

# **Design of a Tissue Sample Preparation Device for Biochemical Analysis**

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**Abstract:** The tissue sample grinder will process surgically removed tissue by freezing, grinding, and finally collecting the ground sample. Our design thus far has been primarily focused on the freezing and grinding components. We conducted initial testing of the grinding mechanism, which showed that the most effective method involves a rounded, hemispherical pulverizing head that deliver impulses and rotates the tissue sample. To drive the motion, two solutions are being considered: a motor-driven design and a pneumatic design. The ability of each design to follow the desired grinding pattern (pounding followed by a continuous downward force with simultaneous spinning) in addition to size, cost, and noise considerations are still under evaluation. Two designs for the delivery of liquid nitrogen to the sample were discussed and developed at the end of the semester. These include a pressurized valve system and a commercially available liquid nitrogen pump. Currently, no decisions have been made concerning the design options for either the grinding or freezing component. We are still carefully weighing the advantages and disadvantages of each alternative and will make design decisions next fall when the project is continued.

**Design Problem:** To design a device that completes the manual process of preparing tissue samples for biochemical analysis. The device should freeze the tissue (with liquid nitrogen), grind it to a powder, and collect it for storage.

## 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 B). 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. The sample should 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. The device would be used 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° C). The device should fit on a laboratory bench. Also, the device should have removable components to aid in cleaning between sample processing. Finally, the total cost of the prototype should not exceed \$1,500. 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).

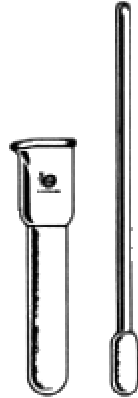


**Figure 1: The BeadBeaterä .** The top unit spins the metal or glass beads to create collisions with the tissue particles. The collision force breaks apart the tissue. Beads must be cleaned after each use (Meyer, 2002). Image from <<http://www.biospec.com>>.

### *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

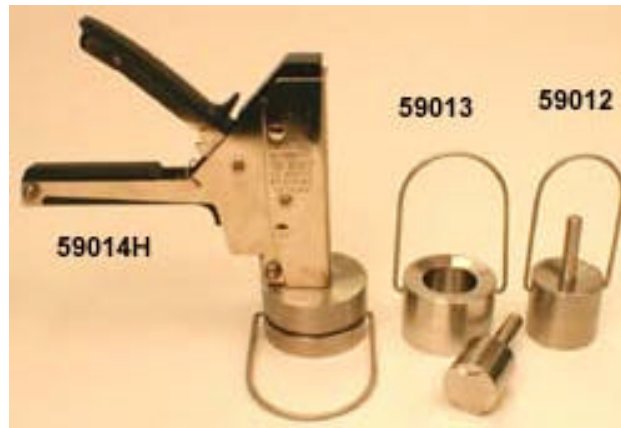
pushed downward and the sample can only pass the spinning pestle if it is smaller than the clearance between the tube and pestle (Seetharam and Sharma, 1991). The tube is typically placed in a beaker of ice to keep the tissue cold. The main problem with homogenizers is that the tissue must be prepared ahead of time by grinding or shearing with a scissors (Burgess, 2002).



**Figure 2: The Potter-Elvehjem Homogenizer (“LG-10650 Tissue Grinder”, 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).



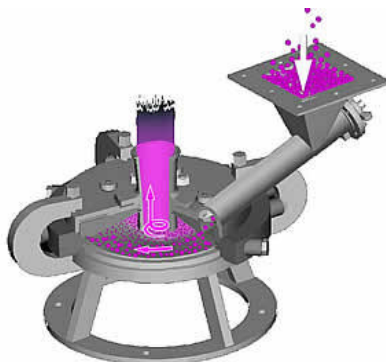
**Figure 3: Images of the Bio-pulverizer.** The spring loaded bio-pulverizer is shown on the left. The mortar and piston are shown individually on the right. This device grinds by freeze fracturing. Image taken from <http://www.biospec.com/Brochures/cryog/BioPulv.html>.

