

High Throughput Quantitative Ex Vivo Murine Brain MRI Capsule

BME 200/300

December 14, 2022

Client:

Dr. JP Yu

Nick Stowe, Ajay Singh

Department of Radiology, UW-Madison

Advisor:

Dr. Kris Saha

College of Engineering, School of Medicine and Public Health, UW-Madison

Team Members:

Team Leader: Joel Nitz

Communicator: Erwin Cruz

BWIG: Ray Steinlage

BSAC: Leo DiCataldo

Co - BPAG: Anna Mercord and Allicia Moeller

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 less 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 leaking, the presence of air bubbles, and the image quality of the scan,

Table of Contents

Abstract	2
I. Introduction	4
A. Problem Statement	4
B. Motivation	4
C. Existing Devices/Current Methods	4
II. Background	7
A. Biological Research	7
B. Material Research	7
C. Client information	8
D. Design Specifications	8
III. Preliminary Designs	9
A. Cylinder Insert Design	9
B. Honeycomb Design	10
C. Integrated Insert	11
IV. Preliminary Design Evaluation	14
A. Design Matrix	14
B. Proposed Final Design:	15
V. Fabrication/Development Process	15
A. Materials:	15
B. Methods	15
C. Final Prototype	22
D. Testing	24
VI. Results	25
VII. Discussion	30
VIII. Conclusion	31
IX. Appendix	34
A. PDS	34
B. Materials	41
C. Testing	43
D. Fabrication Instructions	49
E. Final Prototype Dimensions	50

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. The team plans to streamline this process by working with the client to create an MR-compatible, 3D loading system for rat and mouse 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. Relative to the lab's current system, the capsules will be more efficient, both in terms of cost as well as ease of use. The design must be airtight and resistant to chemicals used during the process. This design must be reusable, 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) [8]. 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 [5]. 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 for 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 [6]. When non-standardized methods are used, lab processes lack efficiency in regards 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 of 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 amount of required scans, meaning if each scan costed the lab \$500, over \$1000 would be required to perform tests on each specimen, due to the required accuracy of the initial modeling scan.

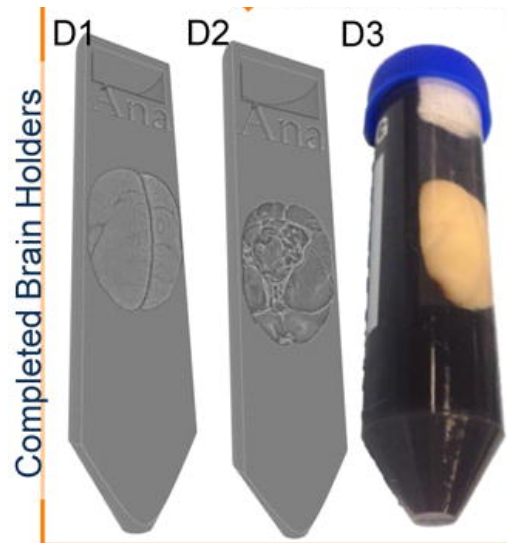


Figure 1. Image of 3D-printed brain cradle design with marmoset brain. [6]

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.

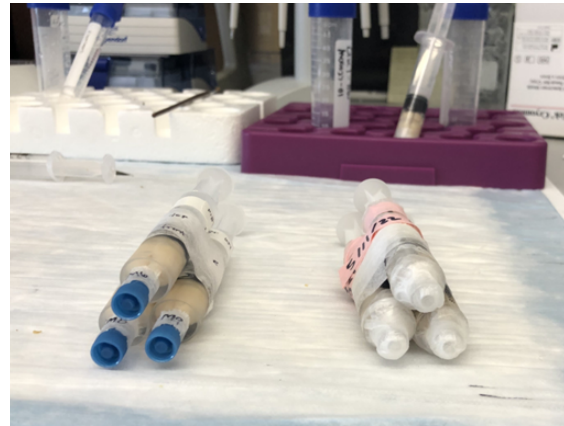


Figure 3. Front view of the modified syringes.

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 [9] and NiftyReg [3]. 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 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 [16]. 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 brain have many notable differences, they are still similar enough to find incredible value in studying them and comparing them to the brains of humans [12].

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. [14]

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 [10]. 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 in 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 [4].

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*)

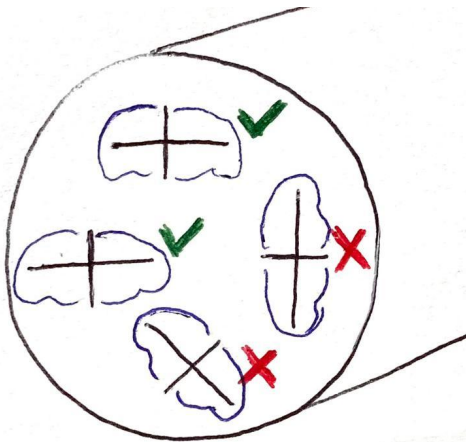


Figure 5. This illustration shows how the brains should be oriented parallel with one another in the MRI.

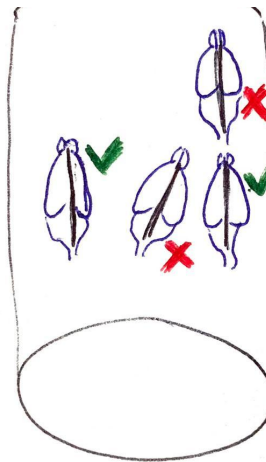


Figure 6. This illustration shows how the brains must be aligned parallel to one another with respect to their longitudinal axis. They must not be slanted. All the brains must be in the same cross-sectional plane, so they can not be in front of or behind one another.

III. Preliminary Designs

A. Cylinder Insert Design

The cylinder insert design is a combination of 3D-printed circular capsules and a 3D-printed complimentary insert. The capsules would hold the brain and have a fin to go in between the hemispheres of the brain down the longitudinal fissure (Figures 7 and 8). This would secure the orientation of the brain. The individual capsules would be filled with Fluorinert after the brains are inserted, and sealed with a cap that has a rubber O-ring to prevent leaks. The capsules would then fit into a larger insert with more notches that would hold the capsules and prevent the capsules from rotating once inserted (Figure 10). This insert would be the size of the MRI bore.

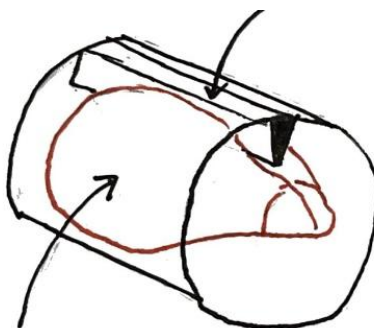


Figure 7. Visual representation of the function of the fin.

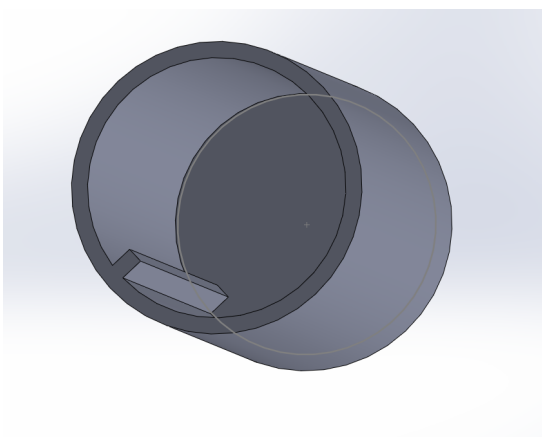


Figure 8. Individual brain capsule.

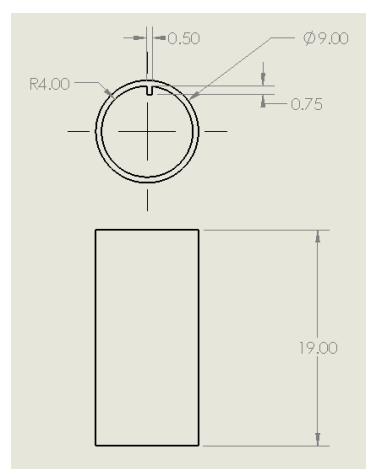


Figure 9. Individual brain capsule dimensions (mm).

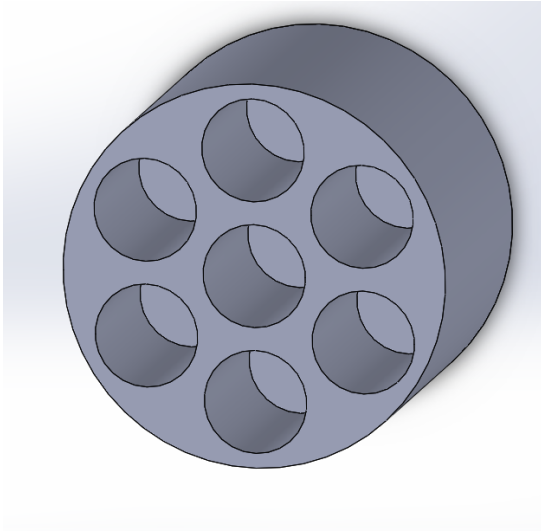


Figure 10. Insert to hold capsules.

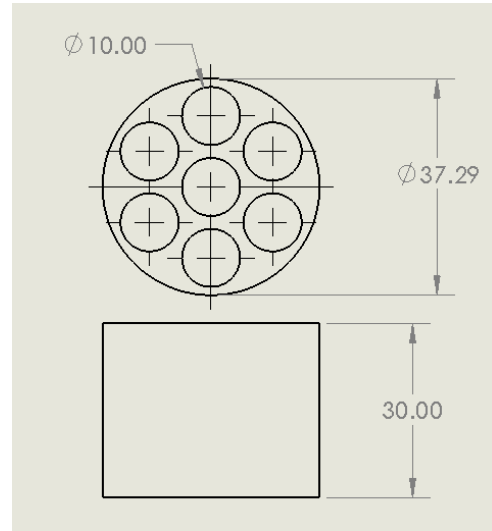


Figure 11. Dimensions of insert to hold brains (mm).

