client wants a fine powder, we found qualitative observation an acceptable method of analysis, especially since each tool set had clear differences in resulting particle size. Another 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 then tackled the overall grinding system design. It was determined that there were two plausible methods of powering the grinder: motor driven or pneumatically driven.

### *Grinding System Design #1: A Motor Driven Grinder*

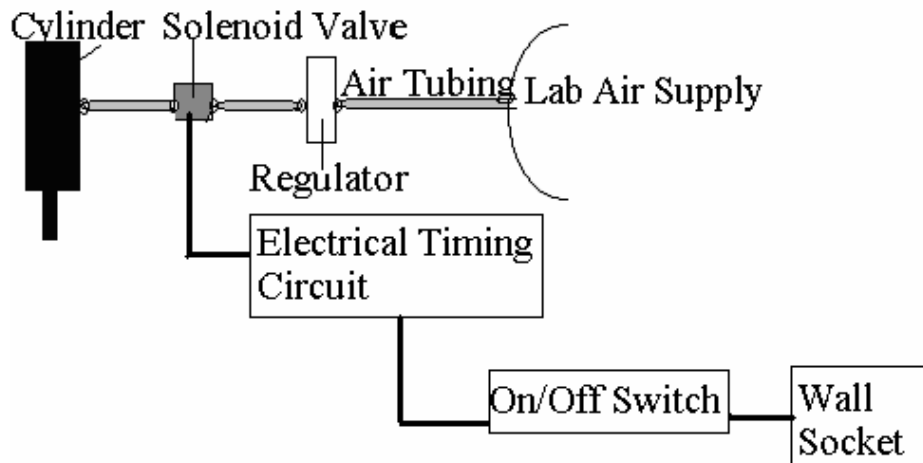
The motorized design operates by using a motor to provide impulses to the sample (Figure 8). The impulses are driven through a spiral gear. A spring lifts the pulverizing head after each impulse to reset the pulverizing head into position for another impulse. A second motor rotates the pulverizing head to distribute the sample evenly within the sample chamber as it is pulverized. This motor is connected through a gear, which allows for both pulverization and rotation to occur simultaneously. The process is fully automated, and all motors are controlled by a microprocessor. This automated design is ideal for eliminating problems due to manual processing such as cold hands and repetitiveness. The design is also somewhat simple. The most complicated component is the gear that allows the motor to spin and distribute the sample while the other motor delivers the impulses. The motors are also isolated from the cold chamber allowing sufficient cooling without excess liquid nitrogen.



**Figure 8: Motorized Design.** 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 our client's lab air supply to drive the overall grinding system (Figure 9). An electrical timing circuit will control the pounding pattern by supplying an electrical signal to the solenoid valve. The air needs to be filtered with a regulator and controlled with by the solenoid valve. Air will flow into the pneumatic cylinder to provide the linear force to break-up the sample. The mounting for the cylinder must be able to support the cylinder.



**Figure 9: Pneumatic Driven System.** The electrical timing circuit controls the solenoid valve which in turn directs the air supply. When the valve is open the cylinder extends. When the valve is closed the cylinder retracts.

### *Evaluation of Grinding System Designs:*

There are several advantages and disadvantages to each system design which influenced our decision. Each was considered and the overall designs were compared using a design matrix (Table 2).

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

**Table 2:** 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.

First focusing on the pneumatic design, the 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 valve in the air line. Various pounding patterns can be accommodated with timers to control the electrical input to the solenoid valve. The amount of force in the cylinder can be changed two ways, allowing much flexibility in design. First, changing the bore size of the pneumatic cylinder proportionally changes the forces created by the cylinder. A simple way to decrease the force without purchasing a new cylinder is to turn down the pressure on the regulator since decreased pressure leads to smaller forces. Pneumatics also allow for continuous application of force. This will facilitate sample pulverization. Additionally, since our client has a lab air supply, the power source is readily available.

The pneumatic grinder also has several disadvantages. It is difficult to stop the piston during a stroke. This may be a safety issue. Ideally, a safety valve would open the air line to stop cylinder pounding for dangerous situations such as a technician attempting to open the

device and view sample. An extra valve may need to be installed to release pressure from the top chamber of the cylinder to raise the piston and stop pounding in case of misuse. This safety switch could also be accomplished with electrical control. By opening the solenoid valve to vent the air from the cylinder and block air from the air source, the cylinder's action is halted. Also, maintenance and repair of the device will require both electrical and pneumatic experts since the device will operate with both electricity and pneumatic power. This will increase maintenance cost.

The motorized design has several differences from the pneumatic design. The pneumatic design uses compressed air, which decreases in volume as temperature decreases. The proposed device is cooled with liquid nitrogen, which may affect the gas compression. Motor electronics will likely be less affected by the cold despite some possible temperature driven resistivity changes. The changes in resistivity are small compared to changes in gas volume, given the same change in temperature. However, the changes in temperature around either design will be insulated.

The motorized design is also expected to be more cost effective and smaller than the pneumatic design. The smaller size is better for use in clinical or research laboratories where lab bench space is often limited.

The motorized design is not without disadvantages. One disadvantage is the amount of contact time the pulverizing head has with the sample in order to properly spin, grind, and distribute the sample. The pneumatic design can provide a continuous force on the sample while the sample chamber is rotated and the sample is distributed. The contact time allows for sufficient grinding and distribution of the sample. The motorized design is not able to provide a

continuous force on the sample while spinning. The motor delivers impulses, but retracts shortly after. This may be less effective to grind and distribute the sample.

Our final decision to use pneumatic power was based on professor advice and an organized decision process. Initially, in conversations with Professor Roderic Lakes, we found that using an electrical motor is a very indirect way to apply force since it changes electricity to circular motion to linear motion. If we wanted an electric grinder, he suggested seeking out other options. Upon hearing the requirements of our design Professor Frank Fronczak suggested pneumatic power was the way to proceed. Pneumatic clearly outweighs electric motors in cost, simplicity, and flexibility.

### **Cooling and Freezing Tissue Design Alternatives:**

In designing this device, we need to be concerned with cooling on a number of different levels. First, a method of delivering the liquid nitrogen to the sample chamber to freeze the tissue sample needs to be designed. Our group initially focused on this component finding two viable options: a pressurized valve method and an un-pressurized tank system. Secondly, before the tissue grinding process can begin, all materials that contact the tissue must be cooled and stay cold throughout the grinding process. Therefore, the temperature inside the grinding chamber needed to be kept cold enough to prevent thawing of the sample during the grinding process. This was taken under consideration when the sample chamber component was designed.