### *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 (“How Jet Pulverizers Work”, 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. (“How Jet Pulverizers Work”, 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 (“How Jet Pulverizers Work”, 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 (“How Jet Pulverizers Work”, 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 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

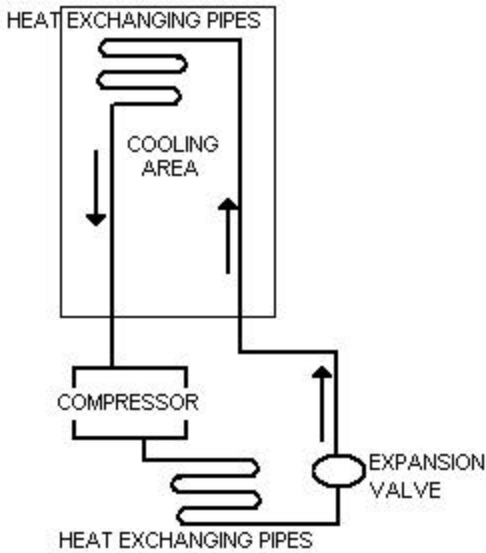
**Table 1: Properties of Liquid Nitrogen (N<sub>2</sub>).** Information from the following sources \*("Periodic Table", 2002), § (MacNeil, 2002).

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 ("Cryogenic Cooling", 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>P = pressure  V = volume  T = temperature  M = mass of gas</p>
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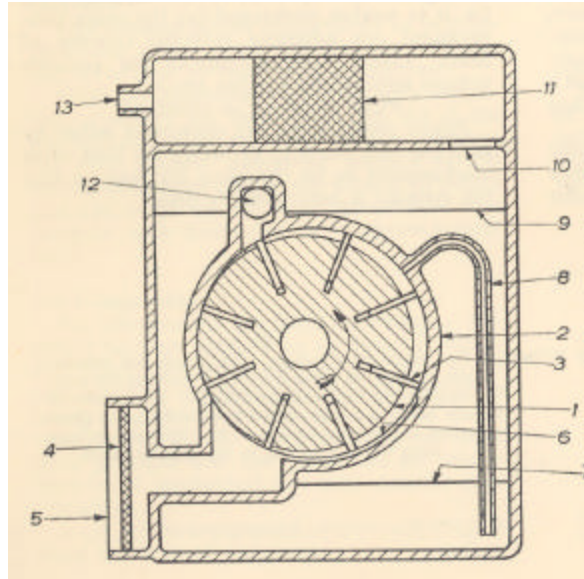
**Figure 6: Ideal Gas Laws .** These equations are used with providing values in different pneumatic situations.

Air pressure is frequently measured in bars where  $1 \text{ bar} = 10^5 \text{ N/m}^2$ . 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  $20^\circ\text{C}$  (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  $90^\circ$  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 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:

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

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

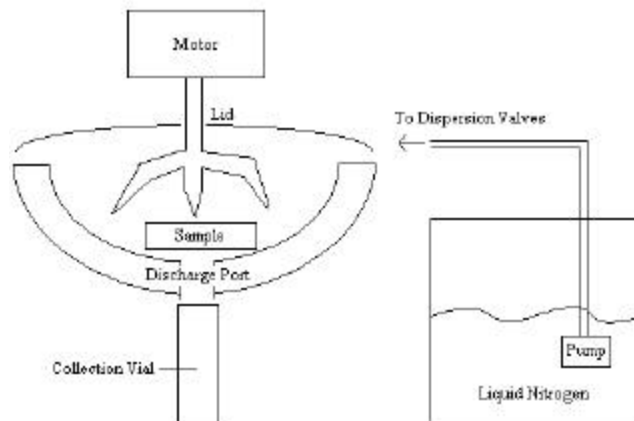
To simplify our task of designing the tissue grinder, we divided the process into three components: cooling/freezing, grinding, and collection. Initially we focused on the mechanism for grinding the sample. After this, our group divided into two subgroups to further progress. One group continued to focus on the grinding system while the other group switched focus to the cooling of the device as well as the freezing process.

## Design Alternatives for Grinding Mechanism:

During the first half of this semester, our group focused on brainstorming potential mechanisms for grinding. 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. Then, based on test results on the effectiveness of the grinding mechanism, as well as other criteria, the grinding mechanism was determined.