B. Honeycomb Design

The Honeycomb design is a honeycomb-like lattice of 3D-printed, separable hexagonal capsules which have a locking mechanism on the outside (Figure 12). The main inspiration for this design was the guaranteed orientation of the brains: the hexagonal shape would secure the orientation of the brain within the capsule. The interlocking mechanism would secure the orientation of the capsule as a whole, and allow for a modular design that could be changed for any size of MR-scanner.

One additional idea was to have a drawer that slides in and out of the hexagonal form. This idea was for ease of loading and unloading.

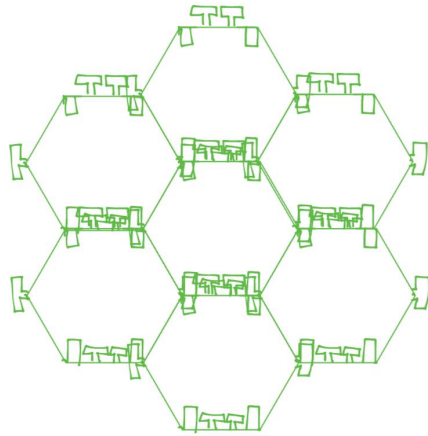


Figure 12. Honeycomb lattice: illustration of interlocking mechanism. Height of internal hexagon 8.27mm. Wall thickness 1.2mm.

C. Integrated Insert

The integrated insert is a 3D-printed cylinder that perfectly fits the bore of the MRI coil. The holes for the samples are integrated in this plastic cylinder. Measurements used for this design were the averages of the sizes of the rat brains from the client-donated samples plus their standard deviation plus any other factors (*Table V, See Appendix*). For example, the depth of the hole is the average length (28.75mm) + standard deviation (0.785mm) + 5 mm for the stopper to enter. Ovular shapes were drawn with a minimum wall thickness of 0.8mm which was found to be the absolute minimum thickness for 3D printed nylon, the previous material of choice [15]. These outlines were then copied and pasted and aligned by eye.

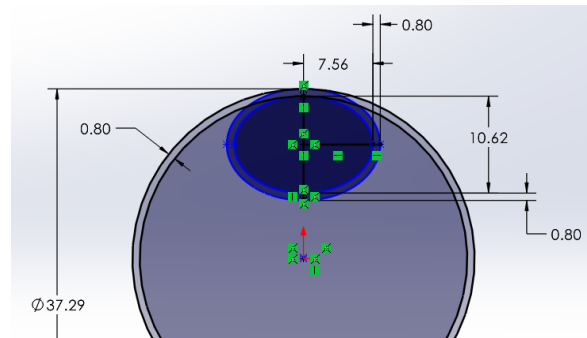


Figure 13. Overall diameter of the insert and dimensions used for rat brains.

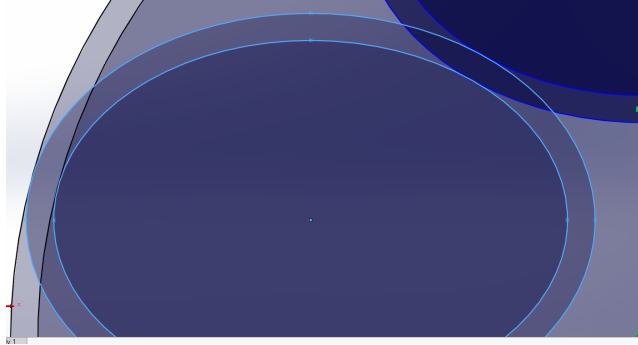


Figure 14. Aligning of the cells by eye and drag dropping with mouse, not using computer software.

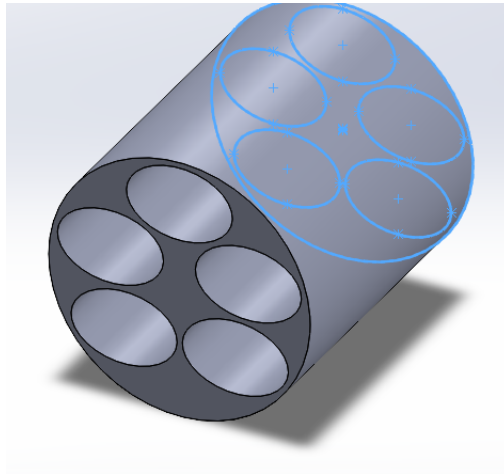


Figure 15. Preliminary “empirically based” design which would hold 5 brains.

The orientation is guaranteed in the Integrated Insert because the oval would fit the brain width and height of the brains stopping rotation around the axis of the MRI bore. There is also the added idea of implementing a ceiling ramp that would press against the front of the brain on its cerebrum with space for the olfactory bulb to fit under this ramp. The holes would then be sealed by individual stoppers or corks made of rubber. The holes would be filled to the brim and when the stopper is inserted will cause the fluorinert to overflow. This overflowing methodology is meant to guarantee that no air bubbles are left in the hole.

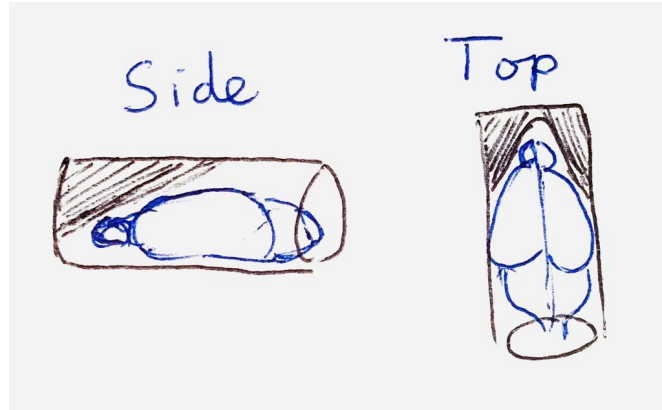


Figure 16. Ramp conceptual visualization.

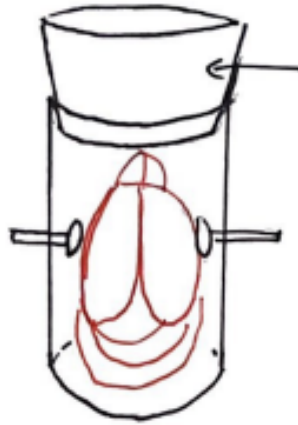


Figure 17. Stopper concept for sealing the holes.

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 is required, in addition to the necessity of reproducibility. Nylon was originally chosen because of its mechanical properties, but due to difficulties of the printed design in practice, the final prototype is printed in FormLabs clear resin. (see *Fabrication/Development Process*).

IV. Preliminary Design Evaluation

A. Design Matrix

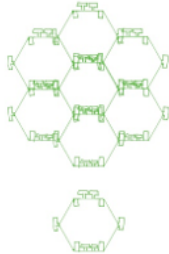
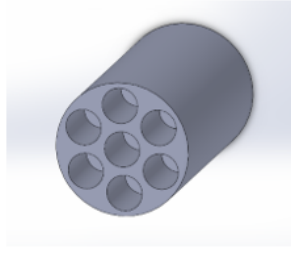
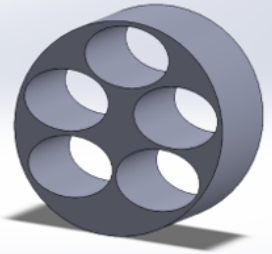
Design Matrix		points out of 5		adjusted weight			
Criteria	Weight	Honeycomb:		Cylinder Insert Design		Integrated Insert	
							
Packing and Unpacking Efficiency	30	5	30	4	24	5	30
Standardization and Consistency of Orientation	20	5	20	4	16	4	16
Airtight	15	3	9	5	15	3	9
Ease of Use	15	4	12	3	9	3	9
Durability	10	3	6	4	8	5	10
Time and Cost of Manufacturing	5	3	3	2	2	5	5
Safety	5	5	5	5	5	4	4
Total	100	80		79		83	

Figure 18. Design matrix of three preliminary designs.

When evaluating the three proposed designs, the two most heavily weighted criteria were packing efficiency and consistency of orientation. The integrated insert and the honeycomb design scored well on packing efficiency because they both minimize the amount of plastic used in between brains. The honeycomb design scored highest in the consistency of orientation category because its hexagonal shape would prevent the brain from freely rotating. The airtight category accounts for both the degree to which the design would resist leaks as well as minimize air bubbles; the cylinder insert design won in this category because its individual capsule and its cylindrical shape would allow for efficient sealing. Ease of use was another criterion used to evaluate the designs; the honeycomb design won because it has fewer components than the cylinder design and it is stackable, which allows for easy assembly. All the designs scored the same in safety because they would all be made of 3D-printed plastic or FormLabs resin, which are both MR-safe. The integrated insert 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 integrated insert. The team plans to make a version of the integrated insert for mouse brains and a separate version of the integrated insert for rat brains. This design incorporates the most important elements while also being simple to use. It also allows for the largest number of brains to be scanned at a time, which provides maximum efficiency.

V. Fabrication/Development Process

A. Materials:

The first material that was considered was 3D-printed nylon. This material fits the criteria specified in the PDS (*See Appendix A*). Nylon is both non-magnetic and non-polar, so it would not be reactive in an MRI. This, therefore, poses no safety concerns. It is non-biodegradable, so there would be minimal concern for the longevity of the product. Additionally, nylon is available to use in a 3D printer, which will be the primary mode of fabrication (*See Appendix B*).

