

Development of a pediatric brain phantom for the investigation of combined intracranial electroencephalography and transcranial magnetic stimulation

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Highlights

- We successfully characterized 6% gelatin material with 1.2% saline to represent electrical conductivity of 0.62 S/m.
- Utilizing real brain scan data, a physically accurate pediatric hydrogel brain and matching 3D-printed skull were fabricated.
- Initial testing was completed by applying single pulse transcranial magnetic stimulation of varying intensities through a MagStim² coil.

Abstract

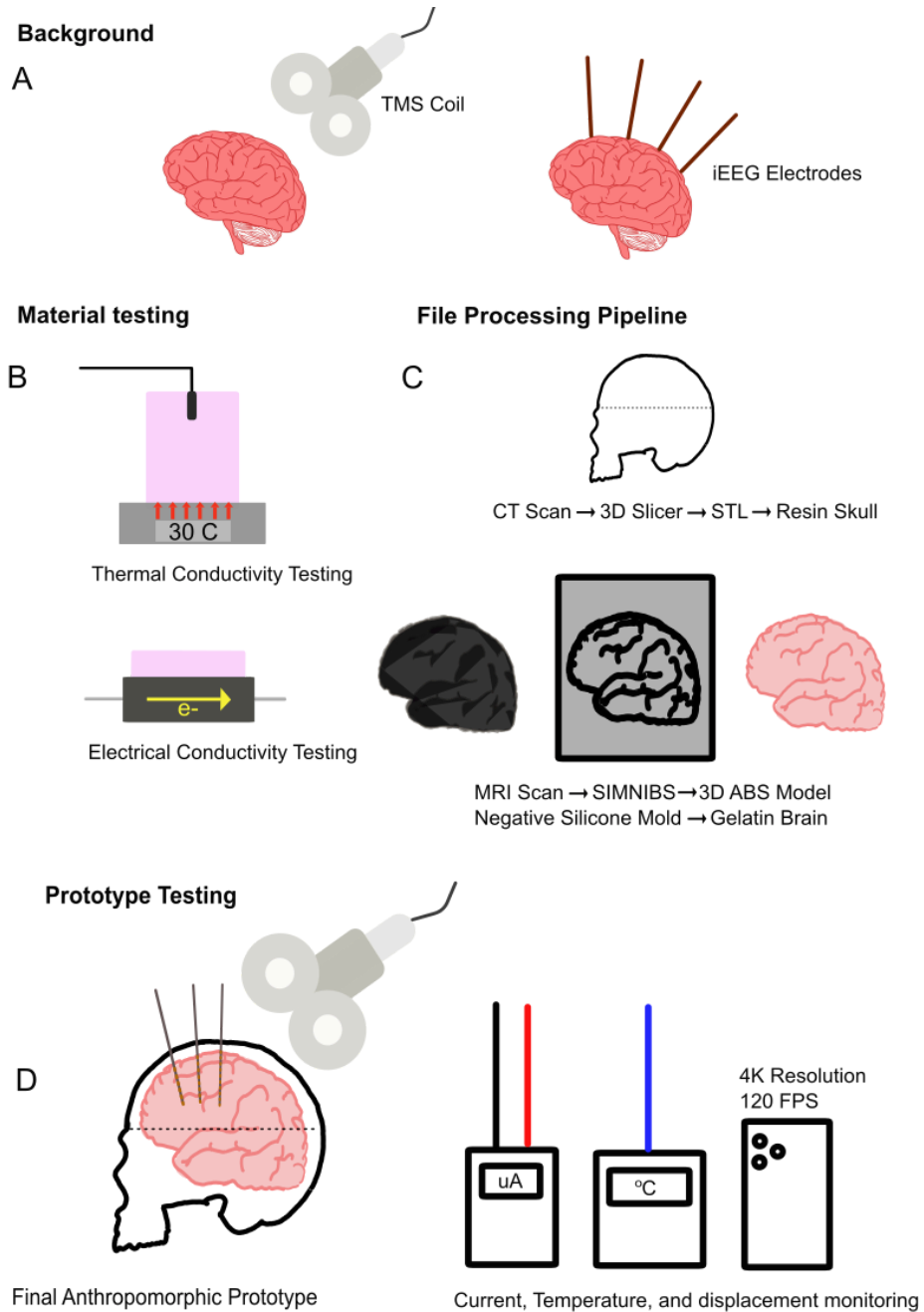
Epilepsy is a prevalent neurological condition marked by the occurrence of repeated, uncontrollable seizures. This disorder can present in individuals of all ages, but commonly manifests in children. One main treatment method is that of surgical intervention, in which neurosurgeons identify and disconnect cranial regions involved in seizure generation. Before operation, brain mapping techniques such as intracranial electroencephalography (iEEG) and transcranial magnetic stimulation (TMS) are utilized to delineate brain connectivity. We investigated the safety of using these methods in tandem via the fabrication of a brain phantom model: a hydrogel brain encased in clear resin. The model was then stimulated to depict any effects of TMS pulses on iEEG electrodes. Preliminary testing was performed to characterize the hydrogel of choice in terms of thermal and electrical properties. We aimed for anatomical accuracy by utilizing both pediatric magnetic resonance imaging (MRI) and computed tomography (CT) scans. The final model was assembled from a gelatin hydrogel tuned with saline, fabricated via a negative silicone brain mold, and a 3D printed resin skull. Final testing, in which the completed model was stimulated with TMS pulses whilst containing inserted iEEG

