High Throughput Quantitative Ex Vivo Murine Brain MRI Capsule

BME 301

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Abstract

Magnetic Resonance (MR) scanners are machines that are often used to produce high-resolution images of organic tissue such as brain tissue. Dr. JP Yu uses MR-scanning in order to learn more about the human brain by conducting research on murine brain samples, and translating how his findings compare. The current techniques used to conduct this research are relatively crude, requiring extended time, and unnecessary expenses and risks. This process includes mouse and rat brains being inserted into modified syringes and can take days to scan a large number of samples, not including post-processing time, as well as thousands of dollars per year on MRI scans. The client uses fluorinert, an expensive inert fluid, to improve image quality. This necessary substance is currently unavailable on the market, which poses another concern. The client wishes to reduce the time spent loading samples and post-processing, and seeks an efficient and reproducible loading system that is air-tight, improves imaging quality and accuracy, conserves fluorinert, and requires fewer resources. The team proposes two capsules: one with holes for rat brains, and one with holes for mouse brains. These holes will be sealed with a custom-sized cap featuring a rubber o-ring. Once fabrication is complete, the team will test for leaks, the presence of air bubbles, and the image quality of the scan.

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I. Introduction

A. Problem Statement

Dr. JP Yu's lab currently takes Magnetic Resonance (MR) scans of ex-vivo rat and mouse brains by loading the samples into modified syringes. This method of imaging involves individually loading and processing each brain separately which is inefficient and expensive. While the main goal of the previous semester was to streamline this process and be able to fit as many samples per scan as is feasible, the team's current goal is simply to overcome the obstacles encountered previously, and provide the lab with an effective, working product in order to begin reducing time and material costs as soon as possible. The main focus of this new goal is maintaining consistent orientation in the product and reducing the introduction of bubbles to the system. Relative to the lab's current system, however, the team will still ensure that the capsules will be more efficient, both in terms of cost as well as ease of use. To guarantee this, the design will be MR-compatible, airtight, and resistant to chemicals used during the process. This design must be reusable and reproducible, and it must establish a standardized, scientific procedure.

B. Motivation

Over 5 million people around the United States are estimated to have autism spectrum disorder (ASD) [6]. Dr. Yu's lab studies neurocognitive, neuropsychiatric, and neurodegenerative diseases such as ASD, which often goes hand in hand with illnesses such as schizophrenia and strokes. In order to do this, the lab tracks the biomarkers across rodent brains with these various neurodegenerative diseases [4]. When these biomarkers are observed, they can be connected to genes, environments, and gene-environment interactions in order to develop diagnosis and treatment. In Dr. Yu's lab, as well as many other labs across the country, the approaches to this are expensive, laborious, and cannot be reproduced. Thus, aiding the efficiency of his work will indirectly help the cause of learning more about not only psychiatric illnesses but the brain in general. Researchers specializing in neurodegenerative diseases run into similar problems of needing to find an efficient way to scan animal brains. One lab even makes 3D models of each individual brain in order to create the perfect template [5]. When non-standardized methods are used, lab processes lack efficiency in regard to time, money, and resources. If these imaging techniques can be better standardized, it will be much easier to compare and contrast results from different studies.

C. Existing Devices/Current Methods

3D Printed Brain Cradle

While there is no substantial market for competing devices for ex-vivo rodent brain holders for an MRI coil, numerous laboratories that perform MR imaging of ex-vivo brains have reported their methods within research papers. One such research paper reported their solution to holding ex-vivo marmoset brains in place while putting them through an MRI. First, they took an initial MRI of a specific marmoset brain by submerging the brain in an MR-compatible fluid (Fomblin) in a 50 mL syringe and padding the brain with gauze. This image was used to create a 3D-printed brain cradle (Figure 1) which held the same marmoset brain exactly in place inside a 50 mL syringe for a second MRI [6]. While this method is ideal for creating a perfectly-dimensioned brain holder, it wastes time and money because it requires an additional MRI for dimensioning and it is specified to the exact dimensions of an individual brain, therefore, it is not reusable. Although the paper did not specify costs, this technique could add hundreds of dollars and many hours of time, as it doubles the number of required scans, meaning if each scan cost the lab $500, over $1000 would be required to perform tests on each specimen, due to the required accuracy of the initial modeling scan.

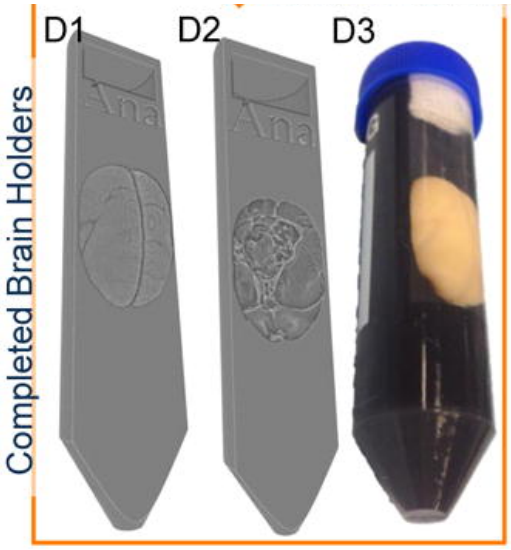


Figure 1. Image of 3D-printed brain cradle design with marmoset brain. [5]

Modified Syringes

Another important existing design for imaging mouse and rat brains is the system currently in place at Dr. Yu's lab, which involves holding brains in modified syringes (Figures 2 and 3). First, the ends of the syringes are cut so that they do not take up excess space in the MRI coil; 35 mL syringes are used for the rat brains and 10 mL syringes are used for the mouse brains. The brain is placed in the opening of the syringe, then, a small rod is used to gently push the brain to the back end of the syringe. Next, fluorinert is poured into the syringe to fully submerge the brain. The syringe plunger is then inserted into the tube to seal it and expunge any

air bubbles out of the syringe opening. When the syringe stopper is pushed up against the brain, the opening of the syringe is sealed with parafilm or a screw-on cap to prevent Fluorinert from leaking out of the tube. Once the brains are individually loaded into separate syringes, the centerline of the brain is marked on the outside of the syringe with a marker and the syringes are bundled into stacks, allowing them to scan 6 mouse brains at a time or 3 rat brains at a time.

Figure 2. Bundles of rat and mouse brains in modified syringes provided by Nick Stowe and Ajay Singh.

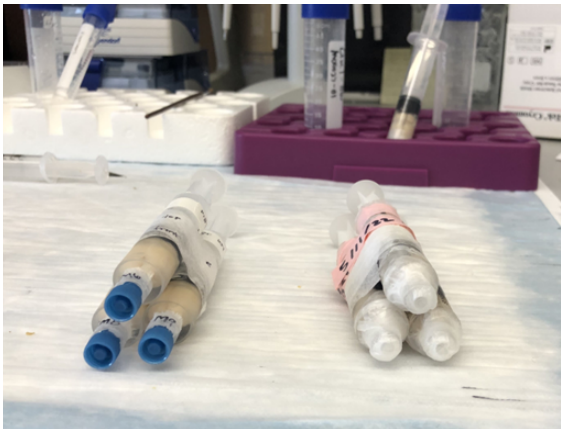


Figure 3. Front view of the modified syringes.

Computational Post-Processing

Dr. Yu's lab currently uses software written by a former employee of the lab to separate the multiple samples scanned at a time in the MRI. This software allows the lab to reorient the individual samples after they are scanned so that they exhibit a consistent orientation with each other. Other versions of this software that allows users to manipulate and rotate MRI images can be found online, such as Reorient [7] and NiftyReg [2]. While this software allows the lab to adjust the orientation of the brain samples, it does not solve the problem of how to load the brains into the MRI without damaging them, while also minimizing the use of Fluorinert, a very expensive fluid. Computational post-processing is an effective tool to reorient the brains if they are misaligned, but it takes a significant amount of time. A more streamlined approach to scanning rat and mouse brains is to maintain a consistent orientation amongst all samples as they are scanned in order to minimize post-processing time.