After the first print, it was discovered that nylon and PLA would have microscopic space between all of the layers, meaning that it would absorb and leak water. For this reason, the team chose to print the prototype using FormLabs resin instead, which is waterproof.[10] However, size testing was still conducted using PLA due to cost efficiency. Another problem was the discrepancy between the size of FormLabs resin and PLA prints. The size for the holes that worked with the test prints in PLA were too small when printed in resin. To avoid any more errors, the team continued to print everything, including test prints, in 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.[10] PLA is not polymerized between the layers.[10] This information was primarily received through informal interviews with technicians at the UW-Madison Makerspace, but see also [10] and [16].

B. Methods

To begin the fabrication process, the team printed the integrated insert design for rat brains in FormLabs black resin and clear PLA. When tested with real rat brains, however, the brains did not fit in the elliptical holes of the capsule (Figures 19 and 20). An integrated insert capsule was also printed for mouse brains in FormLabs black resin, but the mouse brains did not fit into these holes either (Figure 21).

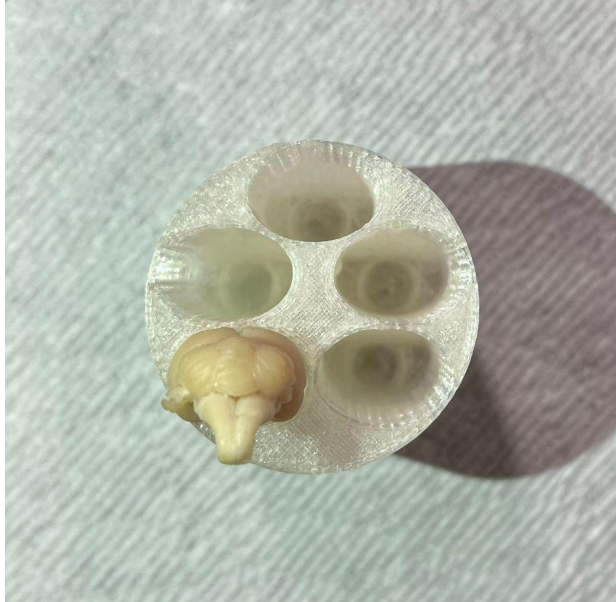


Figure 19. Rat brain not fitting in the hole of the initial prototype clear PLA print.

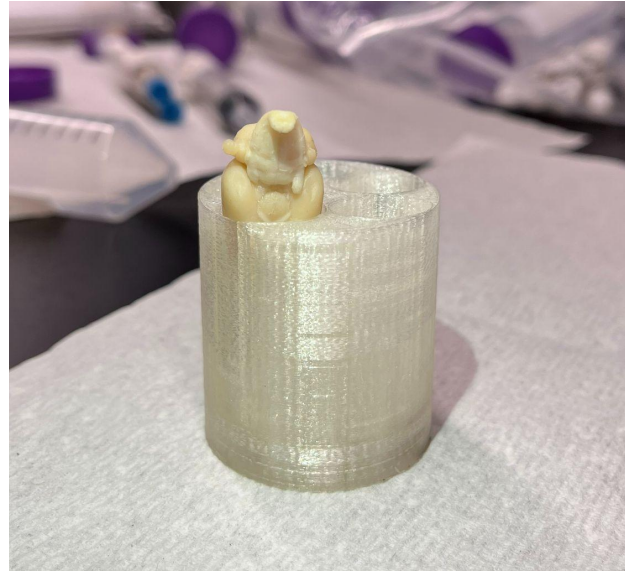


Figure 20. Side angle of brains not fitting.



Figure 21. Mouse brain not fitting in initial mouse capsule prototype in FormLabs black.

This prompted the group to reassess the optimal hole shape by designing and printing a brain sizing array in PLA (Figures 22 and 23). This array features four different hole shapes, each incremented in size five times.

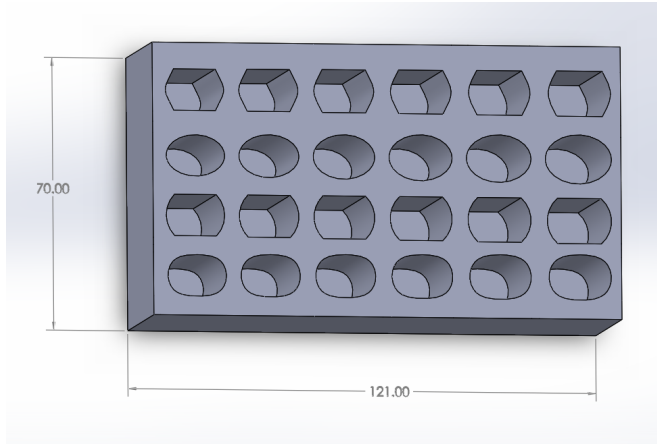


Figure 22. SolidWorks image of rat brain sizing array.

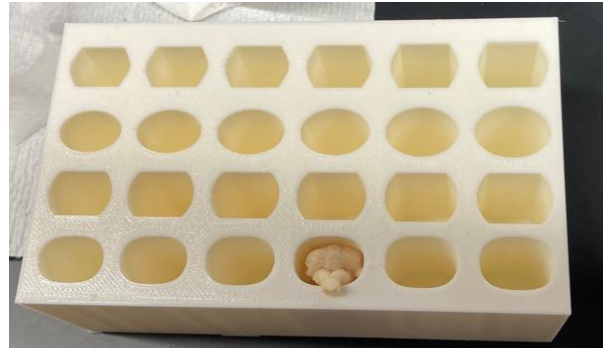


Figure 23. White PLA print of sizing array.

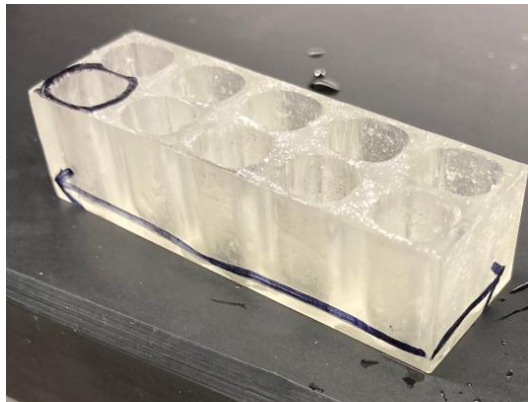


Figure 24. FormLabs clear mouse brain sizing array.

This rat brain sizing array was tested for fit with rat brains and an optimal hole size was found.

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. The prototypes were then updated with the dimensions from the sizing arrays (Figures 25, 26, and 27). While the original design for the rat brain capsule was designed to fit five brains, this had to be decreased to four brains when the dimensions of the holes were changed. The mouse brain capsule was also updated with new dimensions from the sizing array (Figures 28, 29, and 30), however, it could still hold 11 brains. A 2.5mm diameter hole was added to the rat and mouse brain capsules to act as a watermark that would be filled with water and sealed with a rubber plug for each use.

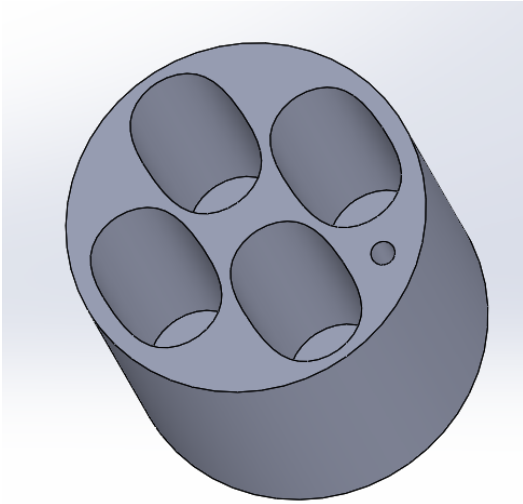


Figure 25. Solidworks rat brain capsule with updated hole dimensions and 2.5 mm diameter hole for watermark.



Figure 26. FormLabs clear resin rat brain capsule.

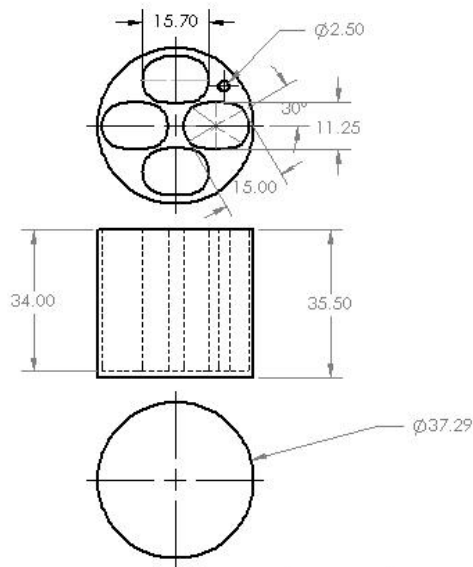


Figure 27. Dimensions of rat brain capsule in mm.



Figure 28. FormLabs Clear mice brain capsule with updated hole dimensions and 2.5mm diameter watermark hole.

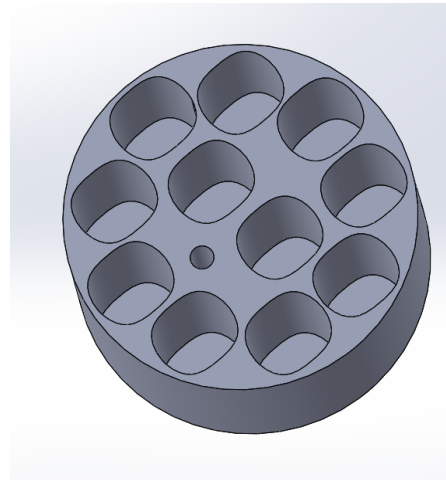


Figure 29. Solidworks image of mice brain capsule.