### *Grinding Mechanism # 1: The Blender*

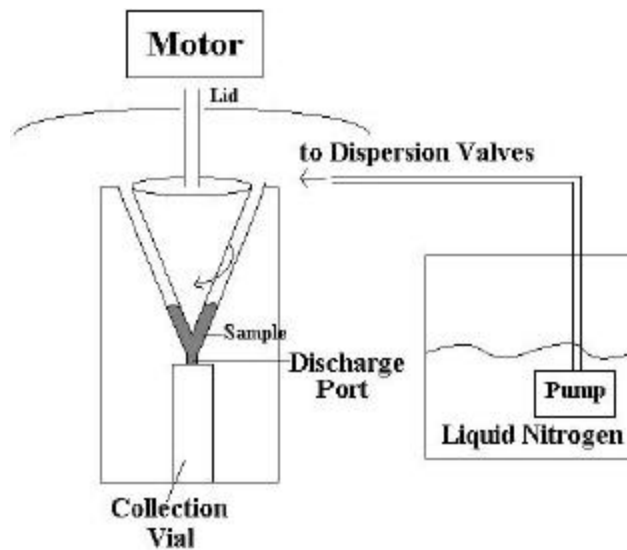
One design option explored was the blender. It operates similar to a household blender by using sharp blades to break the frozen sample into fine particles. The blades are connected through a metal shaft to a motor, which supplies the energy needed to break the sample. By covering the blending area with a lid, sample loss is prevented. After the sample is broken, it can be washed through the discharge port with small amounts of liquid nitrogen into the collection vial (see Figure 8). The discharge port is selectively opened when the sample has finished blending.



**Figure 8: The 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*

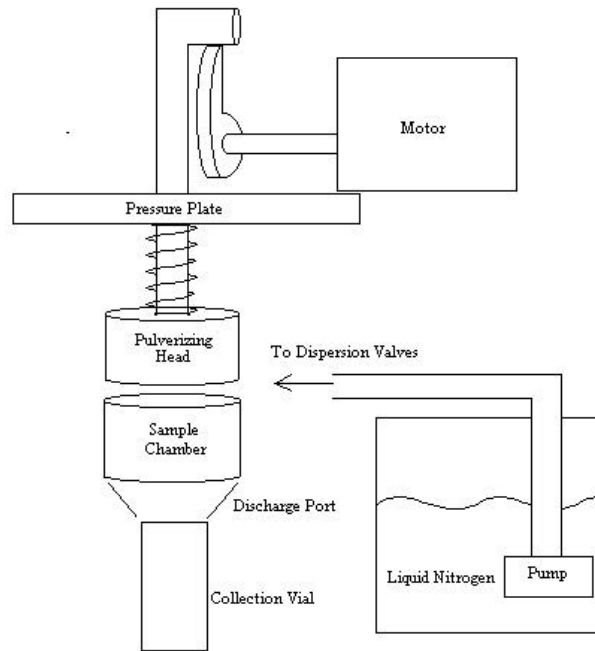
This design uses a metal cone that rotates around its central axis to grind the sample. After a sample is inserted, the motor rotates the cone creating a shear force against the sample. After the sample is completely ground, the discharge port opens and the sample is collected in the vial (Figure 9).



**Figure 9: The Funnel Grinder.** The motor spins a cone shaped grinder to break apart the sample by shear forces. When the sample reaches the appropriate size it enters the collection vial through the discharge port.

### *Design Option 3: 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 10). The sample is first placed inside the sample chamber. It 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 10: The Impulse Sampler.** The single-sampler freezes tissue samples with liquid nitrogen and processes them with impulses delivered by the motor. The processed tissue is collected in the collection vial.

## Evaluating of Early Grinding Designs:

### *Initial Testing:*

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. For our final design, it is necessary that we address this concern.

#### *Evaluation Process:*

Each of these grinding designs had its advantages as well as disadvantages. We used the following criteria to judge each design: grinding efficiency, simplicity, sample collection efficiency, durability, noise acceptability, ease of cleaning, processing time, and effectively pressure release. Grinding efficiency, due to its importance, was weighted by a factor of 4. The results of this evaluation are summarized in our design matrix shown below in Table 2.

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

**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 as the basis for our final design. The exact grinding sequence incorporated a few initial hard impact pounds to break up the sample. After this, the head would rotate around its central axis, further breaking up the sample by shear forces. It was this combination that most effectively produced a homogenous fine powder. Next, the group conducted further testing to determine the most effective grinding head shape and grinding container.