#### *Possible Freezing Mechanisms:*

There are a few different mechanisms available for freezing the tissue sample including 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 can be

beneficial since it would prevent the material from sticking to the sides of the container (Agnelli and Mascheroni, 2000). This mechanism would need to include a method of lowering and raising the sample plate that would be complex due to the attached motor designs already described.

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 (Felizszaz *et al.*, 2000).

Flooding the chamber with liquid nitrogen would be the easiest of the three and is the mechanism chosen by the group 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 that will provide feedback. This feedback will trigger the valve to open once the temperature reached a certain warmth threshold to re-cool the chamber.

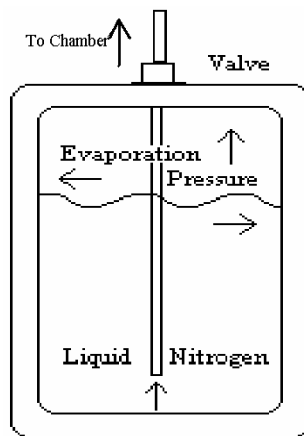
#### *Delivery of Liquid Nitrogen to Sample:*

Currently, only two alternatives for liquid nitrogen flow are available in the cryogenics industry: using an expensive, high-flow-rate pump or utilizing the pressure due to nitrogen gas evaporation that builds up in a sealed tank (“Cryocare Report”, 1996). To deliver liquid nitrogen to this device’s sample chamber, these two system designs were developed and are currently being evaluated for use in the overall design.

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

The pressurized valve design utilizes the normal evaporation pressure build up of liquid nitrogen (Figure 10). Pressure will build once the container is sealed. A liquid discharging

device, such as a valve and tubing can then dispense liquid nitrogen (MVE Form 2195, 2002). The valve used in this design could potentially be coupled to a temperature feedback loop from the grinding chamber. This would better regulate the flow of liquid nitrogen once the temperature reached a certain warmth threshold. The amount of fluid dispensed with this method could vary slightly if flow is not constant; however, since this project is not concerned with having a specific quantity, but rather just enough liquid nitrogen to freeze the sample sufficiently, this should not be a major concern for this design.



**Figure 10: Pressurized System.** 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*

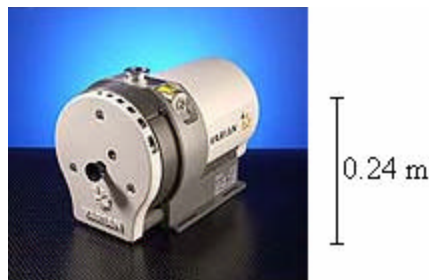
Using a pump to deliver liquid nitrogen to the sample chamber before and during the grinding process is the second alternative to consider for this device. A cryogenic pump is defined as a pump that cools a surface to approximately  $-255^{\circ}\text{C}$  in order to produce a very low vacuum with pressures 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 11, were

incorporated into our device, ideally, we would try to modify it so that the device would control the operation of the pump instead of requiring the user of the device to control it.



**Figure 11: A Manual Pump.** This pump is manufactured by Brymill Cryogenic Systems. ([http://www.brymill.com/catalog\\_4\\_nitro.htm](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 12 minimize the transfer of ambient heat from the motor to the cryogen using a vacuum system.



**Figure 12: 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.

*Evaluation of Cooling Delivery Systems:*

The main advantage of using a pump to deliver liquid nitrogen is the fact that very few or no modifications to the pump itself would be necessary to incorporate it into our device. The pump would simply be connected to a dewar for the source of the liquid nitrogen. The liquid nitrogen could then be pumped through copper tubing or other tubing into the sample chamber. This option allows the liquid nitrogen to be stored separately from the cylinder of our device, which would increase the chances that the pneumatic system could function properly at a reasonable temperature. If the manual pump were used in our device, however, additional modifications would be necessary to more completely automate the entire process. In addition, a pump is a reliable way to transfer liquid nitrogen which is currently commercial available for us to order.

A pressurized valve system would be a simple, comparatively cheaper delivery method. It could easily be used with a temperature gauge, thereby keeping the temperature in the sample-grinding chamber below a temperature threshold during the entire preparation process. It is currently not commercially available, so it would need to be developed specifically for our device. The design has been used for manual liquid dispensing devices, but would need to be modified in order to be automatic. A valve that would function at 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 in the future to better

develop this design. The overall cost involved with this component made it unrealistic to obtain for the prototype based on our limited funds.

### **Sample Chamber Design:**

Next, the group focused on designing a sample chamber that met the temperature needs of the device. When designing our sample chamber, two other requirements besides the temperature concerns governed our design. The chamber needed to be rounded in shape to match the grinding head. Also, the chamber needed to provide some means of sheltering the sample and minimize the amount of sample spray during grinding. Secondary to these was 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 flips the sample chamber upside down into a funnel. When building the prototype, our group didn't consider this design constraint as important and was not incorporated. Currently, the lab technician would need to manually place the sample back 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 that will build 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 began to thaw. In order to calculate the amount of pressure that will build up in the sample chamber during use, a testing Mouse liver was cut into sample pieces of approximately 1 cm x 1 cm x 1cm, the typical size of a tissue sample that needs to be ground to a powder. Each sample 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

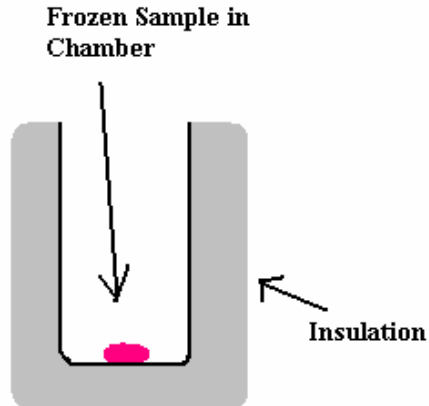
was noted. Also, the time between the addition of liquid nitrogen and the first sign of sample thawing was recorded. Thawing was determined by a change in the color of the sample. It was observed that at least 15 mL of liquid nitrogen is required to completely thaw a tissue sample. Three repetitions per sample using liquid nitrogen volumes of 15 mL and 20 mL were completed, and the times for sample thawing were averaged. A one-tailed t-test assuming independent samples was performed on the thawing time data, with a p-value of 0.09. Since the p-value was greater than 0.05 (for 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 used to freeze a sample can be in the range of 15-20 mL. The data and calculations for this testing protocol are included in Appendix C.