II. Background

A. Biological Research

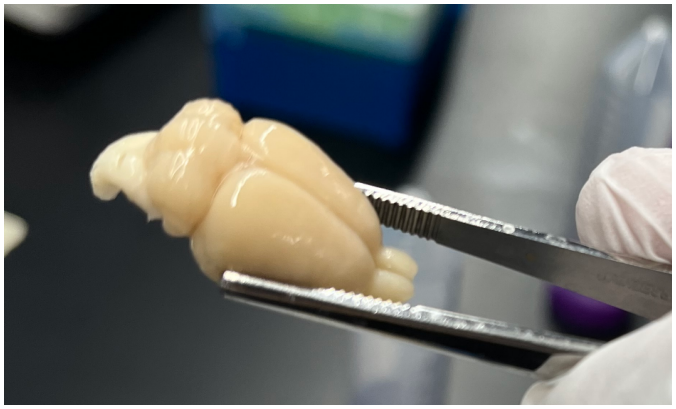


Figure 4. Rat brain donated by the client for measurements and testing.

Mouse brains have a similar structure to humans with a cerebral cortex, brain stem, and olfactory bulb. However, these components differ from humans in size proportionally, with the cerebral cortex and olfactory bulb being proportionally much smaller and larger respectively; the olfactory for example makes up 0.01% of human brains but 2% of mice brains [12]. Mouse brains also have significantly fewer and smaller gyri and sulci than a human brain, which reduces neuron interactions and higher-level thinking. Nonetheless, even though the human and mouse brains have many notable differences, they are still similar enough to find incredible value in studying them and comparing them to the brains of humans [10]. In addition, it was recently noted by the client that the brain samples are stored in paraformaldehyde (PFA) in order to dehydrate, or "fix," the brains while they are not in use. When removing the samples from PFA, they are submerged in phosphate-buffered saline (PBS) solution, in order to rehydrate them, preparing them for analysis.

B. Material Research

MRIs are made of several large, powered magnets that surround organic material. The MRI's strong magnetic field causes the polar and magnetic water molecules in the organic material to be realigned, which produces faint signals. These signals allow for cross-sectional imaging. These cross-sectional images are 3D topographical images that are able to image deep in tissue that would be nearly impossible to obtain through other means. [11]

Viable materials that can be used to make the design include FormLabs resin from the MakerSpace at Wendt Commons. The FormLabs clear resin is made from a photopolymer that is initially liquid and is cured to become hard plastic. The FormLabs materials library states that the

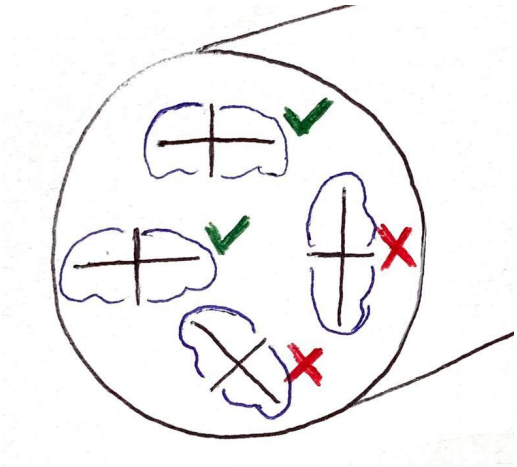
features and applications of standard clear include: polishes to transparency, internal channels, working with light, and semi-gloss surface [8]. Seeing through the plastic will allow the brains to be visible when they are inside the design, and this will allow the researchers to check for air bubbles and correct orientation.

C. Client information

Dr. JP Yu is an Assistant Professor in the Department of Radiology at the University of Wisconsin-Madison and the Neuroradiology Fellowship Program Director. Currently, Dr. Yu's laboratory at the Wisconsin Institute for Medical Research performs MRIs of rat and mouse brains to examine the impact of genes and the environment on quantitative brain microstructure [3].

D. Design Specifications

This design must fit more than 6 mouse brains per scan and more than 3 rat brains per scan. It must maintain a consistent orientation of the brain, where the brain's midline is parallel to the center axis of the MRI bore cylinder (Figure 5), while having all the brains in the same cross-sectional plane (Figure 6). The materials of the design must be MR-compatible and resistant to chemicals used in the lab, and the structure of the design cannot damage the brains during loading, unloading, or scanning. The system must be reusable and must create a leak-proof seal so that the brains can be fully submerged in Fluorinert without the risk of leaks, while also minimizing air bubbles, which would create image artifacts. Finally, the design must establish a standardized, scientific procedure. (*See Appendix A*)



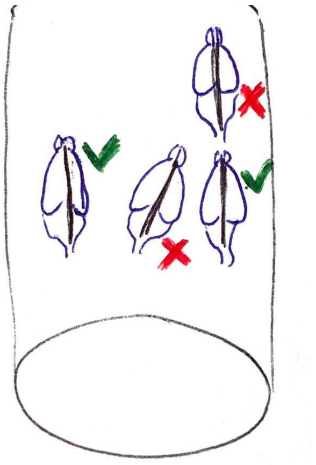


Figure 5. This illustration shows how the brains should be oriented parallel with one another in the MRI.

Figure 6. This illustration shows how the brains must be aligned parallel to one another with respect to their longitudinal axis. They must not be slanted. All the brains must be in the same cross-sectional plane, so they can not be in front of or behind one another.

III. Preliminary Designs

A. Funnel

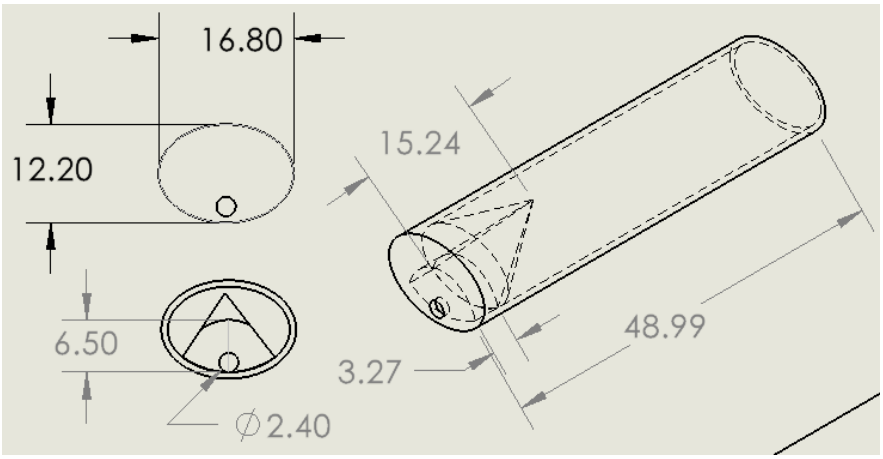


Figure IIIAi. Concept drawing of Funnel design with measurements from last semester's rat brain measurements.

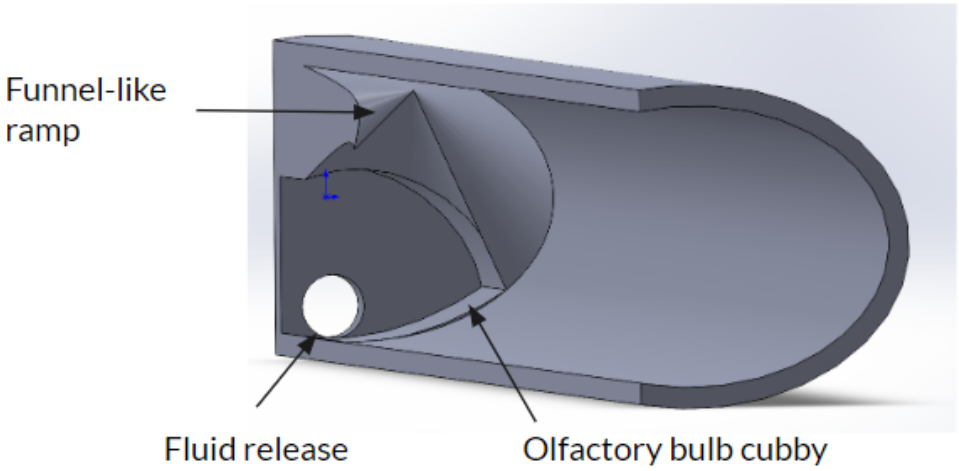


Figure IIIAii. Concept Drawing, SolidWorks design demonstrating the key aspects of the design.

This design is centered around a cone-shaped ramp that would conform to the shape of the brains in two ways; a cubby hole for the olfactory bulb and a funnel ramp for the cerebrum (see figure IIIAii). All measurements shown above are based on the average size of rat brains measured last

semester (Figure IIIAi). One fundamental aspect of this design that is not communicated well in the diagrams is that there would be multiple holes that are continuous with an integrated capsule just like what was done during the last design cycle last semester (see Appendix E).