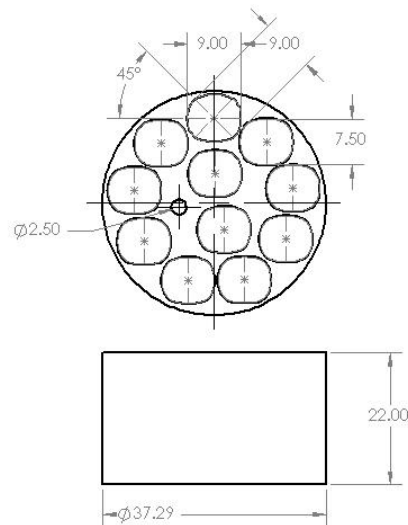
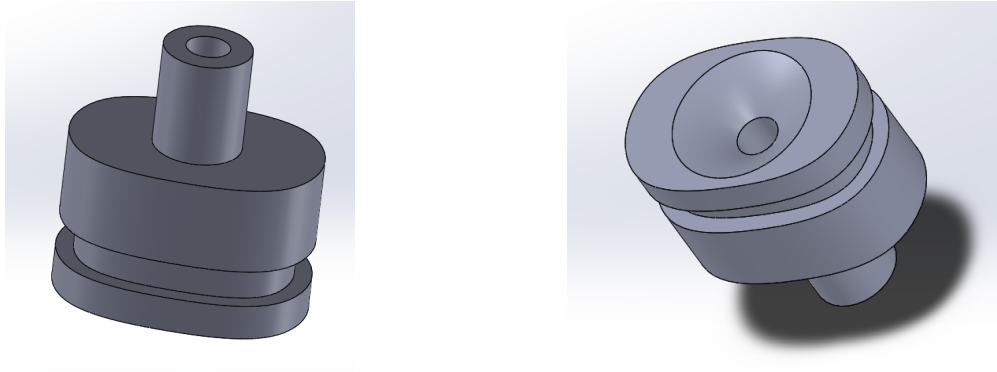


Figure 30. Dimensions of mice brain capsule in mm.

A cap was designed to create a leak-proof seal for each individual capsule (Figures 31, 32, and 33). The cap was designed with a groove for a #12 rubber O-ring (Thickness: 1.78 mm, Inner Diameter: 9.25 mm, Outer Diameter: 12.81 mm) which creates a seal with the walls of the capsule when submerged into fluid[11]. The cap also has a hole that allows displaced fluid and

air bubbles to escape. This hole is sealed manually by a small tapered silicone plug. Four caps were printed in FormLabs clear resin (Figure 34).



Figures 31 and 32. Solidworks rat brain capsule cap from two different angles.

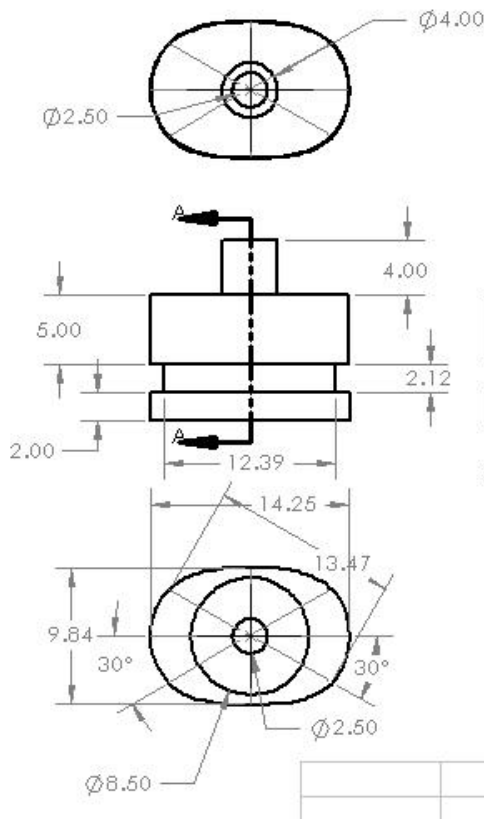


Figure 34. Rat brain capsule cap in FormLabs clear resin with rubber o-ring.

Figure 33. Dimensions of rat brain capsule cap in mm.

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) [11]. 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 35. FormLabs clear mouse capsule cap with o-ring.

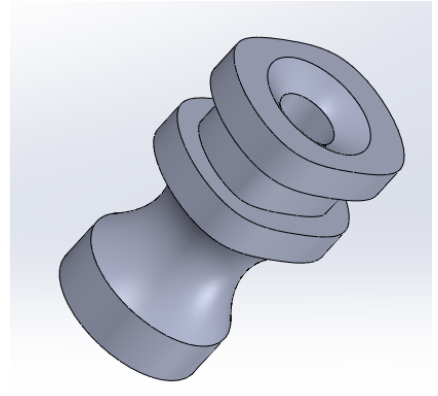
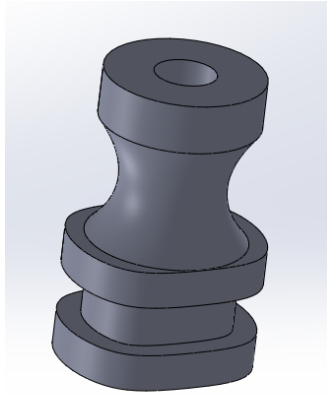


Figure 36 and 37. Solidworks images of mouse capsule caps from two different angles.

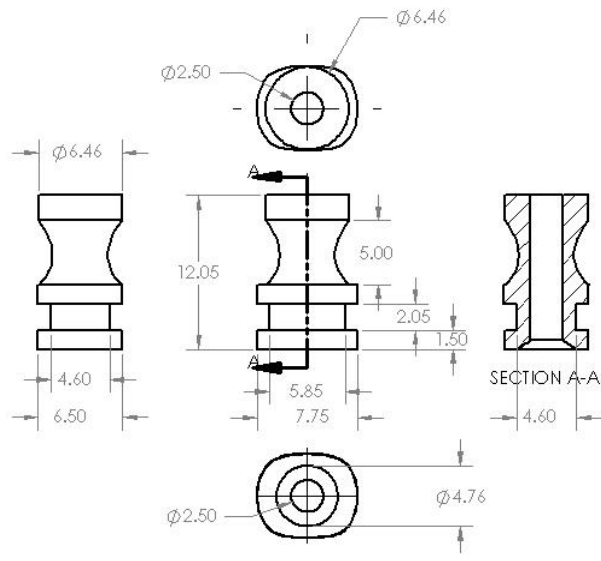


Figure 38. Dimensions of the mouse capsule cap in mm.

C. Final Prototype

The final prototype consists of one capsule for rat brains (Figure 26) and one capsule for mouse brains (Figure 28), as well as four individual caps for the rat brain capsule (Figure 34) and 11 individual caps for the mouse brain capsule (Figure 35). The brains are loaded into the capsule holes, the holes are then filled with fluorinert (approximately 3 mL for the rat capsule, and approximately 1 mL for mice capsule) until a meniscus is formed at the top of the capsule hole. Then, the cap is plunged straight into the fluorinert-filled capsule until the top of the cap is about 1 mm above the top of the capsule (Figure 40). Then, the hole of the cap is sealed with a tapered rubber stopper (Figure 39). The rubber stopper ensures that no fluid escapes the capsule and that no air gets into the capsule. The entire configuration is then placed into the bore of the MRI for imaging.

See Appendix E for final prototype CAD drawings with detailed dimensions.



Figure 39. Assembled rat brain capsule with brains and rubber stoppers.



Repeated Figure 26 for clarity



Repeated Figure 34 for clarity.



Figure 40. FormLabs clear mice capsule with caps.



Repeated Figure 28 for clarity



Repeated Figure 35 for clarity

D. Testing

See Appendix C for I-IV testing protocols.

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 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 at 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 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

Brain-fit Testing

All 5 sample rat brains fit in the final prototype. 4 new rat brains were provided by the client. The new brains also fit, with minimal effort. Rat brains were removed with tapping. There was some difficulty in removing one of the rat samples, and it was damaged in the process.



Figure 41. 4 new rat brains fit in the final prototype.

All six sample mice brains fit in the final mouse prototype. These samples fit and could be removed with ease. Two new mice brains were provided by the client, however, these new brains did not fit the final prototype as the brain was much wider. The client suggested that the sample brains could be younger mice or the structure could have been different due to different experiments being used on the brains.



Figure 42. New mice brains could not fit in holes sized 9mm by 7.8mm. New brains do not have measurements as of now.

Leak Resistance Testing:

The final rat capsule was tested with both water and fluorinert. The capsule did not leak when tipped upside down, nor when placed on its side for the entirety of the 20 minute testing duration.

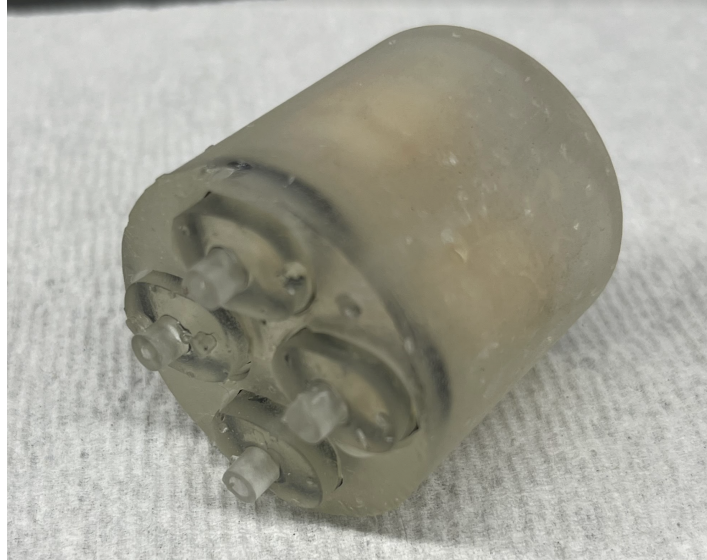


Figure 43. Loaded rat capsule for leak testing.