### **Further Testing: 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 ladles 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

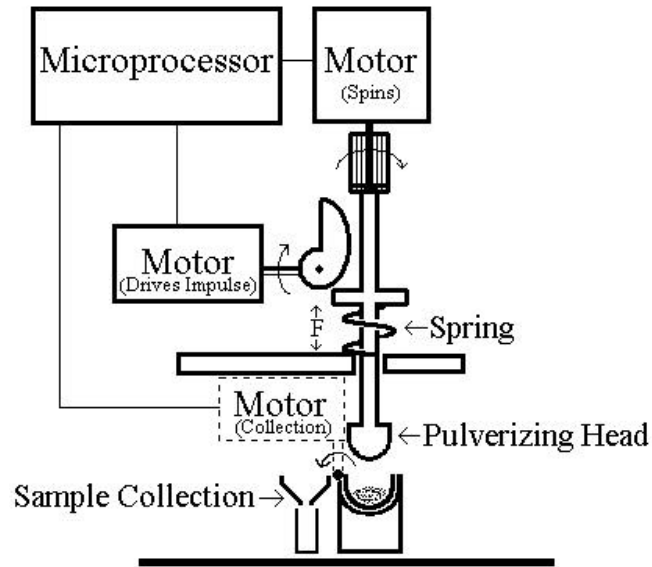
setting (no cooling materials such as dry ice or liquid nitrogen), we did not test frozen materials such as chicken breast.

### **Grinding System Design Alternatives:**

After determining the grinding mechanism as well as the head shape and container, our group worked on 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 11). 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. Collection of the sample is accomplished by a third motor, which flips the sample chamber into a funnel, finally unloading the processed sample in the collection vial. 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 11: 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.

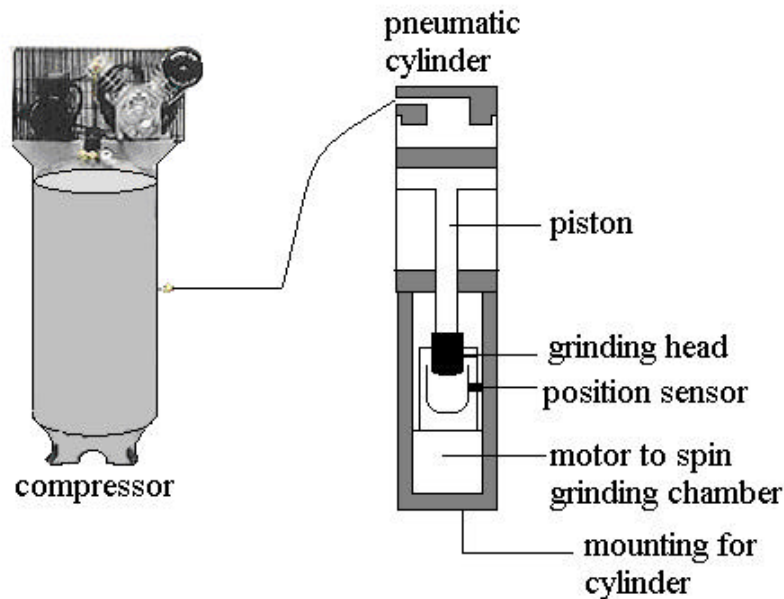
### *Grinding System Design #2: Pneumatically Driven System*

This design uses compressed air to drive the overall grinding system (Figure 12). A compressor will provide compressed air to the pneumatic cylinder via pipe or a hose. Both the cylinder and the compressor will be purchased. A vertical standing compressor will be chosen to save floor space in a clinical lab. The mounting for the cylinder, the grinding head, the grinding chamber, and the spinning mechanism (motor system) will be custom built for this design. The purchased compressor and piston will provide the linear force to break-up the sample. A position sensor will sense when the piston is low enough to apply force to the sample. The triggering of the position sensor will complete the circuit with the motor for rotating the grinding chamber. This will result in the motor only spinning when the grinding head is in the lower phase of its cycle, thus saving energy since it is not necessary for the motor to spin continuously.