The olfactory bulb cubby is to prevent the rotation around the centerline of the cavity. The funnel-like ramp prevents the brain from moving up and prevents some rotation. The concept is to guarantee correct orientation, and then the user will be able to secure the brain by compressing it slightly between the ramp/cubby and the stopper. The fluid release was placed so that air bubbles could more easily be removed from the capsule prior to sealing with a rubber stopper.

B. Conical Plunger

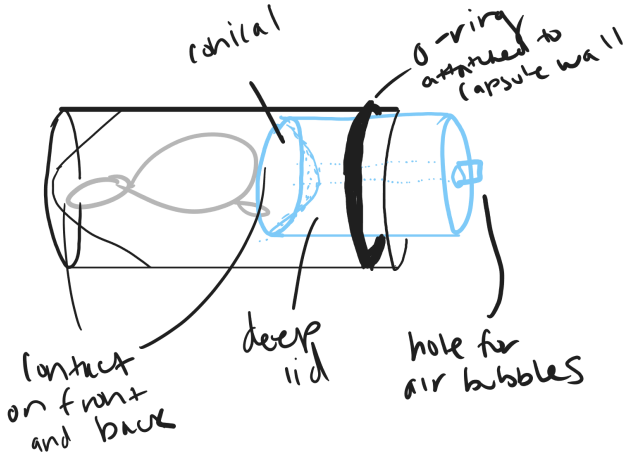


Figure 7. Labeled Drawing of Conical Plunger Design

The conical plunger design consists of three components: the plunger cap, o-ring, and capsule. At the far end of the capsule is a conical cradle for the olfactory bulb and the front half of the brain. The plunger cap is long enough to be adjusted to the different depths of the various brain sizes. There is also a conical cradle at the end of the cap to hold the back half of the brain. The idea is that there will be pressure on the front and back of the brain to keep it in place, but the sides may have no contact with the walls of the capsule. This is to accommodate the range of brain sizes the client wishes to scan. There is also a hole through the plunger cap to let air and excess flourinert out during the loading process. This hole would most likely be closed with a rubber stopper. The o-ring is mounted on the inner wall of the capsule to prevent leaking and air bubbles and to keep the plunger cap in place.

C. One-Way Stopper

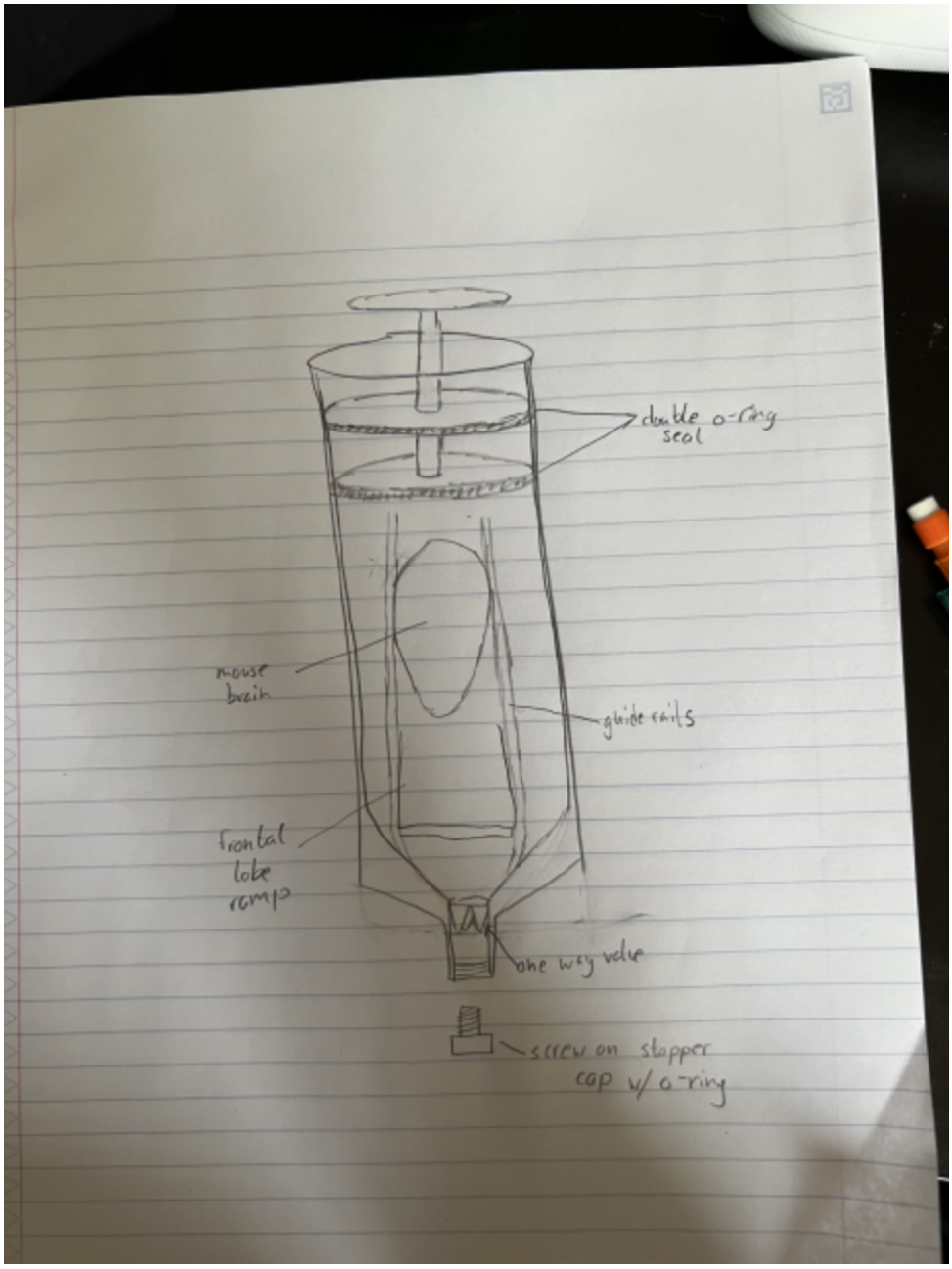


Figure 8. Labeled Drawing of One-Way Stopper Design

The one-way stopper design consists of several parts to prevent bubbles and dislodging of proper orientation. For bubble management, there are two integral features: the double o-ring seal and the one-way stopper valve with a screw-on stopper. The double o-ring seal would be attached to the plunger mechanism and the compartment in between would be filled with fluid, creating a barrier between the outside air and the interior of the capsule. The one-way stopper draws inspiration from the valves of the heart. When fluid is pushed through the syringe (up in the vertical direction), fluid is forced through the flexible stopper. With fluid on the external side of the valve, hydrostatic forces would tightly close the valve on itself. Additionally, a screw-on stopper cap with a small o-ring would be tightened over the filled tube to prevent further leakage

due to movement of the design. As for orientation, there are guide rails that line the edges of the tube in the axial direction. This would prevent the brain from twisting around the syringe axis. Secondarily, a ramp at the bottom of the design would also prevent the brain from straying from the desired orientation. This design would mainly be 3-D printed except for the o-rings and one-way stopper, which would be made of hard plastic material.

D. Basket

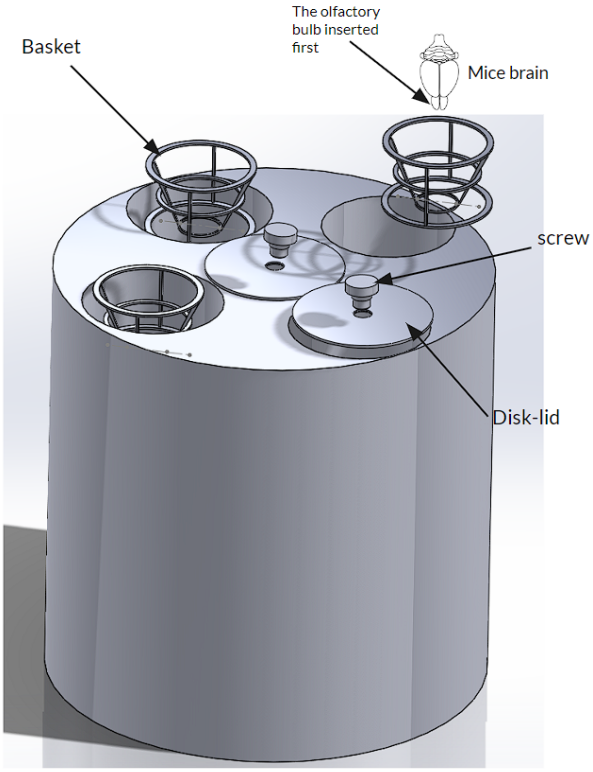


Figure 9. Labeled CAD assembly of Basket design

The basket design is a system made up of four key elements: the integrated capsule, baskets, disk-like lids, and screws. The basket design has been engineered with basket-like extensions that will cradle the brains without touching the wall of the capsule. This feature will provide a stable and secure orientation for the brain. This design can accommodate different sizes of brains, and the basket is removable, allowing for quick access to mice and rat brains. Additionally, disk-like lids with small holes are designed to remove bubbles that may affect the experiment's quality. However, the design may have some leakage issues, which is why screws will be added at the end to prevent any possible leakage. After the screws are added, access fluid will be removed to ensure the product's efficacy. The integrated capsule, baskets, and lids will be 3D printed in Makerspace and then compiled to create the final product.