The final mice capsule experienced leaking out of one of the holes when tested with both water and fluorinert due to a defect from the printing process. This was noticed immediately and the behavior of the individual hole was not considered during the remainder of testing. There was no leaking out of the caps or the rest of the holes. The hole with the leak is marked with a black line (Figure 44).

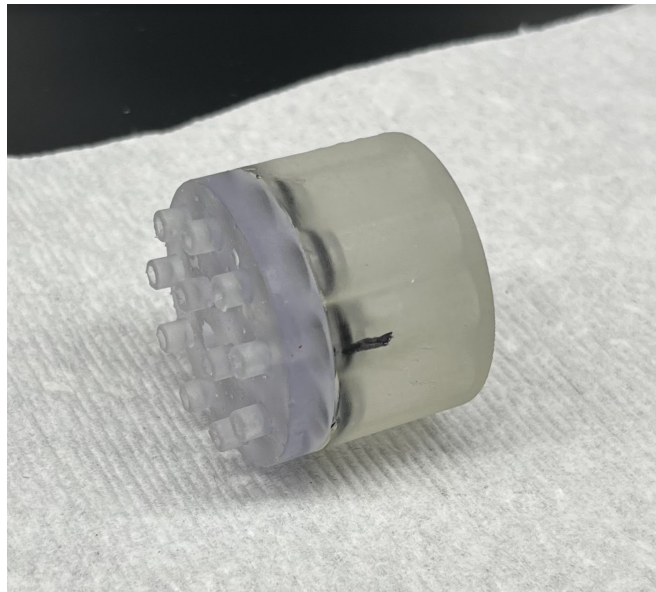


Figure 44. Loaded mice brain capsule for leak testing (no brains).

The amount of fluorinert that was required to use the final rat prototype showed improvement from the current methods used by Dr. Yu's lab. Figure 45 clearly illustrates the reduction in fluorinert needed for the final prototype: about 16mL less overall and 15mL less regained.

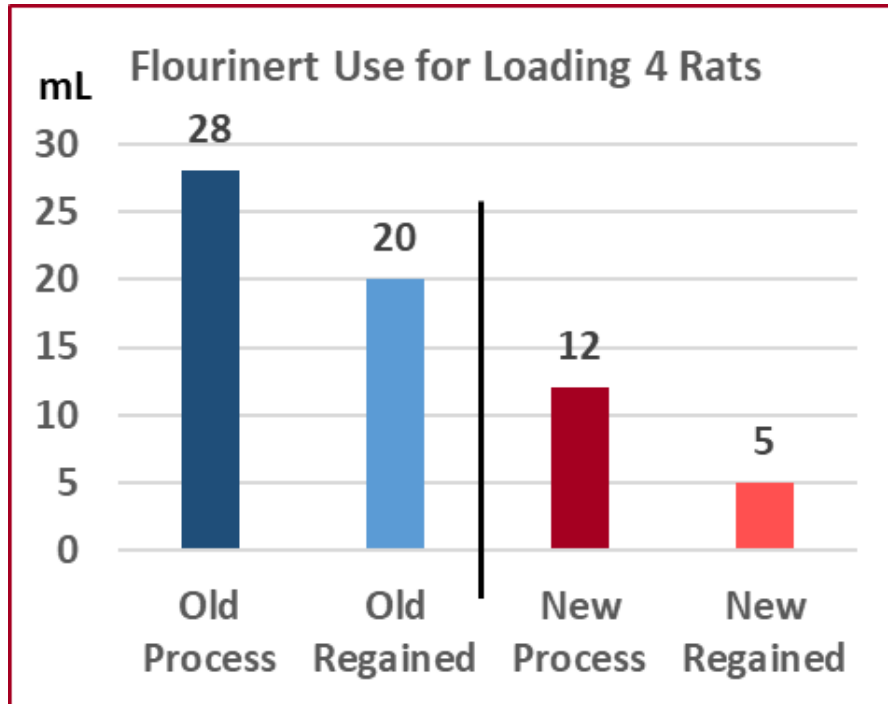


Figure 45. Flourinert used during the process of loading 4 rat brains. The process requires a minimum of 28mL to be used to load four brains. After loading, the overflow that is regained is 20mL. There is a significant decrease for the process using the new product; 16mL less used during the process, and 15mL less regained. It should be noted that regained fluorinert is contaminated because of contact with the brain and must be purified before it can be reused.

Air Bubble Reduction Testing:

After properly loading the rat and mice capsules with water and sealing caps, no air bubbles were observed in any of the chambers. When the capsules were left on their side for 20 minutes, the holes were still free of air bubbles.

When the rat capsule was tested with fluorinert, there were multiple air bubbles approximately 1 mm in diameter in one of the holes of the rat brain capsule. After the scan these holes grew to be around 5mm in diameter due to leaking. While the seal of the o-ring was tight enough to prevent water leakage, it was not tight enough to adequately prevent fluorinert leakage. The rat capsule caps were reprinted to create a tighter seal with the walls of the capsules. After retesting the rat capsule and caps with fluorinert, all four of the capsules formed one air bubble each, about 0.5mm in diameter. There was no leaking and the air bubble did not

grow over the 20 minute observation period. The client informed the team that 0.5mm bubbles would not be a problem for their imaging.

Image Quality Testing

After the client loaded the rat brains into the capsule, a “scout” scan was performed, which is a very short test that allows for positioning and diagnosis of the imaging subject and image, and can be completed in just a couple minutes [7]. When the scan was complete, the client informed the team that the resolution of the image looked very good, relative to their previous technique. This was due to the fact that increasing the amount of signals improves the scan quality, as there is less background noise that can come through according to the MRI technician. However, due to time constraints, a full overnight scan was not feasible and the team was unable to complete the testing protocol to evaluate quantitative image results.



Figure 46. Scout Scan on 4.7T Agilent MRI machine

Although the image quality was good, the scan did show that the capsule allowed one of the brains to tilt, as is evident in the middle image of Figure 45. It was also evident that air bubbles had appeared within the capsule, which appeared as a dark blot on the images. The client had agreed, however, that some of these unfortunate results could have occurred due to the novel loading technique, and could very easily be improved upon in the future. In addition, from leak testing a few hours prior, there was water remaining underneath the o-rings, which caused bright artifacts to appear in the images, as visible at the bottom middle image of Figure 46.

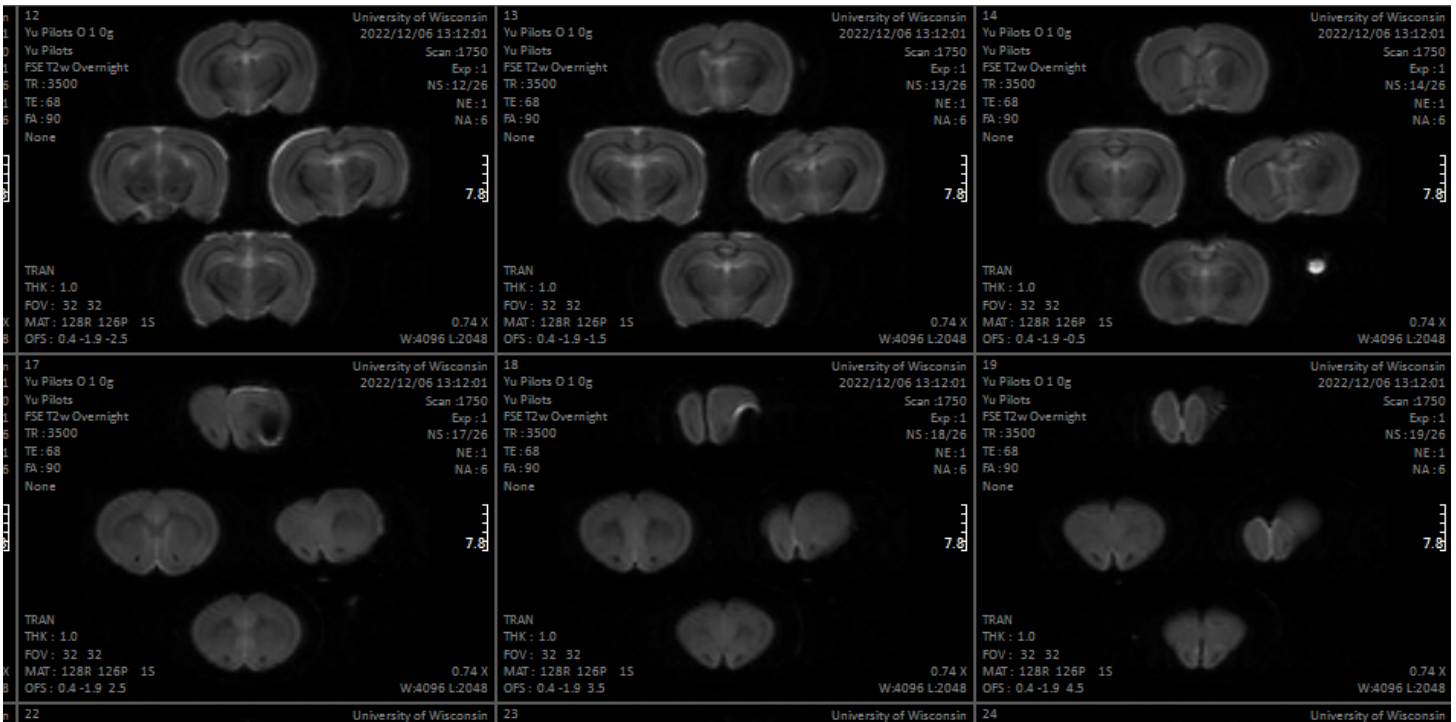


Figure 47. T1 Scan on 4.7T Agilent MRI machine. 6 chosen section images out of the 25 total. Right brain is unsymmetrical which indicates tilting. Black marks on the top brain on bottom sections are caused by air bubbles being in contact with the brain. SEE Appendix Figure 49 for all section images.