#### *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*

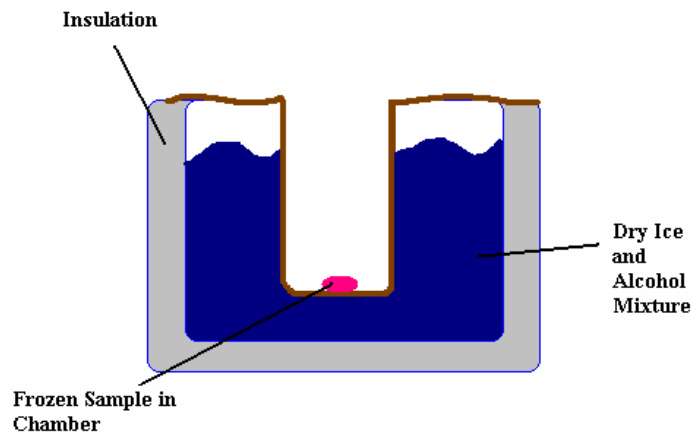
In this simple design, the grinding sample chamber is surrounded by a thick layer of insulating material (Figure 13). When liquid nitrogen is placed in the sample chamber, the chamber will be cooled and the sample frozen. Because of the insulation, the speed at which the sample chamber is able to warm up over time will be lengthened. Depending on the thickness and properties of the material chosen, the exact time it takes before the sample thaws can be changed within a range.



**Figure 13: 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 is an insulated container that can hold a dry ice and alcohol mixture. This coolant mixture will act approximately like a  $-80^{\circ}\text{C}$  freezer, significantly increasing the time the sample chamber is sufficiently cold enough to keep the sample frozen.



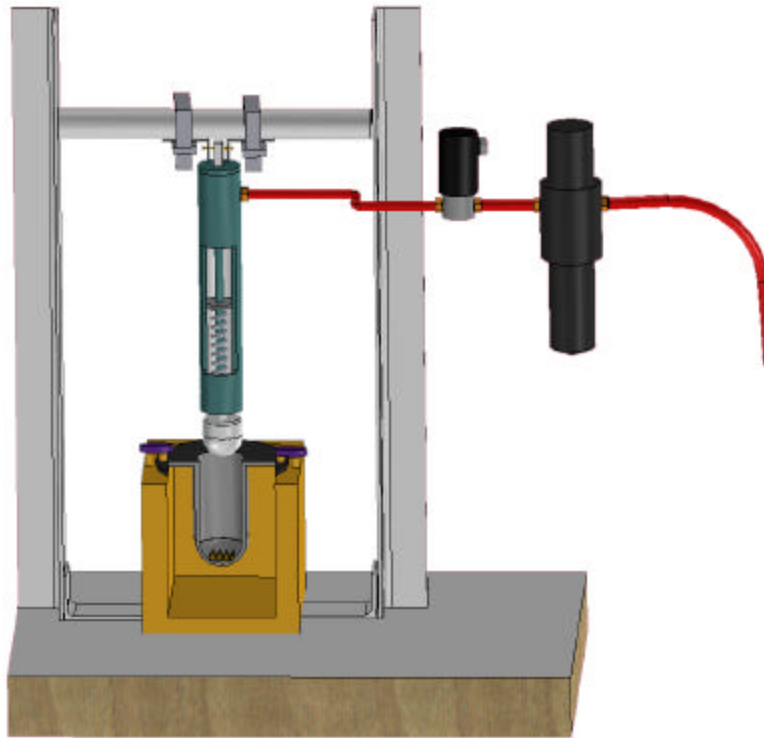
**Figure 14: Dry Ice and Alcohol Bath Insulation Unit.** An insulated chamber containing dry ice and alcohol is placed underneath the sample chamber to keep the unit cool.

### *Evaluation of Insulation Designs:*

By simply surrounding the device with insulation, the increased time that the sample remains cold most likely will not be significant enough. The dry ice and alcohol bath would drastically improve the time the sample remained below the desired temperature. 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: A Pneumatic Driven Grinder**

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). We also would like to include an automated method of delivering liquid nitrogen through a valve release system. This semester, our group focused on the specifics of the pneumatic grinding component as well as the sample chamber. A prototype was built this semester using funding from the biomedical engineering department. See Appendix D and E for Ironcad drawing with dimensions and our budget.



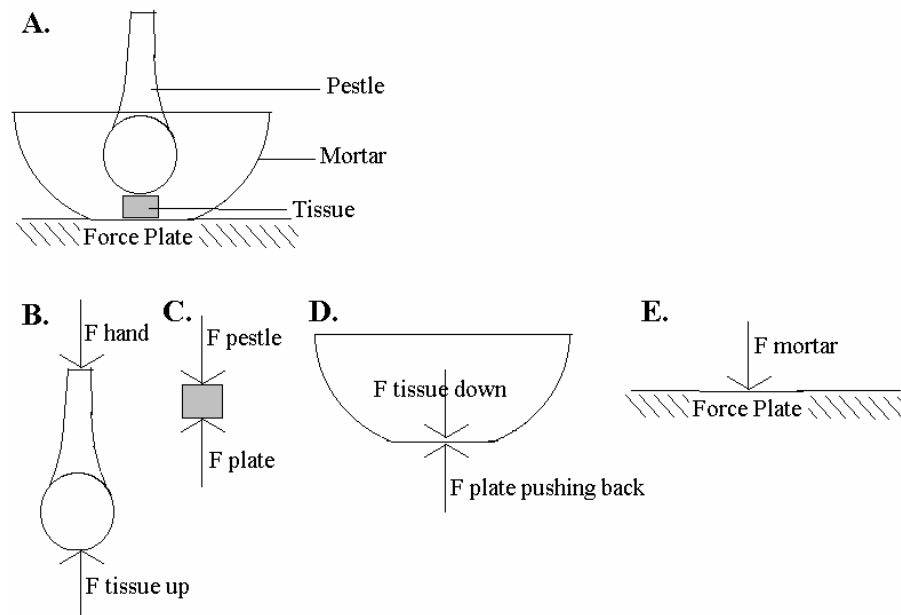
**Figure 15: Drawing of the Pneumatic Driven Final Design.** A pneumatic cylinder will pound into a grinding chamber. The grinder chamber is surrounded by another chamber which will be filled with a dry ice and alcohol coolant mixture to ensure that the sample chamber remains cold.