electrodes, was performed over an array of pulse intensities with recordings taken of electrode positioning, induced current, and temperature change within the model.

Keywords

Epilepsy, neuroscience, neuromodulation, phantom, hydrogel, pediatrics.

Graphical abstract



1. Introduction

Epilepsy, the fourth most common neurological disorder, is characterized by the regular appearance of uncontrollable seizures. These seizures occur as a result of short, excessive electrical discharge in neurons and can either be focal in nature, involving a local neuronal network, or generalized, engaging a larger bilateral network. People of all ages can be affected by epilepsy, but it often manifests before the age of one year; not only is the disorder detrimental to patients' ability to thrive, with a strong correlation to increased injury and accident rates, but it is also associated with a higher risk of depression and death [1].

An area of exploration tied to epilepsy is that of various treatment methods. Aside from the use of medication, surgical management is an oft-investigated treatment tool in the control of epileptic seizures. Procedures such as temporal lobectomies, or removal of certain portions of the brain, are preceded by a variety of brain mapping techniques such as iEEG and TMS. iEEG, routinely used in surgical planning, utilizes electrode systems that are either connected across the surface of or implanted into the brain. This method provides high spatiotemporal resolution and is especially advantageous for epileptogenic foci localization [2]. TMS assesses brain circuit excitability through electromagnetic induction, inducing currents to produce action potentials and painlessly activate brain networks [3].

Both TMS and iEEG provide complementary information for mapping out critical brain regions that should be avoided during surgery. However, there are several safety concerns around the simultaneous use of TMS in patients with iEEG: that of secondary electrical currents, heating of the implanted electrodes, and electrode array displacement, all of which would have severe consequences for the affected individuals [4]. There has been prior research on potential interactions between the techniques, in which representative pulses have been administered on phantom models, before being applied to human subjects to certify their level of risk and effectiveness. For example, researchers at the University of Iowa created a gel-based brain phantom to prove that TMS and iEEG can be safely used in tandem, specifically in adult patients. The brain of this phantom was made of poly(acrylic acid) (PAA) saline gel, and the skull was made of poly(methyl methacrylate) (PMMA) [4].

While there exist brain phantoms that have been used for similar research, none have addressed pediatric populations, contributing to a significant treatment knowledge gap. There are complicated ethical challenges surrounding the participation of children in human studies, such

as informed consent, enforcing strict safety standards, and incorporating in-depth risk assessment. In part due to these more rigorous expectations, there is a clear lack of research and clinical explorations in the pediatric population [5].

Focusing on this underrepresented population introduces different physiological properties for consideration in addition to extensive safety demands [5], [6], [7]. We chose to address this specific demographic in the creation of our own phantom model, opting to consider these added constraints, mainly because of the urgent need for a solution: the mortality rate in children affected by epilepsy is 5 to 10 times higher than the rest of the population, so properly treating and controlling these unprovoked seizures is paramount [8].

We structured our approach similarly to the research team at the University of Iowa, by constructing a detailed brain phantom, inserting iEEG electrodes and applying single-pulse TMS (spTMS), and finally analyzing the implanted electrodes' change in temperature, displacement, and secondary electric current buildup. Our ongoing goal has been to verify that these brain mapping techniques can be used in tandem for cases of pediatric epilepsy.