The mounting for the cylinder must be able to support the cylinder. Since mountings are also commercially available, we will need to look into the design of these to determine

appropriate materials and support structures. The design for the mounting will need to be included with the overall design shape and insulation of the grinding chamber. This will be a part of integrating the separate components of grinding, cooling, and overall shape together into one device next semester.

The sample collection will be either manual with a removable grinding chamber or automated with a motor that will rotate the grinding chamber upside down to empty the contents into a funnel to drain into the client's desired collection vial. This is the same collection format as for the motorized design.



**Figure 12: Pneumatic grinding design.** The image of the compressor is adapted from a Ingersoll-Rand vertically mounted compressor ([www.air.ingersoll-rand.com](http://www.air.ingersoll-rand.com) 2002). The compressor drives a pneumatic cylinder that drives the impacts of the grinder. A position sensor determines the level of the grinding head for applying force while the grinding chamber spins.

### Sample Collection Methods:

The sample collection methods have not been investigated thoroughly. Two options have been 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. The collection efficiency for either method is not known. Future testing is required in order to determine the appropriate method.

*Preliminary Evaluation of Grinding System Alternatives:*

There are several advantages and disadvantages to each system design. First, focusing on the pneumatic design, the pneumatic actuators tend to be lighter and less complicated than electrical actuators since there are no mechanical parts such as gears, shafts, or cams. Varying air pressure will change the force produced by the piston. Assuming a compressor with a switch for controls output, the force applied to the sample can be easily increased or decreased. Changing pressures within the cylinder controls the speed of the piston. This is accomplished by changing valve and pipe sizes in the initial design of the cylinder. Pneumatics also allow for the continuous application of force that will be required to occur simultaneously during spinning. Additionally various pounding patterns can be accommodated with timers that control extra air reservoirs within the cylinder.

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 stop could be implemented to stop the pounding if something goes wrong during the grinding process, such as a technician attempting to open the device and view sample. Since the cylinder is running on air pressure and not electricity, a safety switch in a circuit will not suffice. 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. Also, maintenance and repair of the device may require both electrical and pneumatic experts since the device will operate with both electricity and pneumatic power. This will increase maintenance cost. Additionally, the cost of this device is unknown as of this semester, but it is predicted to be higher than the electrical motors design. The

compressor will take up a relatively large floor space. The size of the compressor needs to be determined before this size can be considered in choosing the design. Compressors on the market vary from tabletop models to room-size industrial models.

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.

Noise is also a consideration for the design. The impulse noise will be the same for both grinding methods. The difference between the two methods is mainly the timing of the noise. The pneumatic design can store up the compressed air resulting in distributed noise intervals.

The compressor may possibly not run at all when the device is in use. However, an unexpected compressor may startle or disrupt a laboratory worker if the desired pressure drops below the expected value while not in use. The motorized design will be noisier while operating, but it has no unexpected noise while not in use. The actual noise of the compressor compared to the motors is unknown.

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

In designing this device, we need to be concerned with cooling on a number of different levels. There are overall cooling concerns for the product since the temperature inside the grinding chamber should be kept cold to prevent thawing of the tissue samples. Also, the liquid nitrogen will be used to freeze the tissue sample placed in the device. This means that a method of delivering the liquid nitrogen as well as freezing the sample needed to be designed.

#### *General Cooling:*

Before the tissue grinding process can begin, all materials that contact the tissue must be cooled. If the tissue will be stored in the device for any extended amount of time longer than 5 minutes (as determined by testing), the device must have an additional system to keep the tissue frozen. Pre-cooling and cold storage of the device could be accomplished by either periodic liquid nitrogen application or an additional cooling system. Two options exist for cooling systems, dependent storage of the device in a freezer or an independent cooling system with refrigerant, heat exchanging pipes, compressor and expansion valve. Also, good insulation is necessary in order to more effectively keep the device cold for longer periods of time.

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

There are a few different mechanisms for freezing the tissue sample 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 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 (Felizaz *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 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.

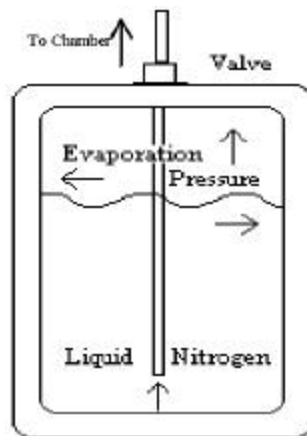
### *Delivery of Liquid Nitrogen to Sample:*

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 13). 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 13: 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.