Unfortunately, again, due to time constraints it was found just prior to the team’s scanning appointment that the chosen mouse samples did not fit in the capsule, and thus the team was unable to perform image testing on the mouse capsule. However, a T1 scan showing image slices of the rat brains could be performed, and showed that the capsule itself did not impose noise on the image, meaning a proper material was chosen, and that the positional water marker was done correctly (see top right section’s white circle of Figure 47).

VII. Discussion

The potential impact that this product has for the client and all labs at the University of Wisconsin-Madison is not trivial. The cost of the analyses limit the number of samples used which decreases the accuracy of the results. Marek et al. found that neurological studies of humans have a median size of 23 participants, but do not have robust results when compared to much larger datasets having hundreds or thousands of human samples [13]. Thus, it can be implied that maximizing the number of murine model brains will improve the significance of the client’s research results for the medical field.

As the results indicate, the final rat design can load, unload and snugly contain each of the sample rat brains without causing damage. However the results do not account for the potential of abnormally large sized brains that may not be able to be loaded into the design, or abnormally small sized brains that may change orientation during scan as it cannot fit snugly. Another potential source of error can occur over time with water potentially seeping into the capsule from filling the watermark. Having trace amounts of water absorbed through the capsule could potentially lead to inaccurate or unclear images taken from the MRI. Due to the lack of a comprehensive removal mechanism an error that could potentially arise, is that larger brains that fit especially snugly may get stuck in the capsule. This could lead to damaging the brain and making further analyses impossible (these are often done by the client, especially on the cerebrum).

This design can fit one more rat brain per scan which saves the lab \$41.60 per brain per scan and saves time as more brains can be scanned with fewer scans. The team's design also saves the client money by requiring less fluorinert to be used per scan (approximately 15mL less). The client can use the additional time and money the design saves to focus on more research on neurological disorders, which will more actively contribute to the scientific world.

The most important consideration is the lack of quantitative data. Because of the timeline of fabrication, testing was not possible. In the near future, higher quality testing can be done and can be compared to previous research the lab has done to verify the quality. There are no MRI imaging testing results for the mouse capsule, and in addition, the newly provided brains could not fit, so much work needs to be done to report on the success of the mice capsule.

VIII. Conclusion

Dr. JP Yu's lab wants a capsule that can easily load and unload rat and mouse brains and hold them during an MRI scan. The team's rat capsule was found overall to be successful, with the final design being able to comfortably fit all of the rat brains with good quality images being taken from the MRI. Overall the O-ring sealing cap worked well with minimal leaking in the final design, and minimal air bubbles forming in the capsule. The watermark also worked well as it was clearly visible on the MRI scan and acts as an identifier for the various brains. Overall, the design did not work well in holding the brains to a consistent orientation as the average degree of rotation was 13.16 degrees.

If this project were to be continued, more MRI image quality testing would be done to compare with previous research. The team would change the design to further stabilize brain orientation utilizing a ramp or depression in the shape of the brain to prevent tilting. More work would be done to guarantee that no air bubbles are touching the brain. A method to catch excess fluorinert during loading would also be created. In addition, a more streamlined watermark and a more secure stopper would be created. Finally the mice capsule would be redesigned to create wider holes to fit the larger brains.

References

- [1] A. S. Shatil, K. M. Matsuda, and C. R. Figley, “A Method for Whole Brain Ex Vivo Magnetic Resonance Imaging with Minimal Susceptibility Artifacts,” *Front Neurol*, vol. 7, p. 208, Nov. 2016, doi: 10.3389/fneur.2016.00208.
- [2] D. Visscher and W. Leidenthal, “DICHTOMATIK O-ring Handbook,” p. 225.
- [3] H. E. Holmes *et al.*, “Imaging the accumulation and suppression of tau pathology using multiparametric MRI,” *Neurobiology of Aging*, vol. 39, pp. 184–194, Mar. 2016, doi: 10.1016/j.neurobiolaging.2015.12.001.
- [4] John-Paul (JP) J Yu,” *Department of Radiology*. <https://radiology.wisc.edu/profile/> (accessed Oct. 12, 2022).
- [5] J.-P. Yu, “High throughput quantitative ex vivo MRI of the mouse brain,” BME Design Projects. [Online]. Available: <https://bmedesign.engr.wisc.edu/selection/projects/943013f3-278d-4fa5-abba-3bbd74da54c1>. [Accessed: 12-Oct-2022].
- [6] J. R. Guy *et al.*, “Custom Fit 3D-Printed Brain Holders for Comparison of Histology with MRI in Marmosets,” *J Neurosci Methods*, vol. 257, pp. 55–63, Jan. 2016, doi: 10.1016/j.jneumeth.2015.09.002.
- [7] J. Y. You *et al.*, “Readability of extraspinal organs on scout images of lumbar spine MRI according to different protocols,” *PLOS ONE*, vol. 16, no. 5, p. e0251310, May 2021, doi: 10.1371/journal.pone.0251310.
- [8] “Key findings: CDC releases first estimates of the number of adults living with autism spectrum disorder in the United States,” Centers for Disease Control and Prevention, 07-Apr-2022. [Online]. Available: <https://www.cdc.gov/ncbddd/autism/features/adults-living-with-autism-spectrum-disorder.html>. [Accessed: 12-Oct-2022].
- [9] K. Heuer and R. Toro, “Reorient: A Web tool for reorienting and cropping MRI data.,” *Journal of Open Source Software*, vol. 5, no. 55, p. 2670, Nov. 2020, doi: 10.21105/joss.02670.
- [10] “Materials Library,” Jul. 2019. [Online]. Available: https://formlabs-media.formlabs.com/filer_public/ac/89/ac8963db-f54a-4cac-8fe9-fb740a7b06f1/formlabs-materials-library.pdf

- [11] “O-Ring Size Chart | USA AS568 Standard O-Ring Sizes.”
<https://www.marcorubber.com/o-ring-size-chart-as568.htm> (accessed Dec. 13, 2022).
- [12] S. Lafee, “Mapping the mouse brain, and by extension, the human brain too,” UC Health - UC San Diego, 06-Oct-2021. [Online]. Available:
<https://health.ucsd.edu/news/releases/Pages/2021-10-06-mapping-the-mouse-brain-and-by-extension-the-human-brain-too.aspx>.
- [13] S. Marek et al., “Reproducible brain-wide association studies require thousands of individuals,” *Nature*, vol. 603, no. 7902, Art. no. 7902, Mar. 2022, doi: 10.1038/s41586-022-04492-9.
- [14] S. Pruthi, “MRI,” Mayo Clinic, 04-Sep-2021. [Online]. Available:
<https://www.mayoclinic.org/tests-procedures/mri/about/pac-20384768>.
- [15] “Wall Thickness: Understanding the Best 3D Printing Wall Thickness,” rapiddirect, Feb. 25, 2022. <https://www.rapiddirect.com/blog/3d-printing-wall-thickness/> (accessed Oct. 01, 2022).
- [16] “3D Printing Materials for Professionals,” *Formlabs*.
<https://formlabs.com/materials/standard/> (accessed Dec. 14, 2022).

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. Krish Saha

Team:

- Joel Nitz - Team Leader - jcnitz@wisc.edu
- Anna Mercord - BPAG - mercord@wisc.edu
- Erwin Cruz - Communicator - ecruz9@wisc.edu
- Allicia Moeller - BPAG - aamoeller@wisc.edu
- Leo DiCataldo - BSAC - dicataldo@wisc.edu
- Ray Steinlage - BWIG - rsteinlage@wisc.edu

Date: Sep 21, 2022

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. Packing efficiency

Must be able to fit more than 3 rat brains and more than 6 mice brains per MRI scan. These numbers are based on the amount that they are currently able 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 all the time is spent during post processing to adjust and fix any problems with the scan.

2. Orientation of murine brains

Must have the brain's midline parallel with the center axis of the MRI bore cylinder, and all brains must be in the same plane (plane being perpendicular to the axis). At the moment, the client loads the brains into syringes (cylindrical) 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, they can twist or turn within the syringe and the midlines become misaligned when transporting the brains to the MRI from the lab. This does not necessarily decrease the quality of the scan, however, it does significantly increase the post processing time. This background explains why the orientation must be kept consistent.

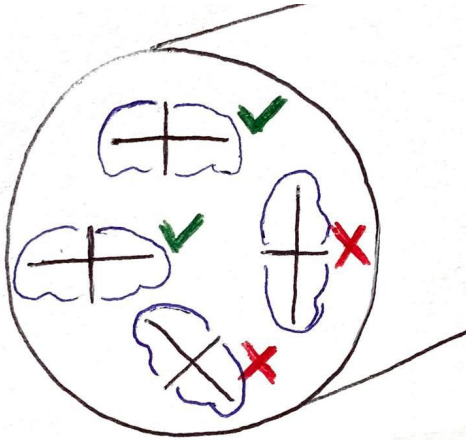


Figure 5. Brain's midline is parallel with the center axis of the MRI bore cylinder.