2. Materials and Methods

2.1 Hydrogel Synthesis

Porcine skin gelatin Type A, 300g bloom (Lot 0000518727), was purchased at 500 grams from Millipore Sigma [9], and combined with sodium chloride (NaCl) powder from Thermo Fisher Scientific [10]. Gelatin was dissolved at 6% w/v in room temperature MilliQ water, and stirred with a stir bar at 400 RPM until the gel was soaked. The temperature was then increased to 80 °C and NaCl powder was added at 1.2% w/v and stirred until the solution reached 65 °C. After dissolution, gels were poured into silicone molds [11] and cooled at 4 °C. Before use, molds were wiped down with 70% ethanol (EtOH).

2.2 File Processing Pipeline

To create 3D models of the brain and skull for 3D printing, a matching set of patient-derived MRI and CT scans were processed. Anonymized MRI scans from 5-7 year old patients were imported into 3D Slicer in digital imaging and communications in medicine (DICOM) format and converted into T1-weighted images (Figure 1A). T1 data was then

processed in SimNIBS 4.5.0, a Python-based program that automates creating a 3D head model of 51 tissues. Using SimNIBS GUI and Gmsh, programs internal to SimNIBS, brain tissues were segmented into a separate model and exported in stereolithography (STL) format (Figure 1B). The brain STL was then used directly for 3D printing.

The CT scans were imported into 3D Slicer in DICOM format (Figure 1C). Voxel size to approximately 0.5 to 1 mm to preserve detail and tissues other than the skull were removed. The skull was segmented into an STL for export. Further cleanup of the model was performed in Autodesk Meshmixer, as well as splitting the skull into a top and bottom to allow for installation of the brain hydrogel. This model was then exported as an STL for 3D printing.