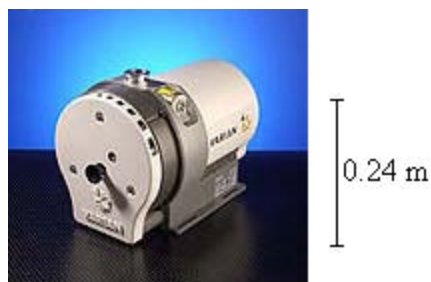
### *Liquid Nitrogen Delivery System #2: Un-pressurized Pump System*

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 14, 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 14: 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 15 minimize the transfer of ambient heat from the motor to the cryogen using a vacuum system.



**Figure 15: 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:*

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 motor of our device, which would increase the chances that the motor 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 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. Also, this system 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.

### **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.

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. 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, the Office of Regulatory Affairs and the Office of Chief Council. The CBER website has many links to the FDA ("CBER Devices", 2002)

Currently the FDA has three classes of regulation for medical devices. Information our group found relevant is included in Appendix D. 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 (“Device Advice”, 2002).

New devices that cannot compare 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 (“Device Advice”, 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 (“Device Advice”, 2002). This suggests we will not have difficulty obtaining FDA approval for our device.

### **Future Concerns and Direction of the Project:**

Since this is only the first of three semesters of design development of this device, there are many questions yet to be answered and decisions to be finalized before a prototype could be built. Next semester, our group plans to answer these questions as we progress with the development of the overall preparation device design.

The important decision between using motorized or pneumatic power to pound our sample is still under discussion. We have developed designs for both types of power. The

decision between these two designs will be made based primarily on cost, size, and ability to follow our desired grinding mechanism (pounding followed by a continuous downward force with simultaneous spinning). The cost of the cylinder and compressor, the main components of the pneumatic design, needs to be determined in order to estimate the overall cost of this design. Once this is chosen, we will evaluate the expected size of each design, considering whether the compressor is a reasonable size for a clinical laboratory and our client's laboratory. Both alternatives need to be evaluated to determine whether they can efficiently and effectively pulverize the sample. This decision will involve considerations of a microprocessor for coordinating the actions (for both designs) and also pneumatic timers (for only the pneumatic design). In addition, we plan to tour the machine shop to evaluate pneumatic tools and compare them to electric tools in order to help us decide between pneumatic and electrical power.

The cooling mechanism of the device (pressurized system versus a pump) is the second main decision that will have to be made. More time will be required to develop the cooling designs as well as weigh each design's advantages and disadvantages. Also, the issue of including a temperature feedback system in the device needs further discussion. A temperature feedback system would deliver additional volumes of liquid nitrogen to the sample during the grinding process if the temperature of the sample chamber became too warm. If the temperature of the sample did increase beyond a certain threshold (to be determined in the future), the sample may thaw, which would prevent any further biochemical analysis. Our group has kept this the feasibility of this feature in while creating our design alternatives, but as of now we have not focused on the specifics.

Once both the specific method of liquid nitrogen delivery and the grinding mechanism have been chosen, more specific details will be need to be finalized such as how each design

component will fit together and how all parts will function in conjunction with one another. The materials for each component of the device and all dimensions for the design will then need to be determined. The volume of liquid nitrogen delivered at once will also be selected. After all these and other details have been worked out, a prototype would be built and submitted to rigorous testing methods, including multiple tissue sample grinding procedures and an evaluation of how easily the device's sample chamber can be cleaned.

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 would be necessary sometime next fall.

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## *Appendix B -PDS*

### **Tissue Sample Preparation Device for Biochemical Analysis Version 4 – May 5, 2002**

#### **Team Members:**

**Sara Alford  
Christine Koranda  
Carla Maas  
Ryan Roth**

**Client: Jeff Ross and Charles Tessier  
UW - Medical School  
Department of Oncology  
Advisor: Mark Nicosia**

**Function:** The device will freeze a tissue sample with liquid nitrogen, grind it into a fine powder, and collect it in a small vial ready for further biochemical analysis. The device will replace the manual mortar and pestle grinding technique currently used.