#### *Force Testing:*

As a preliminary step to choosing a pneumatic cylinder, we needed to quantify force necessary for successful pulverization of tissue samples. We selected to force test by simulating our client's manual pounding motion on a force plate to obtain digital data. The force plate test was chosen over several other potential tests. After consulting with John Dreger and Terry Richards we eliminated a test using a calibrated impact hammer since accompanying analysis would be too complex. A simpler solution of dropping a weight from a known height was disregarded due to the cumbersomeness of repetitively dropping weights until pulverization occurs. The other solution used an analog scale to measure force. We did not choose this test since there exists a large potential for visual error while reading the scale during quick force

impacts. Collecting this data in real time using only our observations would be incredibly difficult.

Prior to force testing, force diagrams were drawn to study the assumed static and rigid system. Since the tissue deforms and the pestle is moving, this analysis is not entirely correct. The analysis did help us realize that the measured force is not truly the force applied by the pestle. This was realized as we tried to equate the forces in Figure 16. Since some energy is absorbed into the tissue pulverization, the measured force is slightly less than the actual applied force. The forces we predicted were between 20 – 100 lbs. so we assumed the force error to be small enough to neglect. Therefore, we used the force measured by the plate as an indirect measurement of how much force we needed to apply to the tissue.

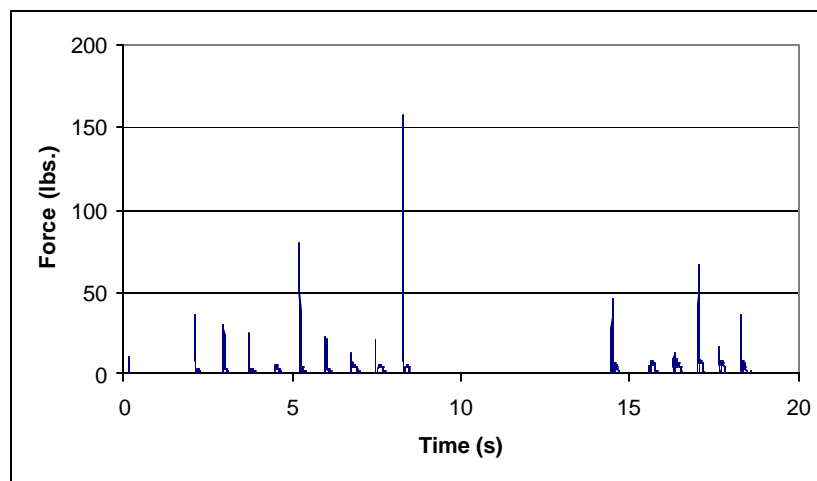


**Figure 16: Free-body diagrams for static analysis.** A. The complete set-up in force-testing B. Pestle C. Tissue D. Mortar E. Force Plate

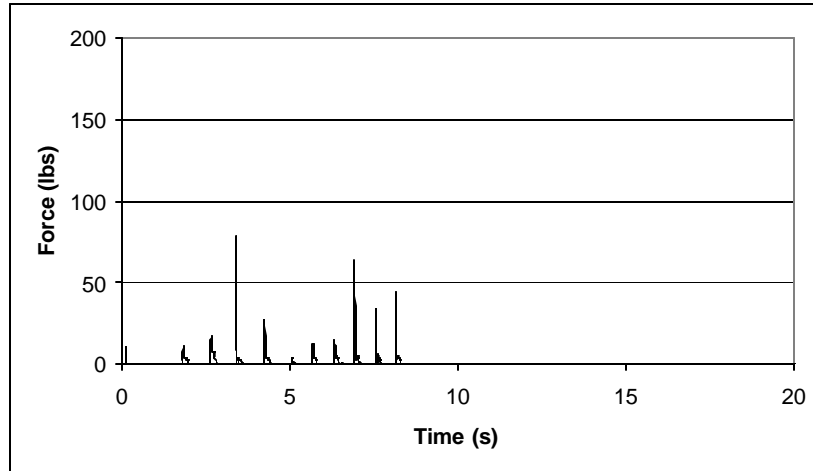
After the mouse liver sample was cut into approximately  $1 \text{ cm}^3$  samples, the tissue was refrozen with liquid nitrogen and placed into labeled miniature vials. These vials were transported from our client’s lab to the Natatorium on dry ice. Calibration data was collected

from a lab performed the previous week. In the Biomechanics Laboratory a plastic tarp was placed over the force plat to protect the plate from cold temperatures and tissue. For each trial, a tissue sample was placed in the pre-chilled ceramic mortar before grinding with a pre-chilled ceramic pestle. One team member was designated to perform the 3 grinding repetitions to maintain as much consistency as possible. He performed 2 tests of approximately 10 sudden impulses and 1 test of approximately 10 extended compressions (Figure 17-19). After the tests, the tissue was visually observed to confirm that it was the consistency of powdered sugar. The additional test of quantifying the tissue size by passing processed samples through a window screen was abandoned since the samples melted too quickly after pounding.

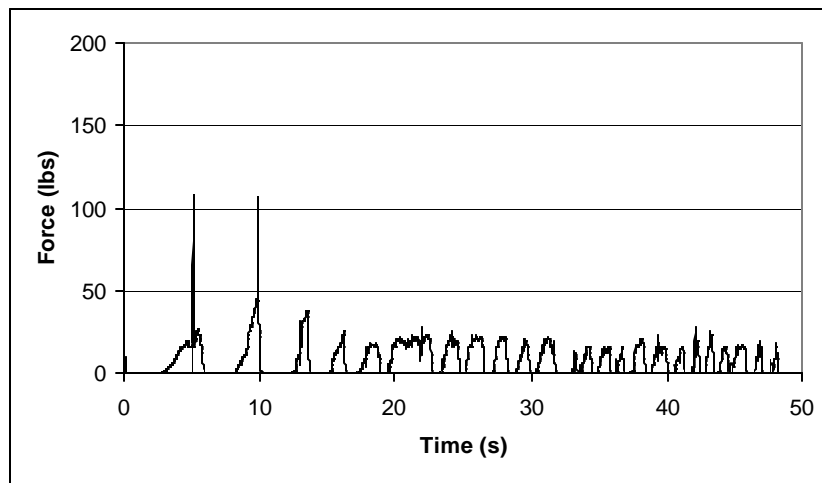
After the data was calibrated, it was converted into English units of pounds and graphed in Excel. The impulses varied between 20 and 150 lbs. The compressions trial (Figure 19) had initial forces larger than 100 lbs; but the majority of impulses were less than 30 lbs. Since Trial 2 (Figure 17) was pulverized with impulses less than 80 lbs, it seems that 80 lbs of force should be sufficient to process our sample.