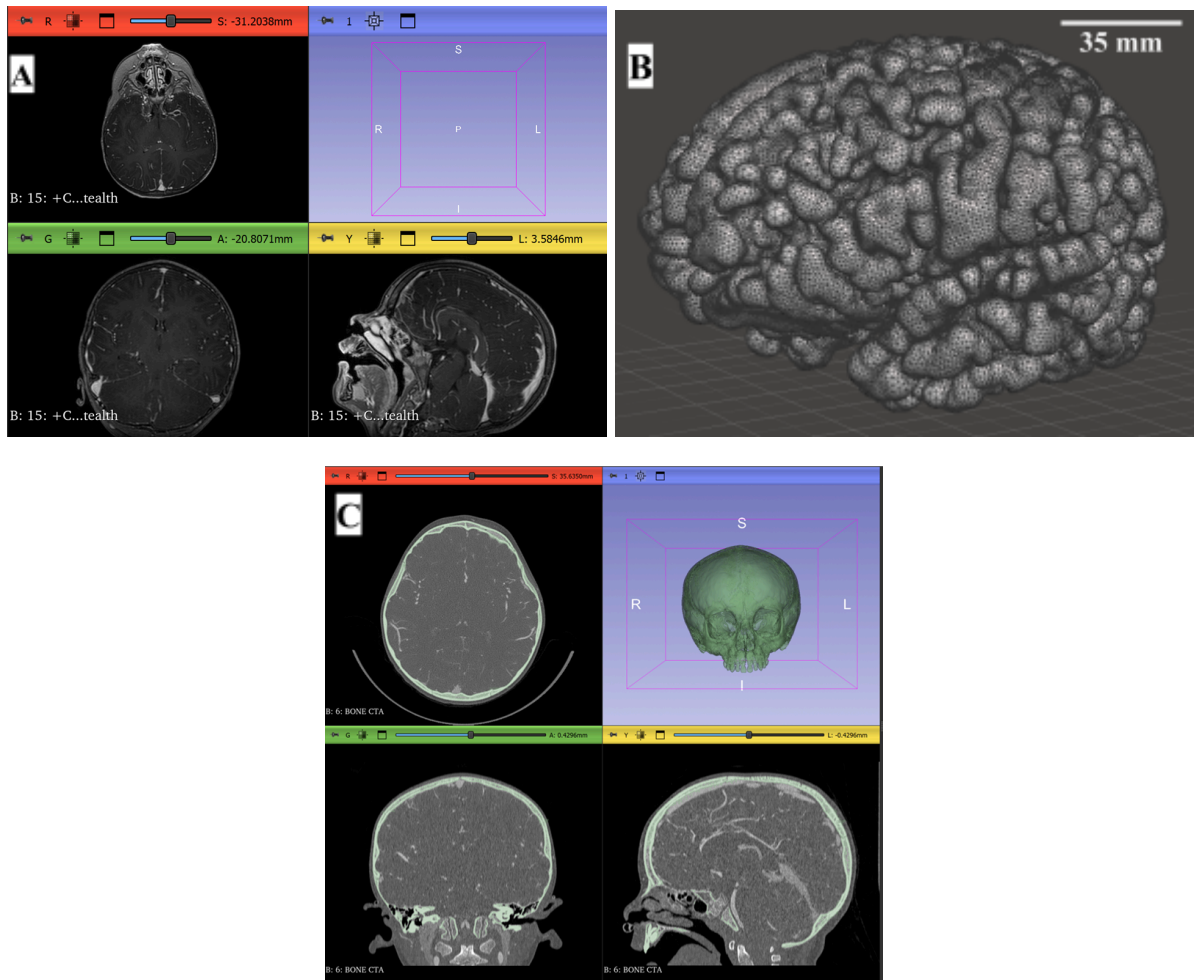


Figure 1: (A) Anonymized MRI scan of a 5-7 year old patient DICOM data in 3D Slicer prior to segmenting T1-weighted MR images and exporting into SimNIBS. (B) 3D model of brain

surfaces in Gmsh created by processing 5-7 year old patient-derived MRI scans in SimNIBS

4.5.0. (C) Segmentation of a pediatric skull model from patient CT scan in 3D Slicer.

2.3 Thermal Conductivity Testing Setup

Thermal conductivity testing was performed to evaluate and tune our brain phantom material to closely match literature values of brain tissue at 0.536 Watts per meter Kelvin (W/m-K) [12]. Equation I was used to calculate thermal conductivity from temperature measurements over a set period of time as one end of the hydrogel sample at room temperature (20°C) and the other was held at 30 °C.

$$k = \frac{m \cdot c \cdot \frac{dT}{dt} \cdot \Delta x}{A \cdot \Delta T} \quad (I)$$

A detailed procedure of thermal conductivity calculations and measurements can be found in the supplementary information, and a schematic of experimental setup, measurements, and circuitry can be found in Figure 2 . Briefly, gel samples were prepared in silicone ice cube trays approximately 2.5 x 2.5 x 2 cm³. Mass, surface area, and height measurements were taken from each gel before beginning testing. Samples were placed on a hot plate pre-heated to 30 °C, whose temperature was periodically confirmed with an infrared thermometer. Initial gel temperatures were recorded, and subsequent measurements were taken every two minutes over a 10 minute period. From the data obtained, calculations of thermal conductivity were made as described in Equation I.