#### **Client Requirements:**

- Sample and tools used must be kept cold at all times during the process
- Must salvage as much as tissue sample as possible
- Processing time should be similar to that for manual preparation (approximately 15 minutes)
- Device to be used for 30-40 samples/day
- Grind tissue to the consistency of powdered sugar

#### **Design requirements:**

##### **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.

**b. Safety:** Liquid nitrogen should not be exposed to flesh or clothing. Instrument may be cold to touch. Cryogenic gloves and safety glasses may be required for the use of the device.

**c. Accuracy and Reliability:** Technique should grind sample to a powdered-sugar consistency. Collection method should gather the majority of the sample (or at least 38 mg) in the vial.

**d. Life in Service:** It will be used 5 days a week throughout the year, lasting at least that year. Around 30-40 samples will be analyzed daily.

**e. Shelf Life:** Stored on a laboratory bench at room temperature indefinitely (possibly several years).

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

**g. Ergonomics:** Sample should be easy to insert, and the user's hand should not be subjected to a cold temperature when inserting the sample.

**h. Size:** The device should fit on a laboratory bench with a maximum volume of 0.91x0.61x0.61 m (3x2x2 ft).

**i. Materials:** Only materials that can be subjected to extremely cold temperature such as metal or Pyrex glass should be used. Regular glass and plastic should be avoided.

**j. Cleaning:** The 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 \$5,000 – 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:**

- Polytron system – a homogenizer that operates at room temperature to grind up sample.
- Biospec BeadBeater – uses glass or stainless-steel balls to break apart sample, operates at room temperature.
- Biospec Cryogenic Pulverizer – uses hammer to crush pre-frozen sample in mortar.
- Jet Pulverizer – use air pressure to pulverize sample.

## Appendix C – 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

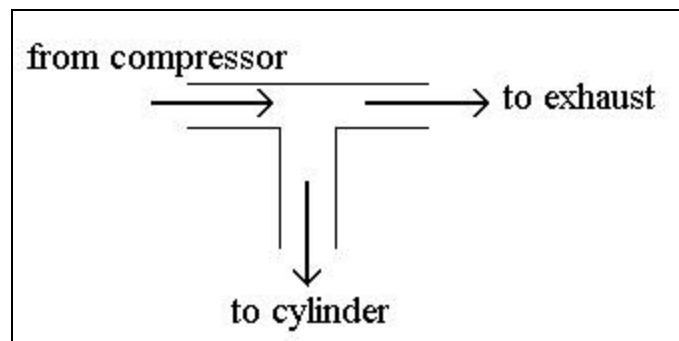


Figure C1: A 3-way valve is the simplest valve for cylinder control.

**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

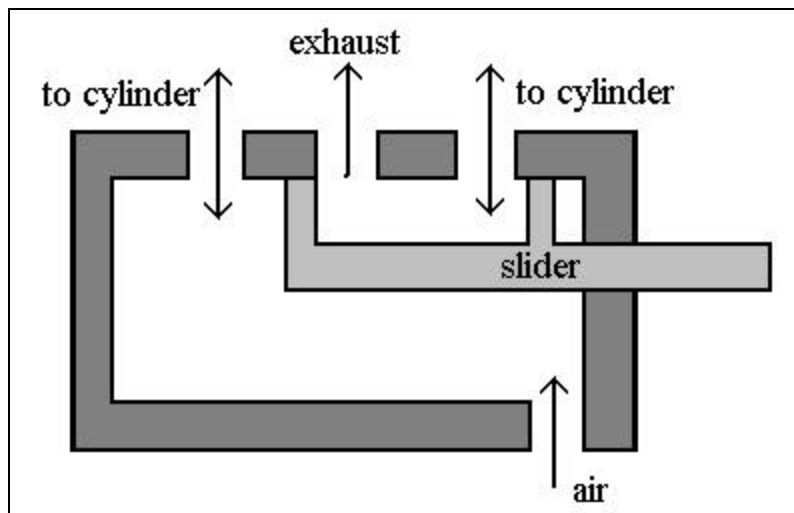


Fig C2: An example of a slide valve.

**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 D – 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.