**Figure 17: Tissue Pulverization Data.** The first trial of sudden impulses measured on a force plate.



**Figure 18: Tissue Pulverization Data.** The second trial of sudden impulses measured on a force plate.



**Figure 19: Tissue Pulverization Data.** A trial of slow compressions measured on a force plate

Biological samples have large differences in shape and composition, so ideally we would perform more trials. But we were limited by tissue supply and melting time. Additional trials could have also reduced variation due to human pounding. As our designated grinder practiced more, he would develop a consistent pounding pattern just sufficient to pulverize the sample.

## **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. We choose a single acting disposable cylinder following Professor Fronczak's advice (Figure 20). The spring operated return stroke allows the cylinder to reset without the use of the air supply. Disposable refers to the lower priced lighter duty cylinders that cannot be serviced if seals deteriorate. The pivot mount was chosen since this minimizes buckling. For the pivot mounting, we ordered Bimba's pivot bracket kit.



**Figure 20: Bimba Single Acting Pneumatic Cylinder.** Picture taken from [www.bimba.com](http://www.bimba.com).

Our ordered cylinder has a 4" stroke length. Only the last 1" of this stroke length will be used for two reasons. First, we need only 1" to clear the maximum sample height of 2 cm, as specified by our client. Second, a pin design will allow the cylinder rod to retract fully to the top of the sample chamber so that the user can remove the sample chamber without the obtrusive long cylinder rod. The pin will be inserted 3" from the bottom of the rod, allowing the cylinder 1" of stroke length. Removal of the pin causes the spring to push back the rod to resting position inside the cylinder.

We made sure to order a circular rod shape. Non-rotating rods come in hexagon shapes. The circular rod shape leaves open the possibility for our 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 lubricants changing consistency, overstressed metal, and damaged and brittle seals (Korane, 2002). These seals extend the operating range of our cylinder from  $-40^{\circ}$  to  $200^{\circ}\text{F}$  for only \$0.80.

We discarded the option of ordering an attached solenoid valve to save money 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).

#### *Solenoid Valve:*

In order to control the cylinder motion we need to control the lab air supply. This is performed with a simple 3-way, 2-position inline valve (Figure 21). The “3 way” describes the 3 ports for the valve including: inlet, vent, and to cylinder. We only need 2 positions to our valve, open and closed, since our cylinder has a spring operated return. Solenoid controls allow the design to be electrically controlled. When the solenoid is energized the valve will open to allow air to flow to the cylinder pushing out the rod to pound. When the solenoid is de-energized, the valve will open to vent the air to the environment allowing the spring to retract the cylinder’s rod to reset the stroke. Most solenoid valves come with spool valves, although a poppet valve is less expensive and provides a better seal.



**Figure 21: Solenoid Valve.** The solenoid valve controls the cylinder motion during grinding. Image taken from Humprey Technical Library.

On the advice of an engineer at Price Engineering, the Bimba Distributor, we selected the 31-E1-120-VAC solenoid valve. With a 1/8" port this valve should align to our cylinder's port without size adaptors. The valve has wires extending out of it which we will have to wire into a timer to control the valves actions, and into an electrical wall socket.

*Filter/Regulator:*

To properly implement the pneumatic system into the device and ensure all working parts are properly maintained, a filter is incorporated into the design (Figure 22). The filter removes debris and liquid from the pressurized air, which is used to operate the cylinder. The debris and liquid, if not removed, can cause excess wear on the cylinder, limiting its lifetime compared to use with filtered air. This particular filter is coupled with a regulator (Figure 22). The regulator is required to properly deliver an expected amount of pressure to the pneumatic cylinder. It should be incorporated in the device in an upright fashion to work properly. The air pressure from the HVAC system installed within a building is expected to range around 120 psi. The pressure will fluctuate because the number of users of the HVAC system changes with time. A regulator is necessary to control the pressure fluctuations. The regulated pressure drives the

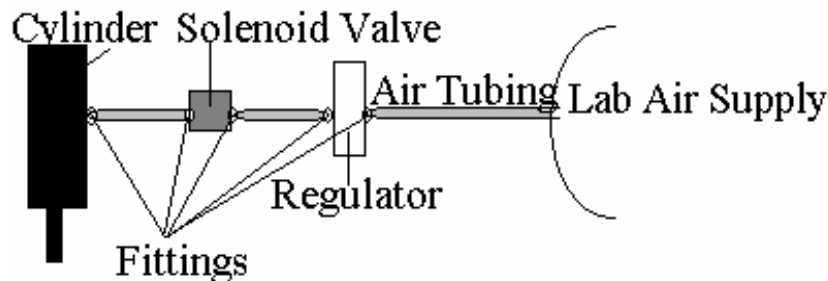
cylinder in regular intervals, which is consistent with the appropriate method for grinding the tissue.



**Figure 22: The Filter/Regulator.** This filters debris and liquid, and it regulates the air flow into the pneumatic cylinder. Image from <<http://www.parker.com/parkersql/series1.asp?id=2765>>

*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. The following schematic shows the order of connection for all of the pneumatic parts.



**Figure 23: Schematic of the order of connection for the pneumatic parts.**

### **Mounting and Three-Point Bending**

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).

$$\sigma := \frac{M y}{I}$$

(Equation 1.1)

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

$$\sigma = E\varepsilon$$

(Equation 1.2)

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

$$I := \left( \frac{\pi}{4} \right) r^4$$

(Equation 1.3)

where I = inertia; r = 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.