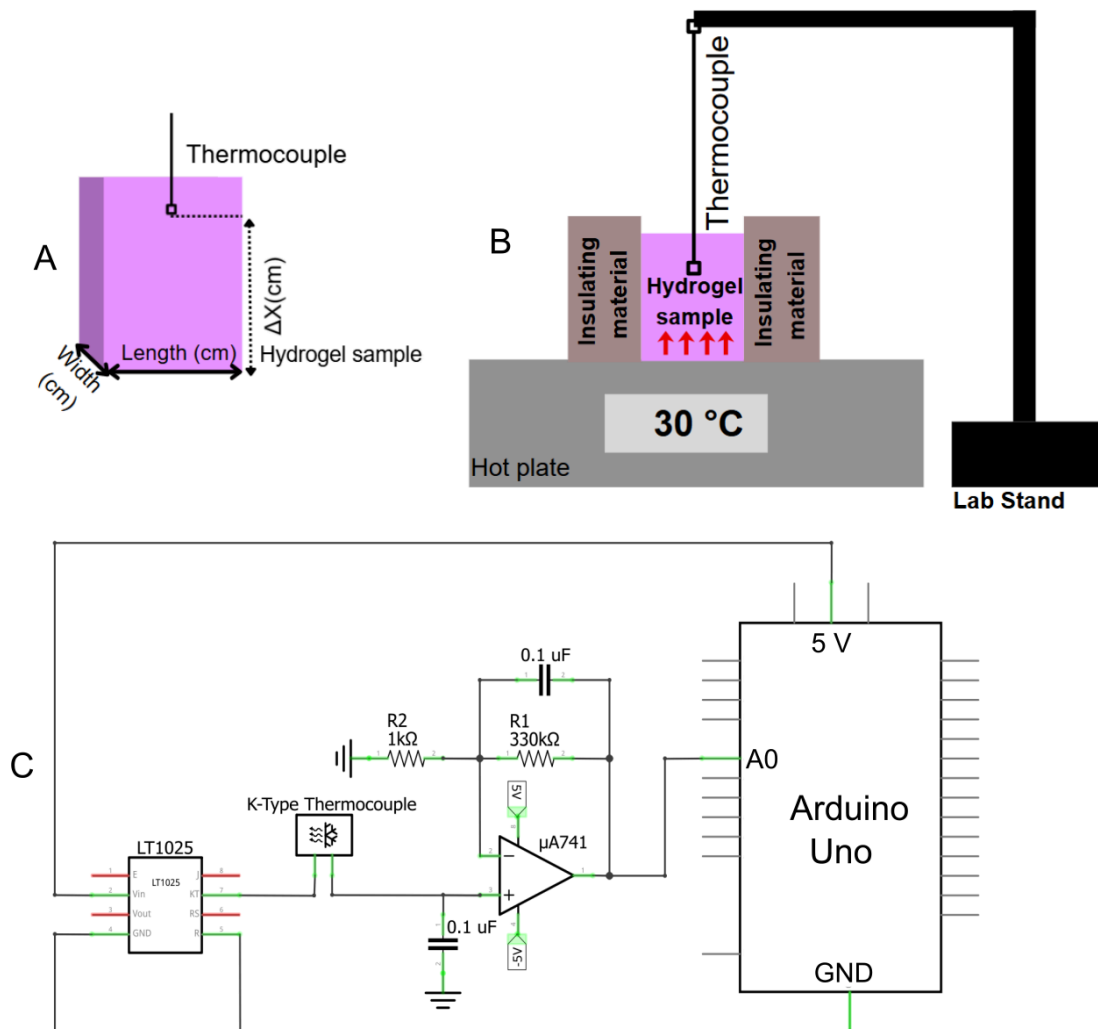


Figure 2: Hydrogel thermal conductivity testing. (A) Gel measurements compared to (B) conductivity testing setup. Panel (C) shows a schematic of the circuit used to take temperature measurements.

2.4 Electrical Conductivity Configuration

Another important consideration for the brain phantom material was that of its electrical conductivity, or its ability to conduct electric current [13]. Measuring the material's ability to allow flow of electric charge is essential prior to final phantom assembly, given the desired testing metric of secondary current build-up. Once again, the goal was to match values similar to those of brain tissue in literature: 0.2-0.5 Siemens per meter (S/m) [14]. To characterize the

various gel concentrations' conductivities, an induced-voltage set-up was configured, pictured in Figure 3, which made use of the relationships displayed in Equations II-IV:

$$V = IR \quad (\text{II})$$

$$\rho = \frac{R \cdot A}{L} \quad (\text{III})$$

$$\sigma = \frac{1}{\rho} \quad (\text{IV})$$

Gel samples of varying saline concentrations and gelatin concentrations, respectively, were formed and set within 3D-printed housings of 3.5 cm x 3.0 cm x 8.5 cm dimensions and left for 12 hours overnight. At the onset of testing, pairs of copper electrodes were inserted within opposite ends of each gel sample and attached to two digital multimeters, one in parallel and one in series. A Keysight 33210A Waveform Generator was used to generate a 10 V_{pp} sinusoidal wave across a frequency range of 100-500 Hz. Subsequent induced voltage and current measurements reported by the digital multimeters were recorded for each sample.

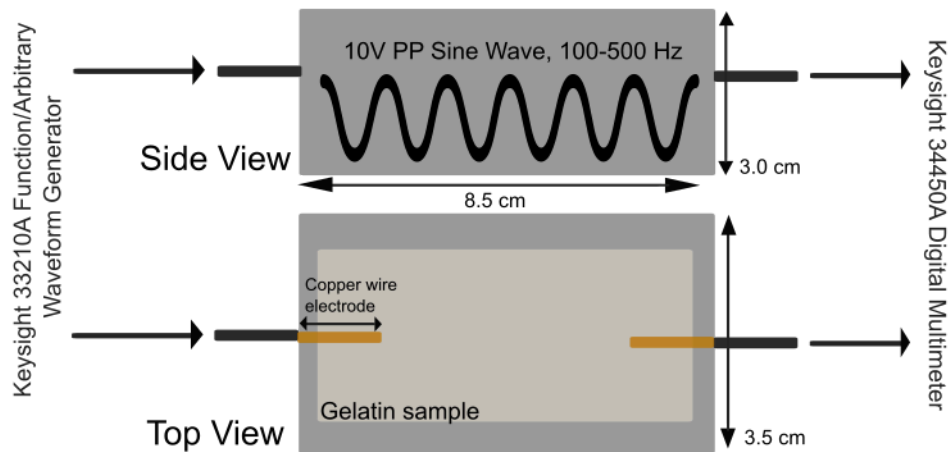


Figure 3: Schematic of electrical conductivity testing. A 10 V_{pp} sine wave was induced across the gels, with a frequency range of 100-500 Hz. Induced voltage and current measurements were used to calculate resistivity and conductivity.