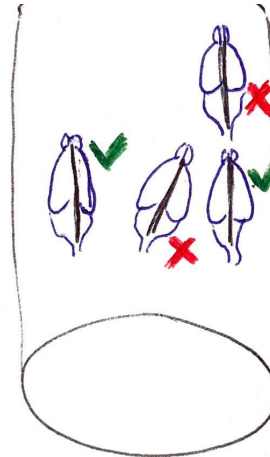


Figure 6. All the brains in the same cross-sectional plane.

Other Requirements

3. Have a complimentary scientific procedure to make the process standardized and reproducible. Because they are doing scientific research, they are interested in having reproducible results that any scientist could verify. Their current method does not fulfill this requirement.
4. MRI compatible: Material must not obscure image. (See design requirements for more details).[informal interview with MRI technician]
5. Reusable: They would like to reuse the device after removing and/or discarding brains.
6. 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]
7. 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 causes a bad 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 MMMMMM minutes to load 1 brain 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

Design requirements

1. Physical and Operational Characteristics

a. Performance requirements:

Must increase loading efficiency to higher than the current rates of 3 rat brains per MRI scan and 6 mice brains per MRI scan, which currently take long increments of time to insert and remove from individual syringes. Shall not increase scanning time to more than 20 hours. The device should also minimize air bubbles introduced to the brain samples.

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 compactly 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. Minimally must be able to be both sealed and reopened once, but preferably can be reused over the course of a year (approximately sealed and opened once per month).

e. Shelf Life:

The client did not specify a specific shelf life; we estimate that the product should have a shelf life of 1 year.

f. 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, and should not move within the scanner.

g. Ergonomics:

Should have the ability to sustain the force of a vacuum seal (exact force to be determined) without deformation. 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.

h. 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.

i. Weight (redundant):

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

j. 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 decrease imaging accuracy. A non-biodegradable, waterproof material that is compatible with Fluorinert is preferred.

k. 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 4 - 8 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 8-16 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

- [1] T. Woods, "MRI Safety and Compatibility of Implants and Medical Devices," *ASTM International*, pp. 82–90, doi: 10.1520/STP11156S.
- [2] "Specifications for a 4.7 Tesla/400MM Actively Shielded Magnet System," 2001. Accessed: Dec. 15, 2022. [Online]. Available:
- [3] S. Yi, B. Barnett, M. Poetzel, N. Stowe and J. Yu, "Clinical translational neuroimaging of the antioxidant effect of N -acetylcysteine on neural microstructure", *Magnetic Resonance in Medicine*, vol. 87, no. 2, pp. 820-836, 2021.

B. Materials

Budget:

The client gave us a budget of \$100 for fabrication as well as the cost for four, 20-hour MRI scans, which cost roughly \$500 each. The team exceeded their fabrication budget by spending \$140.59 (Table I) on the product, however, the team did not use any of the four 20-hour MRI scans for testing, opting for two, 8-minute “scout” scans instead, which did not cost the lab any money.

Table I: Materials.

Item	QTY	Unit Cost	Total Cost	Description	URL to product	Date Purchased
Prototype print 1	1	\$12.41	12.41	FormLabs SLA Black of the rat and mouse capsule and 3 rat capsule caps.		10/25/2022
#12 O-ring	3	\$0.10	0.3	9 #12 O-rings, priced at \$0.10 / 3 O-rings		10/27/2022
Single PLA 5-hole prototype	1	\$1.52	1.52	Test print in PLA of rat brain capsule for leak-proof testing		10/27/2022
PLA rat brain sizing array	1	\$10.80	10.8	Print in PLA of various capsule shapes and sizes to test for the optimal hole dimensions.		11/01/2022
Formlabs integrated cap reprint	1	\$4.02	4.02	1 Formlabs clear integrated cap reprint		11/08/2022
Formlabs integrated cap and capsule, mice array	1	\$21.66	21.66	2 Formlabs integrated caps, 2 versions of rat brain capsule prototype, and 1 PLA mice sizing array		11/08/2022
PLA test capsule and 2 test caps	1	\$0.64	0.64	Printed to test for O-ring dimensions		11/11/2022
O-rings	1	\$0.10	0.1	2 #13 O-rings and 1 #12 O-ring		11/11/2022
PLA mice brain array and cap array	1	\$2.48	2.48	Array to test mice brain sizes and array to test cap groove depths		11/14/2022
PLA Test Capsule and 4 Test Caps	1	\$0.48	0.48	4 PLA caps to test for O-ring groove depth and a single testing capsule		11/15/2022
Formlabs rat capsule	1	\$6.21	6.21	1 Capsule for the rat brains in formlabs clear		11/15/2022
Formlabs rat lids (top and bottom) and mice capsule	1	\$12.54	12.54	Formlabs clear print of integrated top and bottom rat capsule lid and mice brain capsule		11/15/2022
Test cap in PLA and	1	\$1.96	1.96	22 #8 O-rings, 10 #12 O-rings, 3 #1		11/16/2022

Formlabs and o-rings				O-rings, test cap in PLA and Formlabs clear		
FormLabs integrated cap (top and bottom)	1	\$9.29	9.29	Integrated caps for the rat brain capsule in FormLabs Clear		11/21/2022
Tapered Rubber Stoppers	1	\$8.39	8.39	Set of 50 rubber stoppers to plug the holes in our integrated caps	https://www.amazon.com/gp/product/B00V6BYQQO?ref=ppx_pt2_dt_b_prod_image&th=1	11/24/2022
FormLabs White mouse brain capsule and test caps	1	\$4.83	4.83	1 mouse brain capsule in white resin and 3 test caps of different dimensions		11/28/2022
FormLabs rat and mouse capsule and rat and mouse lids	1	\$20.70	20.7	FormLabs clear print of a one-sided capsule and integrated lid for both rat and mouse brains.		12/1/2022
FormLabs Individual Caps	1	\$4.44	4.44	FormLabs Clear 11 individual mouse caps and 4 individual rat caps		12/2/2022
FormLabs BioMed Clear integrated caps reprint	1	\$10.46	10.46	FormLabs BioMed Clear integrated caps reprint		12/2/2022
Mouse integrated cap reprint in FormLabs clear	1	\$3.08	3.08	FormLabs Clear integrated mouse cap with an updated w_c value		12/5/2022
O-rings	1	\$0.30	0.3	O-rings from MakerSpace		12/5/2022
6 FormLabs clear individual rat caps	1	\$3.98	3.98	FormLabs Clear individual rat caps with an updated w_c value		12/6/2022
Total			140.59			

C. Testing

Table II: Leak-resistance testing.

Time Elapsed (min)	Observations Mice (not including defect)	Observations Rat
5	No leaking	No leaking
10	No leaking	No leaking
15	No leaking	No leaking
20	No leaking	No leaking

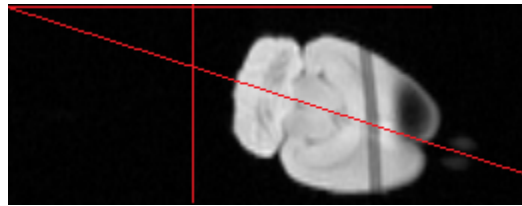


Figure 48. Angle measurement methodology: trigonometric analysis of aerial view.

Table III: Image quality testing.

Brain	Rotation necessary (Degree)	Observations of image
Rat 1 (see right brain in Figure 49)	18.06	Severe obstruction of image by air bubbles on 6/22 sectional images. Mild on 3/22.
Rat 2 (see top brain in Figure 49)	8.25	Severe obstruction of image by air bubbles on 6/22 sectional images
Rat 3	~0	Minimal to no obstruction
Rat 4	~0	Minimal to no obstruction
Average	13.16	

Figure 49. T1 Scan, all 25 section images.

	Rat 1	Rat 2	Rat 3	STDV Rat	Avg Rat	Mouse 1	Mouse 2	Mouse 3	Avg Mouse	STDV Mouse
Max Length	28.7	27.99	29.56	0.78619 33604	28.75	13.72	13.95	15.41	14.36	0.91656 96918
Max Width	14.76	15.1	14.99	0.17349 35157	14.95	8.68	8.64	8.36	8.56	0.17435 59577
Max Height	10.66	10.23	10.46	0.21517 43479	10.45	6.69	7.49	5.99	6.72333 3333	0.75055 53499
Length of cerebrum	15.59	13.98	14.65	0.80876 44898	14.74	8.59	8.6	9.11	8.76666 6667	0.29737 74257
Length of cerebellum	6.32	6.68	5.68	0.50649 11977	6.22666 6667	3.21	3.73	5	3.98	0.92081 48565
Width of cerebellum (no ficculus)	12.03	11.59	11.58	0.25696 95183	11.7333 3333	7.38	7.93	7.16	7.49	0.39661 06403
Height of brainstem	6.36	4.56	5.19	0.91340 02409	5.37	2.52	3.08	2.01	2.53666 6667	0.53519 46686
Length of brain stem	2.95	4.87	6.47	1.76242 2575	4.76333 3333	5.05	2.95	5.15	4.38333 3333	1.24230 9677
Olfactory bulb height	4.38	6.32	5.73	0.99450 15502	5.47666 6667	3.18	3.09	2.83	3.03333 3333	0.18175 07451
Olfactory bulb length	3.98	3.36	2.83	0.57558 66572	3.39	3.2	2.3	2.87	2.79	0.45530 20975
Olfactory bulb width	5.43	5.94	4.97	0.48521 47291	5.44666 6667	3.91	3.18	3.7	3.59666 6667	0.37581 02358

Measurements of the mouse and rat brains provided by the client. Measured using a digital caliper.

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, 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.