### **Sample and Insulation Chamber :**

#### *Pressure Calculations in the Sample Chamber:*

Although our prototype's sample chamber is open to atmospheric pressure, a future design would include enclosing the sample and insulating chambers. In anticipation of this design feature, preliminary calculations were performed to determine the amount of pressure that would build up inside the closed sample chamber due to the evaporation of the liquid nitrogen

added. When liquid nitrogen evaporates, its volume increases; 1 L of liquid nitrogen occupies 700 L as a gas (Liquid Nitrogen: Safety, 2002). To ensure that the tissue grinding device is safe, the pressure that builds up inside the sample chamber must be vented to atmospheric pressure so the evolving gas can escape at the same rate as it evolves. The estimated pressure build-up in the sample chamber when adding 20 mL of liquid nitrogen to the sample allowed the size of the vent hole in the chamber to be estimated. All calculations are included in Appendix F.

#### *Sample Chamber:*

To construct the sample chamber, a stainless steel tube was welded to a stainless steel cap. The proposed design includes two tabs attached to the top rim of the sample chamber (Refer to Figure 15). Each tab would pivot to lock the sample chamber in place inside the insulation chamber. This would allow the sample chamber to be easily detached for sample removal and chamber cleaning between samples. Ideally, there would be two sample chambers available so that while the first was being cleaned, the second could be used in the device. The sample chamber in our prototype does not feature the locking tabs. It is currently held up with clamps attached to a ring stand. An insulated chamber made from polystyrene foam insulation was designed for the dry ice and alcohol chamber. It was 1 inch larger than the sample grinding chamber in all dimensions allowing for adequate room for the dry ice and alcohol mixture.

#### **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 that it would not cause any harm to the user. The outside of the device must be a temperature that can be handled without causing discomfort or frostbite burns due to extreme coldness of the outer surface. If the exterior were found to be too cold to touch, the manufacturer would have to require cryogenic gloves to

be worn whenever the user might touch the device. It would have to be determined if wearing gloves would also be necessary when collecting the ground sample. In testing the device, an attempt to measure the exterior temperature of the device should be made before, during, and after the grinding process. Warning labels for the possible extreme temperatures on the surface 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.

## **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 Counsel (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 F. 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 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:**

#### *Electrical Control:*

In the ideal prototype the solenoid valve would be controlled by a timing circuit where we can specify the time on and time off to determine times for cylinder rod extension and retraction. Several repeat cycle timers found in the Grainger catalog were too expensive (over \$80). The integrated chip 555 timers were avoided since the guidelines at Radio Shack showed a maximum 10V output. Another possibility, an electrical plug-in flasher, commonly used for Christmas lights, did not perform desirably with the solenoid valve. Tested on Christmas lights, the flasher functioned, flashing 14 – 20 times per minute after a brief 20 second warm-up time. Connect to the solenoid valve, rapid switching of the valve occurred for over 20 seconds. The circuit never settled into the calmer alternating rate seen in the tested Christmas light.

Next semester we would like to design a simple circuit with an integrated chip where we can control the duty cycle. This is the most flexible, inexpensive option, although it was too time consuming for the end of the semester.

#### *Temperature Monitor:*

Temperature monitoring in our device could be inexpensively accomplished with a thermistor or thermocouple and simple electrical circuit. We are considering two separate designs to occur after the sensor indicates the sample chamber is too warm. In the simplest

design, either an alarm sounds or an light emitting diode (LED) illuminates informing the technician to add more coolant. A slightly more complicated design could link to a valve in the hypothesized cooling chamber. When the temperature exceeds a certain threshold, the valve would open adding more liquid nitrogen to the sample chamber or more coolant to the cooling chamber. Another sensor may need to be added to prevent overflow in either chamber.

#### *Shearing Forces:*

If after testing our system, we find the sample is not efficiency ground to the consistency of powered sugar we will need to implement shearing forces into our design. When choosing a cylinder, a round rod was selected to allow for the later addition of a rotating rod. The design has not been created yet, but it needs to be similar to a mechanical pen that rotates as the user pushes on the top. Precise control of the rotation needs to be executed in order to achieve consistent grinding results.

#### *Prototype Testing:*

A prototype is nearing the end of construction. Upon completion, the prototype is tested to ensure proper functioning of all components. The components and observations to look for on our design are the following:

- frame stability
- cylinder mount integrity
- cylinder motion accuracy and precision
- grinding head efficacy
- grinding chamber efficacy
- cooling efficacy
- insulating efficacy
- filter/regulator efficacy
- solenoid efficacy

The testing is necessary because although the design appears adequate, factors may be overlooked. To overcome these possible human errors in design, building and testing a prototype is imperative. Testing ensures that the problem is solved as desired.

#### *FDA and Patent Concerns:*

FDA approval would probably be necessary for the device to be marketable. Therefore all materials and aspects of the design would have to comply with FDA standards. Also, we are considering patenting this device; therefore once the design is further along, a meeting with WARF may be necessary sometime next fall.

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## *Appendix A -PDS*

### **Tissue Sample Preparation Device for Biochemical Analysis Version 6 – November 1, 2002**

**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.

#### **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 completions, 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 cm<sup>3</sup> (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. Pressure blow valve should be included to avoid 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 wall 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.

## ***Appendix B – Pneumatic Valves***

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

This information was researched before it was known that pneumatic cylinders are manufactured by a number of companies.