Fabrication and Material Considerations for Design

3D printing was chosen as a means of fabrication for all of the designs due to the precision and detail that are required, in addition to the necessity of reproducibility. The final prototype will be printed in FormLabs clear resin. (*see Fabrication/ Development Process*).

IV. Preliminary Design Evaluation

A. Design Matrix

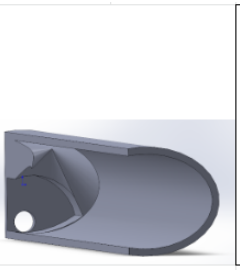
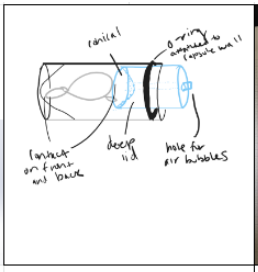
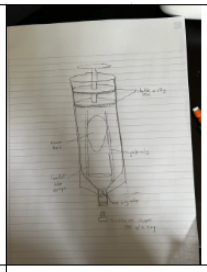
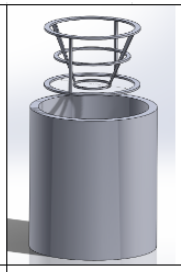
Design Matrix									
Criteria	Weight								
		Funnel		Conical Plunger		One-Way Stopper		Basket	
		Points of out 5	Adjusted Weight	Points of out 5	Adjusted Weight	Points of out 5	Adjusted Weight	Points of out 5	Adjusted Weight
Leakage and Bubble Management	30	4	24	3	18	5	30	3	18
Standardization and Consistency of Orientation	30	5	30	2	12	4	24	5	30
Ease of Fabrication	15	4	12	3	9	2	6	2	6
Ease of Use	15	4	12	5	15	3	9	4	12
Cost of Manufacturing	5	5	5	5	5	5	5	5	5
Safety	5	5	5	5	5	5	5	5	5
Total	**100**	88		64		79		76	

Figure 18. Design matrix of four preliminary designs.

When evaluating the three proposed designs, the two most heavily weighted criteria were Leakage and Bubble Management, and Standardization and Consistency of Orientation. The Funnel and One-Way Stopper scored well on leakage because they both have an intentional component to prevent leaking and air bubbles. The Funnel and the Basket both scored the highest

in the category of Standardization and Consistency of Orientation because they both have advanced components that intend to keep the brain in place. This category is the basis for both of these designs. The Ease of Fabrication category accounts for the risks of complications related to 3D printing and assembly throughout the fabrication process; the Funnel won this category because it consists of one component and no smaller parts. Ease of use was another criterion used to evaluate the designs; the Conical Plunger design won because it has components with fairly intuitive functions. It also resembles the design from the previous team, so the lab would have experience with this general concept. All the designs scored the same in Safety and Cost of Manufacturing because they would all be made of 3D-printed plastic or FormLabs resin, which are both MR-safe. The Funnel design scored the highest overall.

B. Proposed Final Design:

Based on the criteria assigned and the scores given for each design, the final proposed design is the Funnel design. The team plans to make a version of the Funnel design for mouse brains and a separate version for rat brains. This design incorporates the most important elements while also being simple to use.

V. Fabrication/Development Process

A. Materials:

Please note that this section is based on experience from the previous design cycle during the last semester. The prototypes will again be 3D printed. For 3D printing, the two most common types of printing at the MakerSpace are PLA and resin. Formlabs resin is water resistant because it is UV cured during the printing process and is later put in a bath with UV light to further polymerize layers of the print.[8] PLA is not polymerized between the layers.[8] This information was primarily received through informal interviews with technicians at the UW-Madison Makerspace, but see also [8] and [12].

B. Methods

Methods for fabrication are not fully defined at this stage of the project. Historic methods used last semester are as follows: 3D printing sizing arrays, 3D printing array of o-ring caps, 3D printing prototype capsules and caps, and testing them. Please see below for examples of these methods.

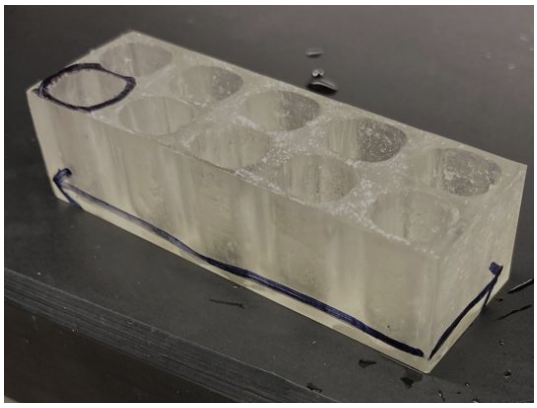


Figure 10. FormLabs clear mouse brain sizing array. Arrows indicate increasing size in either width or height. The circled hole was the most ideal hole: 9x7.5mm

To determine an optimal hole size for the mouse brains, an array was printed in FormLabs clear resin (Figure 24). This array was tested with mice brains and an optimal hole size was determined. This was then used for the prototype below.

Figure 11. FormLabs Clear mice brain capsule for 11 mice brains with 9x7.5mm hole dimensions and 2.5mm diameter watermark hole.

A cap for the mouse brain capsule was designed to seal the holes in the rat brain capsule (Figures 36, 37, and 38). This cap was designed with a groove to hold a #8 O-ring rubber (Thickness: 1.78 mm, Inner Diameter: 4.47 mm, Outer Diameter: 8.03 mm) [9]. This cap also features a hole that allows displaced fluid and air to escape which is plugged by a rubber stopper. 11 of these caps were printed in FormLabs clear resin (Figure 35).

Figure 12. FormLabs clear mouse capsule cap with o-ring.

C. Testing

See Appendix C for I-IV testing protocols from the previous design cycle.

Leak-resistance testing

The PDS specifies that the prototype must be completely leak-proof when sealed because the inserts will be filled with fluorinert. Leaking of this fluid not only creates an inconvenience for the user but poses a hazard as the Fluorinert could leak into the MRI and damage the machine. In order to test the degree to which the prototype is resistant to leaks, the team will fill all holes of the integrated insert with water and secure the caps onto the holes. The tester will then dry the outside of the prototype with a cloth, as some water will spill when the caps are secured. The tester will flip the prototype upside down for ten seconds, and then rest it on its side. It will be placed on a paper towel for 20 minutes so leaks can be observed. Updates will be recorded in *Table II (see Appendix C)*. If no leaks are observed from these tests, the design will be considered sufficiently leak-proof.

Air bubble reduction testing

The PDS states that air bubbles within the capsule must be minimized as much as possible, and air bubbles in contact with the brain must be completely eliminated. Air bubbles in the capsule could cause interference with the imaging of the brain and thus the image may not be usable. To test for air bubbles, the team will 3D print a clear version of the product. Then the team will fill each capsule with water and seal it (*see Appendix C: Air Bubble Testing)*. The team will then observe each capsule and record each air bubble, its size, and its location in the capsule. The team will repeat this process 3 more times with more data being recorded. If there are fewer than 3 air bubbles and they are less than 1mm in diameter, then the product would be considered acceptable for minimizing air bubbles.

Brain-fit testing

The PDS specifies that the brains should not be damaged during loading and unloading, and allow for simple and quick insertion and extraction. Damaged brains can cause image defects and prevent further testing, in addition, excess pressure on the brains will cause inaccurate images. To test for brain fit, the team will create multiple arrays of various shapes and sizes to see which will be best for mice and rat brains respectively. Six brains will be tested in each array. The smallest hole that fits all the brains loosely enough so that it can be removed via tapping will be the one that is used in the prototype.