D. 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]: 1 import math

In [2]: 1 def o_ring_data(w_c, perimeter, inner_d, thickness = 1.78):
2     decimal_stretch = perimeter / (math.pi * inner_d)
3     percent_stretch = (decimal_stretch - 1) * 100
4
5     percent_w_o_decrease = 0.5 * percent_stretch + 2.5
6     decimal_w_o_decrease = percent_w_o_decrease / 100
7     cs_area_initial = math.pi * (thickness / 2)**2
8     cs_area_decreased = cs_area_initial * (1 - decimal_w_o_decrease)
9     w_o = 2*(math.sqrt(cs_area_decreased / math.pi))
10
11     percent_squeeze = ((w_o - w_c) / w_o) * 100
12
13     percent_gland_fill = 70
14     decimal_gland_fill = percent_gland_fill / 100
15     cs_area = ((w_o/2)**2) * math.pi
16     groove_height = cs_area / (w_c * decimal_gland_fill)
17
18     o_ring_data_dict = {}
19     o_ring_data_dict["Percent Stretch"] = percent_stretch
20     o_ring_data_dict["Percent Squeeze"] = percent_squeeze
21     o_ring_data_dict["Percent Gland Fill = " + str(percent_gland_fill) + "% when height is "] = groove_height
22     return o_ring_data_dict

In [43]: 1 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 Dimensions

Mice Final Prototype CAD drawings (cap and capsule):

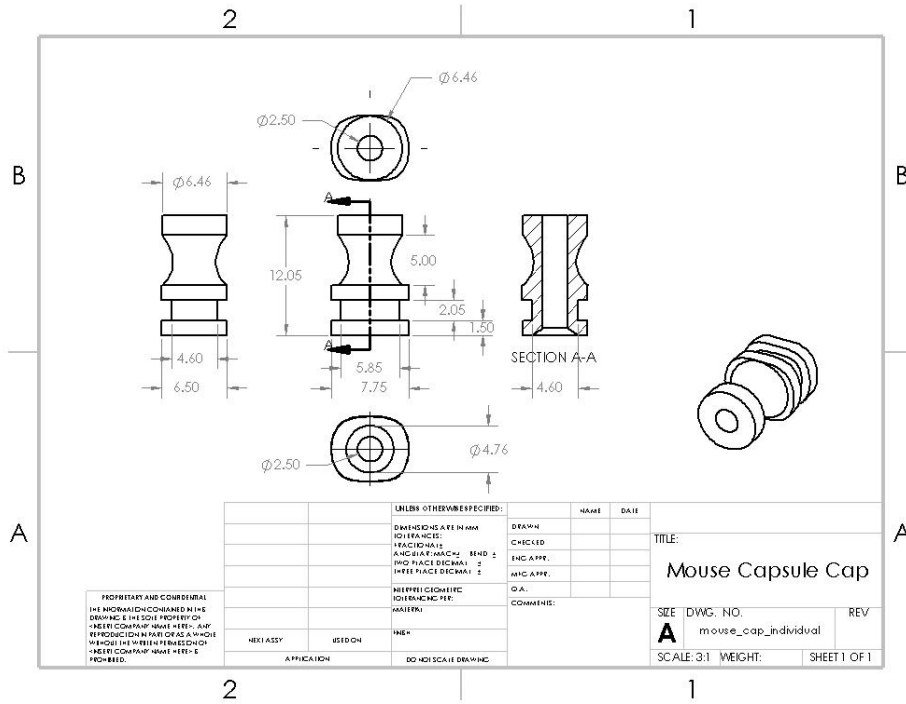


Figure X. Mice cap SolidWorks drawing with dimensions in mm.

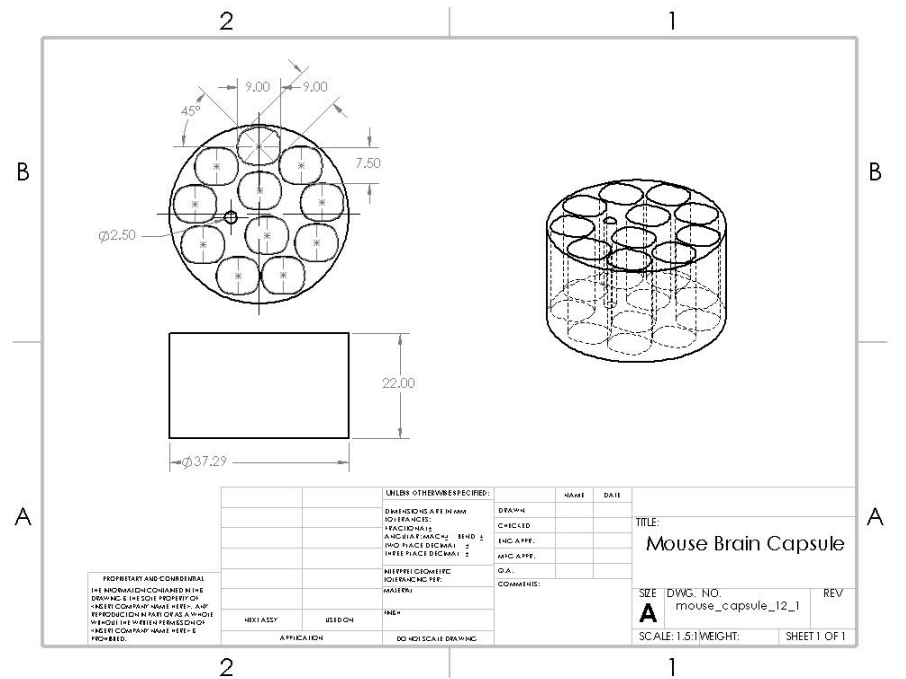


Figure X. Mice capsule SolidWorks drawing with dimensions in mm.

Rat Final Prototype CAD drawings (cap and capsule):

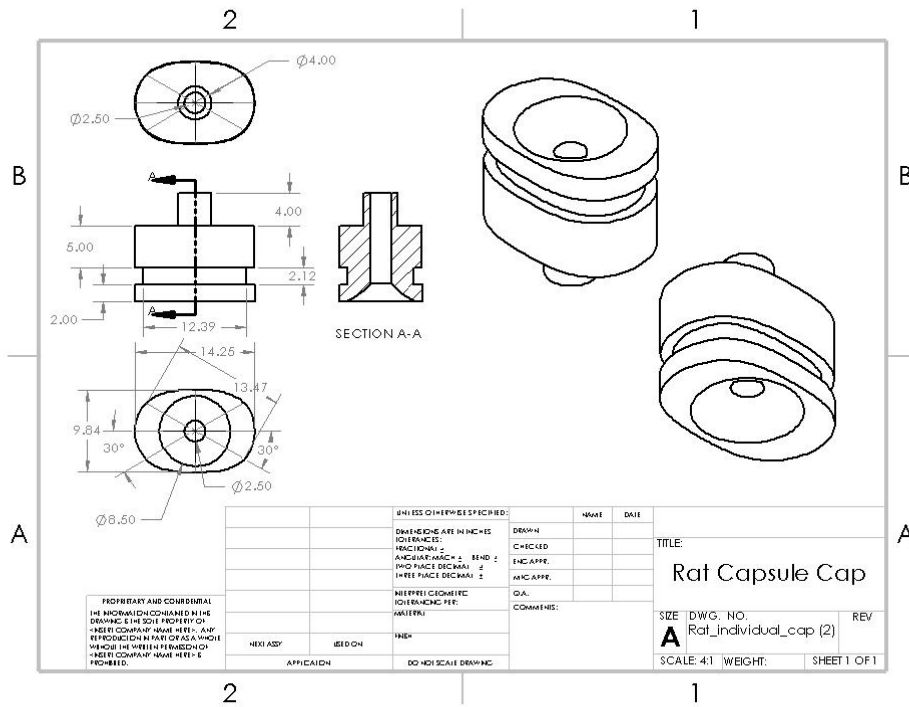


Figure X. Rat cap SolidWorks drawing with dimensions in mm.

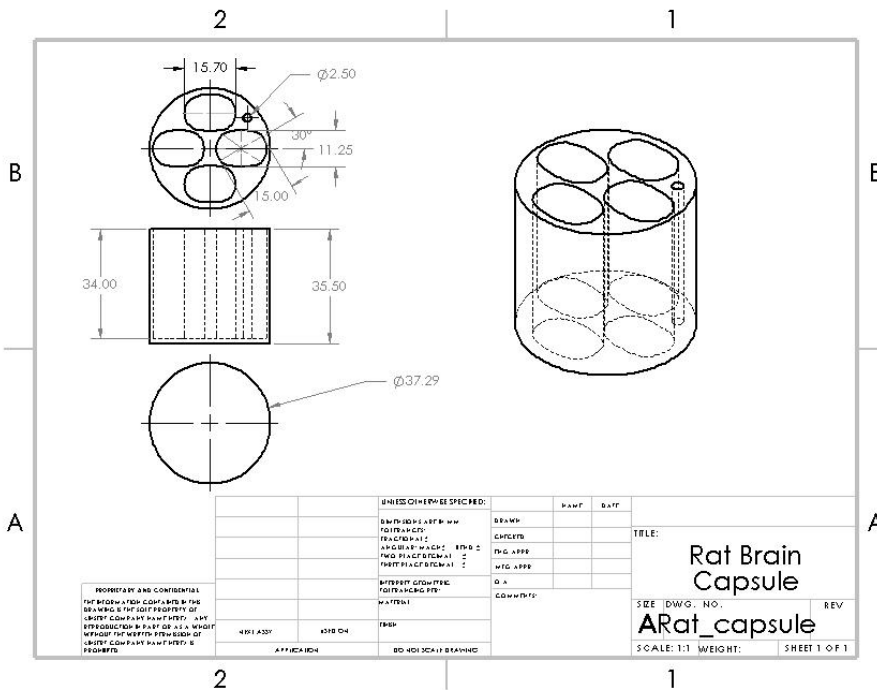


Figure X. Rat brain capsule SolidWorks drawing with dimensions in mm.