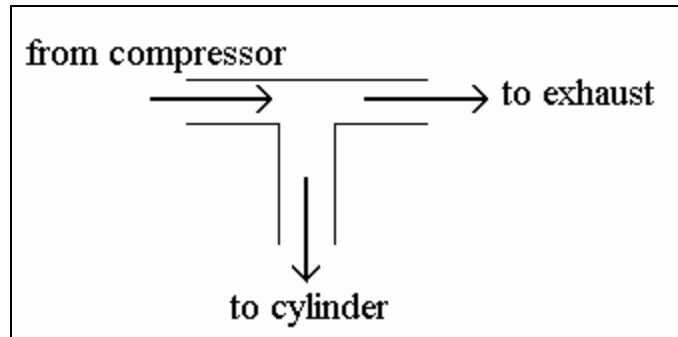
### **Distribution valve**

**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



**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).

## Designs of Control Valves

**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

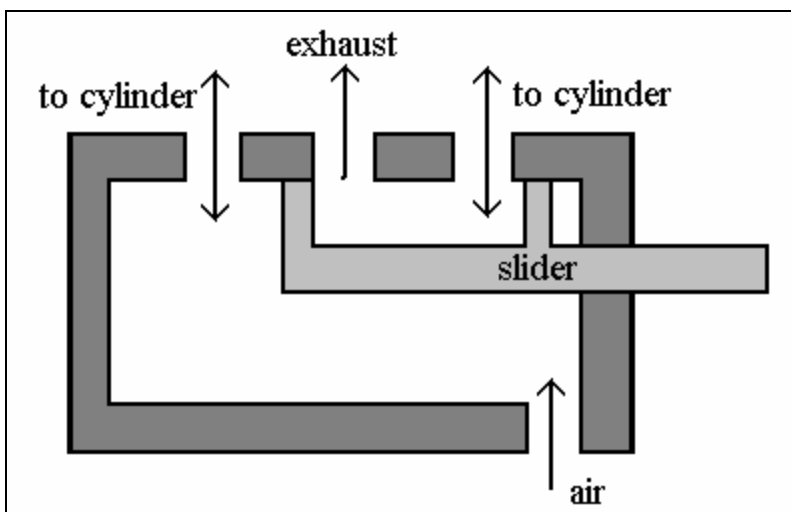


Fig C2: An example of a slide valve.

**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 C – Liquid Nitrogen Testing Analysis of Results:**

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

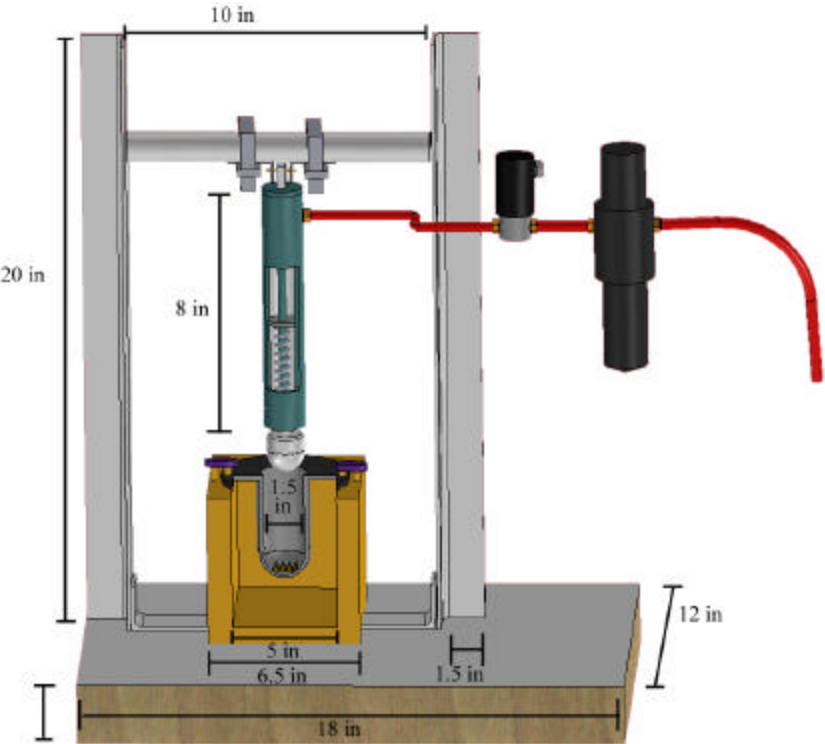
LN <sub>2</sub> Volume (ml)	Average Evaporation Time (min)	Average Evaporation Time (sec)	Average Thaw Time (min)	Average Thaw Time (sec)
15	1.0	60	5.4	330
20	0.83	50	6.1	360
		Difference	0.6	30

**T-test for 15ml and 20 ml LN<sub>2</sub> volume**

t-Test: Two-Sample Assuming Equal Variances		
	15 ml Volume	20 ml Volume
Mean	5.44	6.08
Variance	0.34	0.13
Observations	3	3
Pooled Variance	0.24	
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.61	
P(T<=t) one-tail	<b>0.09</b>	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.18	
t Critical two-tail	2.78	

\*\*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 D: Ironcad Drawing of Final Design with Dimensions*



## Appendix E – Budget for Semester

Part	Quantity	Price	Purchaser
<b>Pneumatic</b>			
Bimba cylinder – 094-PN	1	\$25.85	BME dept.
Bimba pivot brackets – D-167	1	\$2.70	BME dept.
Bimba grinding head mount - D-166-1	1	\$3.10	BME dept.
Parker regulator/filter - 05E -02A18AA	1	\$35.49	BME dept.
Humphrey solenoid valve - 31-E1-120VAC	1	\$45.40	BME dept.
Fittings (1/8") - KQ2-H07-345	5	\$7.50	BME dept.
Tubing (10ft section) - AP-44-N	1	\$1.20	BME dept.
<b>Electrical</b>			
Wall outlet plug	1	\$3.30	Carla
Plug-in flasher	1	\$2.63	Carla
Electrical tape	1	\$1.33	Carla
Switch pushbutton	1	\$3.04	Carla
<b>Sample Chamber</b>			
Sphere (unthreaded stainless steel butt-welded fitting cap)	1	\$20.00	BME dept.
Chamber pipe (stainless steel, 1.5" diameter)	1	\$10.00	BME dept.
Chamber labor (UW-Machine shop)	--	\$50.00	BME dept.
<b>Grinding head</b>			
Grinding head (nickel-plated cabinet knob, 1.25" diameter)		\$2.31	Christine
<b>Support Stand</b>			
Sheet metal	1	\$5.65	Ryan
Wood for base (2"x12"x8')	1	\$8.23	Ryan
Post for support beams	1	\$9.00	Ryan
Threaded rod for cross-beam (5/8")	1	\$2.29	Ryan
U-bolts	2	\$1.58	Ryan
Washers, nuts, and bolts	43	\$6.23	Ryan
L brackets	2	\$3.74	Ryan
Drill bit	1	\$20.97	Ryan
tax	--	\$3.23	Ryan
<b>Cooling Chamber</b>			
Cauk gun	1	\$2.12	Sara
Foam board	1	\$4.78	Sara
Foam board cauk	1	\$1.98	Sara
<b>Miscellaneous Parts</b>			
cabinet handles	2	\$2.78	Sara
soup ladles	1	\$2.65	BME dept
Pneumatic Total		\$123.89	BME dept.