Image quality testing

Image quality testing will test for any image distortions or artifacts present in an MRI scan with the prototype as well as any changes in the orientation of the brains. One of the specifications in the PDS is that air bubbles in the Fluorinert must be minimized because they can cause image artifacts [1]. The PDS also states that the brains must be held in a consistent alignment with each other throughout the scanning process, with a tolerance of 2 degrees. In order to test for these factors, the prototype will be given to Dr. Yu's lab to do a testing scan. One of the team members will show a lab member how to load and unload the brains from the prototype and will help them load the prototype for both rat and mouse brains. Once the test scan is completed, the results will be inspected for image distortions, and the time it would take to undergo post-processing will be evaluated. The degree of rotation necessary for each brain will be recorded in *Table III (See Appendix C)*. If the average rotation necessary in post-processing is less than 2 degrees, the prototype will have met the specifications for orientation consistency. Any image artifacts or distortions will be documented in *Table IV*. If image artifacts are not present or negligible according to the client, the prototype will be deemed sufficient in creating a high-quality image.

VI. Results

No results available at this time.

VII. Discussion

No discussion is available at this stage due to the lack of results.

VIII. Conclusion

In light of the new focus given by the client to shift the project to focus on achieving functionality, during this design cycle the team will attempt to produce a working prototype. The primary challenges from the previous design cycle last semester were large air bubbles, leaking of fluid from the capsule, inconsistent orientation of brains, and large brains not being able to fit in the capsule. To address these challenges, the team will be proceeding with the development of

the “Funnel” design which stabilizes orientation of the brain with a cubby and ramp, and tries to guarantee no bubbles by having an outlet of fluorinert strategically placed. This general design will be used in conjunction with components of other designs. For example, having a double O-ring sealed plunger and possibly a removable stabilizer for smaller brains. The team is optimistic of fulfilling the project given by the client.

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IX. Appendix

A. PDS

The Product Design Specification (PDS)

High Throughput Quantitative Ex Vivo Murine Brain MRI Capsule

Client: Dr. JP Yu, Radiology

Advisor: Dr. Melissa Kinney

Team:

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Date: Feb 10, 2023

Function

Dr. JP Yu's lab currently takes Magnetic Resonance (MR) scans of murine brains by loading the samples into modified syringes. This method of imaging involves individually loading and processing each model separately which is inefficient and expensive.

Our team plans to streamline the process by working with the client to create MR-compatible 3-D loading capsules for murine brains which will hold the samples in the correct alignment for a scan and be able to fit more samples per scan than their current procedure permits. The capsules will be more efficient, both in terms of cost as well as research throughput. It will allow for more reproducible scientific methodology, it will be reusable, and the design will ensure the capsule is airtight and resistant to chemicals used during the process.

Client requirements

High Priority Requirements (Highlighted by client as most important)

1. Functionality and Usability

The primary update to this PDS is the emphasis on producing a usable product by the end of the project deadline. This relates to the quality of the scans, the elimination of leaking and bubbles, and ensuring the product can be reusable. Formerly, the emphasis was increasing the number of brains that can be scanned per scan.

2. Orientation of murine brains

The brain's midline must be parallel with the center axis of the MRI bore cylinder, and all brains must remain oriented in the same plane, perpendicular to the axis. This orientation must be maintained through the scans. At the moment, the client loads the brains into 3 mL and 5 mL syringes (for mice and rats, respectively) and the midlines are marked with a pen on the outside of the syringe. Then directly before scanning, 3 syringes are taped together with the midlines oriented in the same direction based on the marking on the outside of the syringe. However, because the brains do not fit the syringes properly, they can twist or turn within the syringe and the midlines can become misaligned during transport from the lab to the MR-scanner. This does not necessarily decrease the quality of the scan; however, it does significantly increase the post processing time to realign the brains for comparison. This background explains why the orientation must be kept consistent.

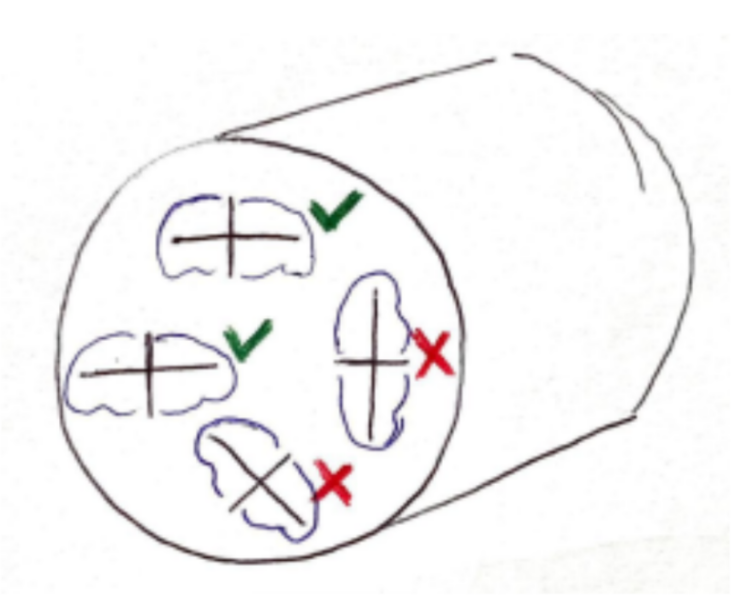

Figure 1. Brain's midline is parallel with the center axis of the MRI bore cylinder.

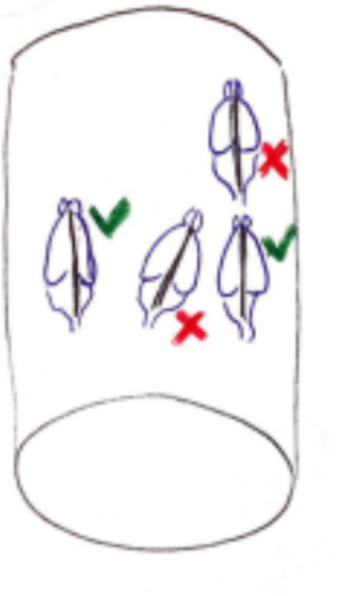

Figure 2. All the brains in the same cross-sectional plane.

3. Packing efficiency

Attempt to scan more than 3 rat brains and more than 6 mice brains at a time. These numbers are based on increasing the amount that the lab is currently able to scan using their current methodology. Each MRI scan takes 24 hours and costs $500, which highlights the importance and value of this requirement. It should be noted that only once in the last 5 years was a brain rescanned due to poor quality of image, therefore a large amount of time is spent during post processing to adjust and fix any problems with the scan. In addition, it takes 75 minutes just to load and unload 12 brains from their syringes.

Other Requirements

4. Have a complimentary scientific procedure to make the process standardized and reproducible. Because the clients are doing scientific research, they are interested in having reproducible results that any scientist could verify. Their current method does not fulfill this requirement.
5. MRI compatible: Material must not obscure image. (See design requirements for more details).[informal interview with MRI technician]
6. Reusable: The clients would like to reuse the device after removing and/or discarding brains.
7. Must not damage brains or deform brains during loading, unloading, and scanning. The lab studies the microstructure of the brain to draw implications on the impact of diseases and drugs.[3]
8. Seal in fluorinert without air. The capsule must have no air touching the brain and there must be a seal so that fluorinert does not spill. When air touches the brain, the barrier going from the magnetic properties of air to the properties of the brain produces a poor quality image.[informal interview with MRI technician]

Non-Essential Specifications

- Be able to retrieve fluorinert. Fluorinert is very expensive and it is currently not on the market due to supply chain issues from the pandemic. (cost consideration)
- Decrease the amount of fluorinert required for submerging brains. (cost consideration)
- Be able to retrieve the brain safely without damage (for further research). There are further tests that they sometimes want to do on the brain, which requires that they are not damaged after the MRI scan.
- Be able to resize the physical device for both mouse and rat brains. The implication was that the device can have notches or some form of physical adjustment to resize for one or the other type.
- Decrease the loading time of the brains. It takes about 1-3 hours to load and unload 12 brains with the current methodology.
- Minimize vibration of the capsules and components to improve MRI scan
- Can contract or restrict the size slightly to secure the brain during the MRI
- Have points of reference to align midlines of brain with the MRI machine bore axis
- Have space for a watermark
- Provide 2-4 usable units at the end of the semester

Design requirements

1. Physical and Operational Characteristics

a. Performance requirements:

Within a timeline of 18 months, the lab has been able to scan ~163 mice brains and ~63 rat brains. The product must be able to perform this many scans in this timeframe. It can be reusable or a product that can be mass produced, so long as the client can themselves replace the product when it stops functioning and perform this many scans per year.

The following standards should be maintained or improved upon. Current method of loading results in approximately 2 mL fluorinert loss per brain loaded, 12 brains loaded per hour, 12 brains unloaded in 15 minutes, and the loss of another 1 mL of fluorinert, in addition to the destruction/loss of a 3 mL and 5 mL syringe for mice and rats, respectively, when unloading each brain. With the lab's current loading method, they are able to scan up to 6 mice brains or up to 3 rat brains at a time. Each scan costs $500 and takes about 20 hours.

b. Safety:

MRI machines and rooms cannot contain any magnetic elements or metals which will react dangerously with the magnetic fields produced by the MR-scanner.[1] The product should not have sharp edges as the client will be loading the specimens by hand.

c. Accuracy and Reliability:

Brain samples should remain in correct alignment during the MRI scan within a margin of error of 2 degrees. Samples should fit snugly within the capsules in order to minimize shaking from mechanical vibrations caused by the MR-scanner, which could cause imaging issues. The capsules themselves should allow for simple alignment within the scanner to allow for standardization of image location. The capsules should also be easily reproducible for mass production.

d. Life in Service:

Must be in service for approximately 24 hours minimally, however, should be able to be used as a storage device for the brain samples for at least one year. Must also be able to be both sealed and reopened once, but preferably can be reused at least 50 times (approximately sealed and opened once per month).

e. Operating Environment:

Strong magnetic fields will be applied to the device in the MRI machine, which require high voltage and current to power the device, possibly causing high temperatures, however, the MR-scanner itself has its own cooling system to mitigate this.[2] Nonetheless, while the machine is powered the device will be exposed to high noise levels as well as vibration, and will likely be handled often. In addition, the device will be in contact with Fluorinert often. Thus, the device itself should be sturdy, waterproof, and should not move within the scanner.

f. Ergonomics:

Brain should not be damaged during loading and unloading, and allow for simple and quick insertion and extraction, and should also protect brain samples during scanning.

g. Size:

The device(s) must fit in a cylinder bore with a diameter of 37.29mm and a length of 50.35mm. The brains must be positioned within the relatively small scanning length of the coil (50.35mm), however, other parts of the device can extend outside of the coil.

h. Weight (redundant):

The device should weigh less than 15 kg when combined with brain samples as well as fluorinert.

i. Materials:

Magnetic metals should not be used since the product will involve MRI imaging. The product must not contain polar molecules that would be affected by the magnetic coil and consequently decrease imaging accuracy. A non-biodegradable, waterproof material that is compatible with Fluorinert is preferred.

j. Aesthetics, Appearance, and Finish:

The capsule will preferably be transparent for ease of visualizing the brain positioning. Texture should be smooth to avoid damage to the brain and coil when loading and unloading.

2. Production Characteristics

a. Quantity:

The client wants 2-4 units of the product and the ability to reproduce the product. Since the client performs experiments on both mouse and rat brains, this quantity will double to 4-8 total.

b. Target Product Cost:

The target product cost should not exceed $30. Additional costs from test printing prototypes should not exceed $50, for a target total cost of $80. Currently, the Yu lab is using 3.5 mL and 10 mL syringes to hold each rodent brain. This is likely costing them approximately $2.00 per syringe. This does not include costs from fluorinert and imaging film, which should be reduced by our design.

3. Miscellaneous

a. Standards and Specifications:

ASTM STP1438-EB is the standard for determining whether a device or material is safe for a Magnetic Resonance environment. The most critical factor of determining whether a material is MR safe is that the material does not contain any metallic or magnetic components. [1]

b. Customer:

The customer and user is our client.

c. Patient-related concerns:

There is no patient interacting with the product, and thus this section is not applicable. d.

Competition:

No competing devices or patents were found. One study that was cited numerous times by other articles showed scans with 4 brains in one array. The brains were oriented with the top of the cerebrum toward the center axis in a radial formation.

Sources

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Testing Protocols
I) Fit Testing:

The minimum requirement for a functioning product is that the brains fit inside the product. With 3D printing, this is not guaranteed and must be tested, even if careful measurements are taken and used.

Materials Needed:

- Printed, assembled prototype after post processing: filing and buffing.
- Sample brains

Procedure:

1. Inspect prototype for burs from 3D supports
    a. Remove burs as necessary
2. Take brain and insert it into hole using tweezers
3. Record how tight the hole is and how much perceived scrapping against the side of the hole was present
4. Remove brain
5. Record difficult of removing brain
6. Repeat steps 2-5 with all samples brains

II) Leak-proof Testing:

It is important to test for leaking in our prototype because fluorinert is the fluid that will be used in practice. Fluorinert is extremely expensive and difficult to obtain. One of the goals for this project is to conserve fluorinert as much as possible.

Materials Needed:

- Printed and assembled prototype
- Water

Procedure:

Pt I:

1. Fill each well with water.
2. Gently place lids appropriately in the wells.
3. Once lids are secure, hold vertically with lids facing down for 10 seconds.
4. Record observations.

Pt II:

1. Fill each well with water and apply the lids.
2. Let sit for 20 minutes.
3. Observe presence or absence of leaking.

III) Air Bubble Testing:

It is important to test for air bubbles within the capsule because air bubbles can create distortions to MRI images. These errors can increase post-processing time and, and in some cases, require another $500 scan.

Materials Needed:

- Printed and assembled prototype
- Water

Procedure:

1. Fill each well with water.
2. Gently place lids appropriately in wells.
3. Observe overflow of water.
4. Tilt prototype and observe the presence of absence of air bubbles.

IV) MRI Image Quality Testing:

MRI testing is the most important testing for verifying the usability of the final product. It also involves loading brains. It is done in three different levels, with increasing measurement time. It will demonstrate how the brains are kept in correct orientation, whether the seal is successful, and whether air bubbles are present or have formed. It will also provide quantitative

results about the quality of the scan compared to previous scans in the confidence levels of post processing research/tests of previous versus new scans.

Materials Needed:

- Prototype
- Ex-vivo murine brains
- 9.4-T Bruker MRI machine, with associated coil and computer software etc.
- Fluorinert (approx. 40ml).
- Tweezers

Procedure:

Preparation:

1. Load brains into the prototype.
2. Fill hole with fluorinert, making sure to remove air bubbles.
3. Seal hole with cap.
4. Repeat step 2 and 3 until all holes of the insert are filled.
5. Verify that there are no air bubbles and no leaking.

Pt. 1:

1. Perform a scout scan of insert (approx. 1 minutes)
2. Save data
3. Verify that no black marks, caused by water marks, are present on scans
4. Analyze orientation of brain in scans

Pt. 2 (if Pt1 successful):

1. Perform T1 scan of insert (approx. 30 minutes)
2. Save data
3. Verify that no black marks, caused by water marks, are present on scans
4. Analyze orientation of brain in scans

Pt. 3 (if Pt2 successful):

1. Perform diffusion velocity scan (approx. 20 hours)
2. Save data

Post Scans:

1. Remove brains and record difficulty and quality of the brain afterwards
2. Verify that no black marks, caused by water marks, are present on scans
3. Analyze orientation of brain in scans
4. Analyze quality of scans compared to previous trials.

B. Fabrication Instructions

Overview Steps:

1. Print the mouse brain capsule, 11 mouse capsule caps, the rat brain capsule, and four rat capsule caps in FormLabs clear resin.
2. When the prints are finished, the resin supports must be removed with metal wire cutters.
3. Some resin supports will leave small extrusions on the exterior of the prints when they are removed. These extrusions must be filed down with sandpaper or small metal files.
4. If any parts of the print still feel tacky, this can be solved by scrubbing the tacky area with isopropyl alcohol with a toothbrush. Let the part dry, and it will feel smooth and not sticky.
5. Finally, stretch the rubber O-rings around the grooves of caps.