Electrical Parts		\$10.30	Carla
Sample Chamber Total		\$80.00	BME dept.
Support Stand Total		\$60.92	Ryan
Grinding Head Total		\$2.31	Christine
Cooling Chamber Total		\$11.66	Sara
Miscellaneous Total		\$5.43	combo
<b>Total</b>		<b>\$294.51</b>	
BME dept paid		\$206.54	
team members paid		\$87.97	
<b>Total</b>		<b>\$294.51</b>	

## Appendix F – Pressure Calculations

### Liquid Nitrogen Properties and Pressure Calculations In Sample Chamber

Atomic Mass N (g/mol)	14.00674	Atomic Mass N <sub>2</sub> (g/mol)	28.01348	
<u>Density of LN<sub>2</sub></u>	g/cm <sup>3</sup>	0.807	<u>Vapor N<sub>2</sub> gas evolved per 1 L (0.001 m<sup>3</sup>) LN<sub>2</sub></u>	
	kg/m <sup>3</sup>	804	m <sup>3</sup>	0.7
<u>Heat of Fusion of N<sub>2</sub></u>	J/g	2.57E+04	<u>Atmospheric Pressure</u>	
	J/mol	360.1	N/m <sup>2</sup> , kg/(m*s <sup>2</sup> ), Pa	101,325
<u>Latent Heat of Vaporization</u>	J/kg	1.99E+05	mm Hg	760
	J/mol	2.79E+03	atm	1
<u>LN<sub>2</sub> Melting Point</u>			<u>Gas Constant R</u>	
	F	-345.75	J/(mol*K)	8.3143
	°C	-209.86	<u>LN<sub>2</sub> Boiling Point</u>	
	K	63.14	(°C)	
			°C	-195.8
			K	77.35
			+ °C	
			273.15 = K	
Specific Heat of Nitrogen (at 77.35 K, liquid)			J/(kg K)	2042
Specific Heat of Nitrogen (at 300 K, gas)			J/(kg K)	1042

Volume LN <sub>2</sub> to freeze sample once	Corresponding Vapor N <sub>2</sub> gas evolved LN <sub>2</sub>	Mass N <sub>2</sub>	n Moles N <sub>2</sub>
0.02 L	0.014 m <sup>3</sup>	0.01614 kg	0.5762
20 mL	14 L	16.14 g	

assume T = -78.5 °C = 194.65 K (dry ice temperature)  
 let V=volume of space in the sample chamber  
 actual volume of chamber will be V+1 cm<sup>3</sup> (vol. of sample)

$$P=F/A \quad PV=nRT$$

$$nRT = (0.57615 \text{ mol}) \cdot (8.3143$$

$$J/(\text{mol} \cdot \text{K}) \cdot (194.65 \text{ K})$$

$$nRT = 932.4307051 \quad J = N \cdot m = \text{kg} \cdot \text{m}^2/\text{s}^2$$

Assume chamber shape is hemisphere and cylinder

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

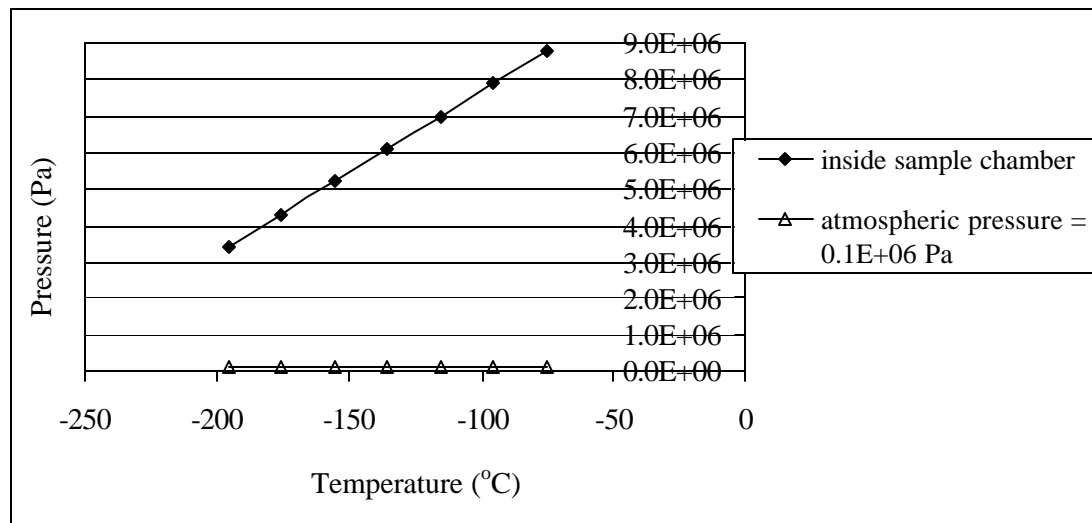
$\pi$					
=	3.14159	0.000001	m <sup>3</sup>	=	1 cm <sup>3</sup>

P/atm P	P - atmP	P (Pa)	V (m <sup>3</sup> )	V (L)	Height (m)	Radius (m)	Radius (cm)
85.53	8.56E+06	###	1.08E-04	1.08E-07	0.083	0.019	1.91

$$PV=nRT$$

$$P/T=(nR)/V$$

V (m <sup>3</sup> )	(nR)/V	T (K)	T (°C)	P (Pa)	Δ P (Pa)
1.08E-04	4.45E+04	77.4	-195.8	3.44E+06	5.34E+06
		97.4	-175.8	4.33E+06	
Height (in)	3.25	117	-155.8	5.22E+06	
Diameter (in)	1.5	137	-135.8	6.12E+06	
		157	-115.8	7.01E+06	
		177	-95.8	7.90E+06	
		197	-75.8	8.79E+06	



The above graph shows how pressure inside a sealed sample chamber will increase as temperature increases. The temperature inside the sample chamber begins at  $-196^{\circ}\text{C}$ , 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 G – 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>

Code of Federal Regulations  
Title 21 - Food and Drugs  
Revised as of April 1, 2001

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From the U.S. Government Printing Office via GPO Access  
[CITE: 21CFR862.2050]

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.