O-ring Calculation Python Program:

When calculating o-ring groove dimensions for the caps, three dimensions must be considered to make an adequate seal: percent squeeze, percent stretch, and percent gland fill. Percent squeeze should be between 10-25%, percent stretch 1-5%, and percent gland fill should be 65-85%. A python function was written to perform these calculations to determine the optimal depth and width of the o-ring groove for the caps [2].

```
In [1]:
import math

In [2]:
def o_ring_data(w_c, perimeter, inner_d, thickness = 1.78):
    decimal_stretch = perimeter / (math.pi * inner_d)
    percent_stretch = (decimal_stretch - 1) * 100

    percent_w_o_decrease = 0.5 * percent_stretch + 2.5
    decimal_w_o_decrease = percent_w_o_decrease / 100
    cs_area_inital = math.pi * (thickness / 2)**2
    cs_area_decreased = cs_area_inital * (1 - decimal_w_o_decrease)
    w_o = 2*(math.sqrt(cs_area_decreased / math.pi))

    percent_squeeze = ((w_o - w_c) / w_o) * 100

    percent_gland_fill = 70
    decimal_gland_fill = percent_gland_fill / 100
    cs_area = ((w_o/2)**2) * math.pi
    groove_height = cs_area / (w_c * decimal_gland_fill)

    o_ring_data_dict = {}
    o_ring_data_dict["Percent Stretch"] = percent_stretch
    o_ring_data_dict["Percent Squeeze"] = percent_squeeze
    o_ring_data_dict["Percent Gland Fill = " + str(percent_gland_fill) + "% when height is "] = groove_height
    return o_ring_data_dict

In [43]:
o_ring_data(1.47, 17.49, 4.47)

Out[43]:
{'Percent Stretch': 24.546754124261728,
 'Percent Squeeze': 10.543955202770631,
 'Percent Gland Fill = 70% when height is ': 2.0610559969160134}
```

Figure X. O-Ring calculations function in Python.

E. Final Prototype From Previous Semester

I) Dimensions

Mice Final Prototype CAD drawings (cap and capsule):

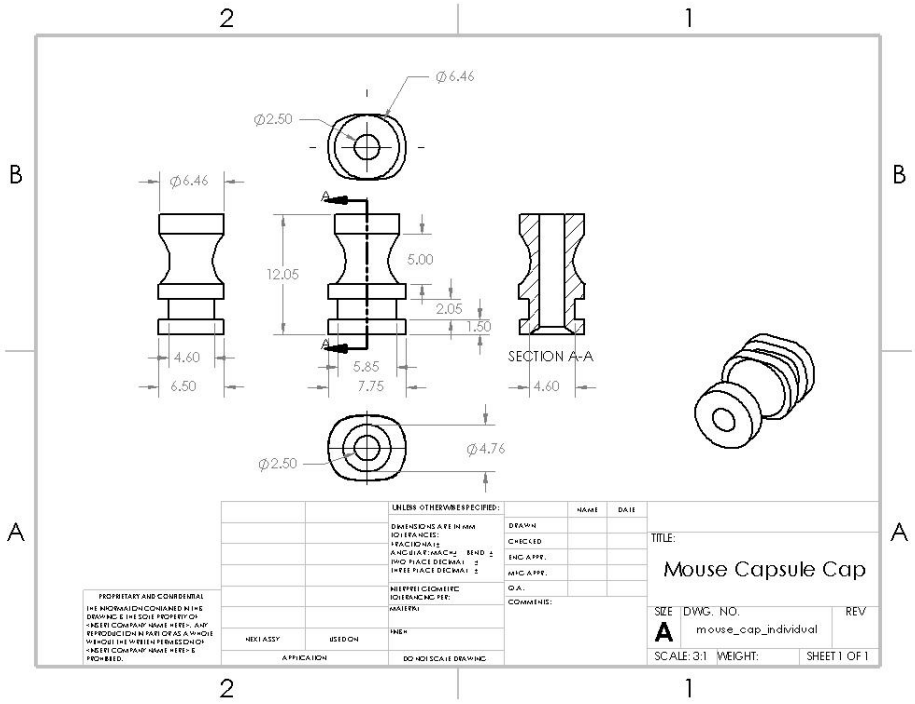

Figure X. Mice cap SolidWorks drawing with dimensions in mm.

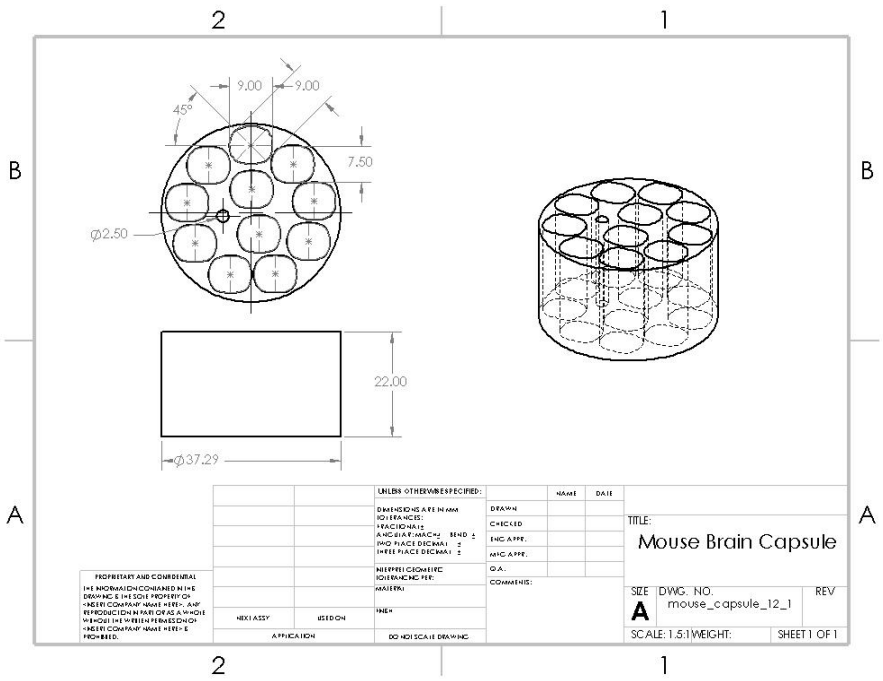

Figure X. Mice capsule SolidWorks drawing with dimensions in mm.

Rat Final Prototype CAD drawings (cap and capsule):

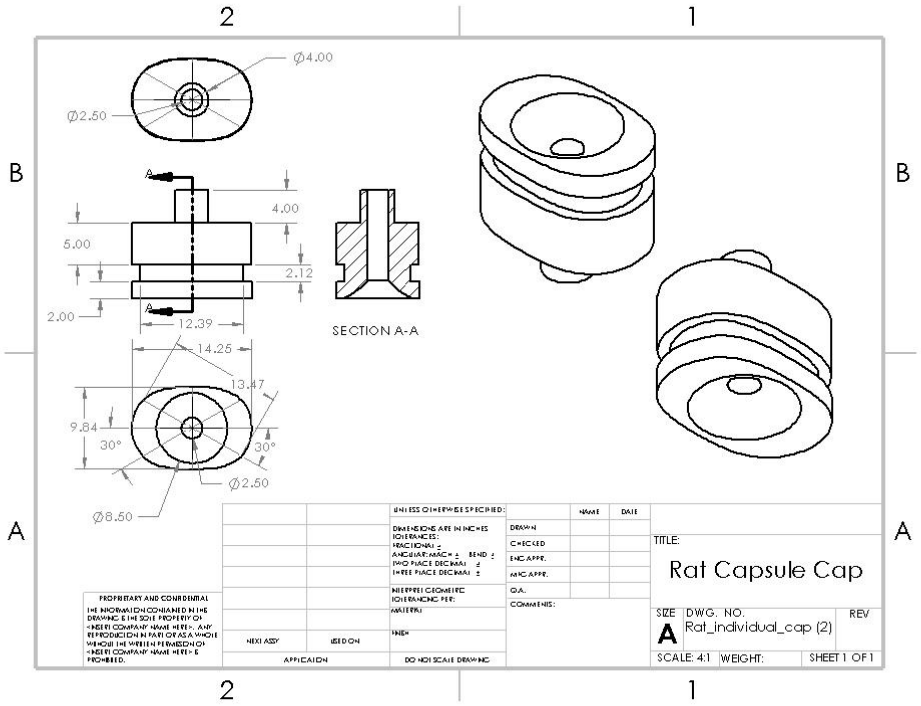

Figure X. Rat cap SolidWorks drawing with dimensions in mm.

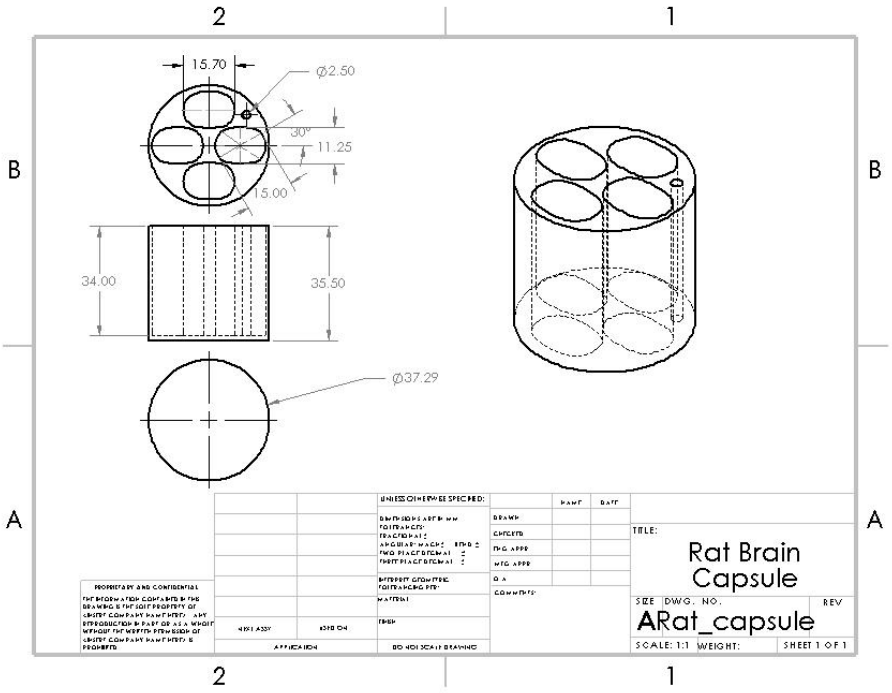

Figure X. Rat brain capsule SolidWorks drawing with dimensions in mm.

II) Images of 3D Prints

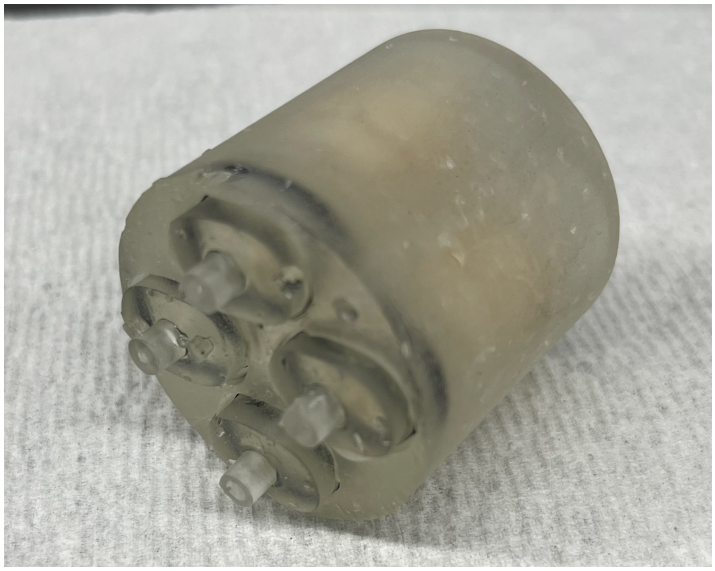

Figure 43. Loaded rat capsule for leak testing.

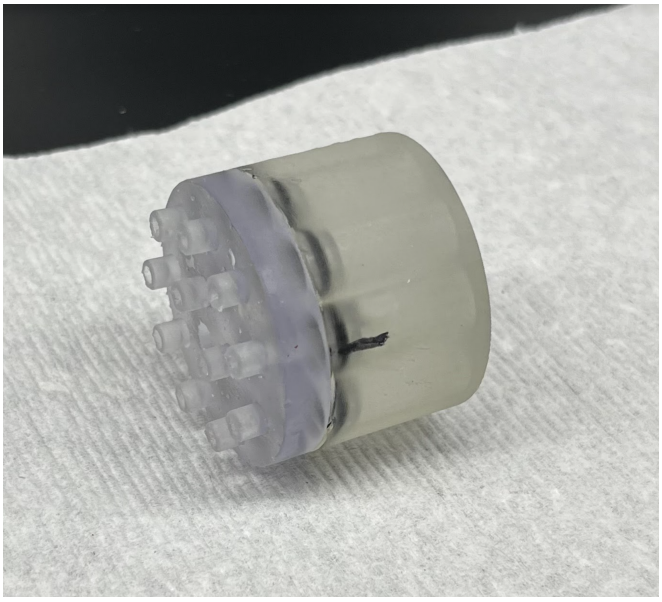

Figure 44. Loaded mice brain capsule for leak testing (no brains).

III) Results from Testing Previous Prototype

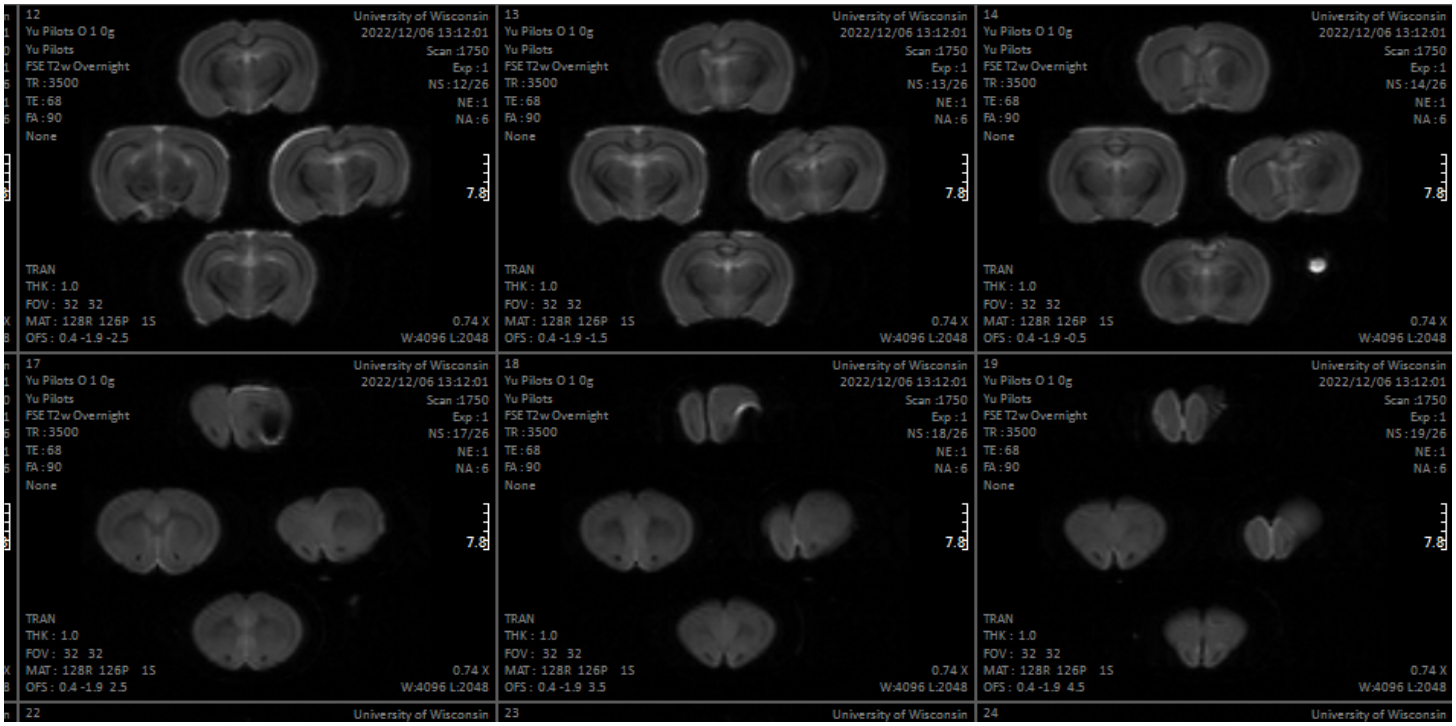

Figure 47. T1 Scan on 4.7T Agilent MRI machine. 6 chosen section images out of the 25 total. Right brain is unsymmetrical which indicates tilting. Black marks on the top brain on bottom sections are caused by air bubbles being in contact with the brain.
SEE Appendix Figure 49 for all section images.