2.5 Final Phantom Assembly

The final model is made up of an anatomically accurate 3D-printed pediatric skull component, based on matching CT and MRI scans of a 5-7 year old patient, and a gelatin-based hydrogel brain tuned with 1.2% saline. To fabricate the skull, CT scans were processed in 3D Slicer and refined in Autodesk Meshmixer. The processed file was printed at the Design Innovation Lab on the UW-Madison campus using a Formlabs Form 4 SLA 3D printer in Clear V5 resin. To create an anatomically accurate brain gel, a model of the brain was processed in SimNIBS from MRI scans and printed on a Bambu Lab X1E 3D printer in ABS. The printed brain model was vapor smoothed using 100% acetone to remove surface texture and a negative mold was fabricated out of silicone so as to form the brain component. After pouring the gelatin-based hydrogel into this mold, it was allowed to set in a 4 °C refrigerator for approximately 12 hours. Final assembly of the phantom model, combining both the skull and brain components into one prototype, occurred at the site of the TMS testing equipment, the Pediatric Neuromodulation Laboratory (PNL) at the Waisman Center. The brain was fitted snugly within the skull receptacle immediately prior to the onset of testing, as displayed in Figure 4. Prior to testing, three holes were pre-drilled into the skull to accommodate the insertion of the iEEG electrodes.

TMS pulses were administered to the final prototype in accordance with preexisting protocols which measure baseline and experimental values of activity by varying pulse intensity. A detailed description of our testing protocol can be found in the supplementary information. To evaluate impacts of varying pulse intensity levels, recordings were taken for TMS output intensities between 80-100%. Two testing methods were employed to see how to best integrate TMS and iEEG application with each technology's physical components: first, iEEG mechanical hardware was screwed within the skull material for electrode insertion, then removed to allow for more proximal pulse application.

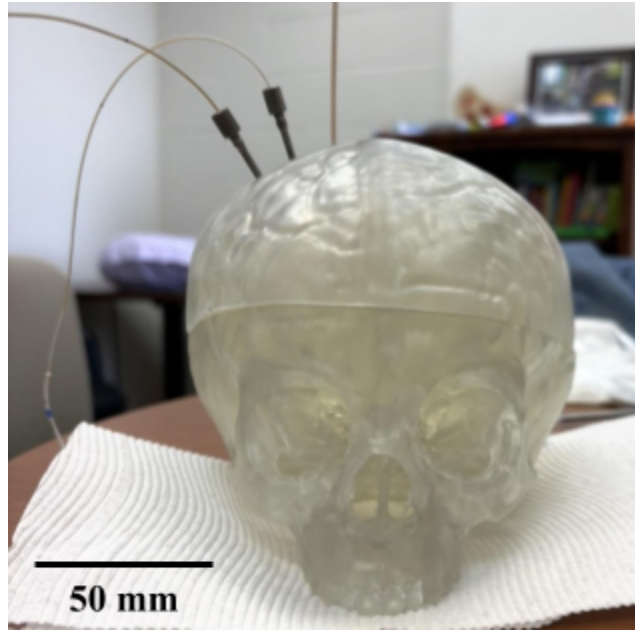


Figure 4: The final assembled brain and skull phantom, complete with inserted hardware and depth electrodes.

3. Results

3.1 Thermal Conductivity

Thermal conductivity testing was conducted on gelatin samples varying in both gelatin and saline percentage, with the setup described in section 2.3. Initial testing revealed average thermal conductivity values of 1.33 (4%), 1.47 (6%), and 1.32 (8%) W/m-K (n=4). Further thermal conductivity testing was then conducted on 6% gelatin samples by varying saline concentrations. These tests revealed average thermal conductivity values of 2.06 (0%), 1.77 (0.3%), and 2.11 (0.6%) W/m-K (n=3 or 4). Results from these experiments are summarized in Figure 5. These results were then compared to physiological values for the thermal conductivity of brain tissue, 0.536 W/m-K [12].

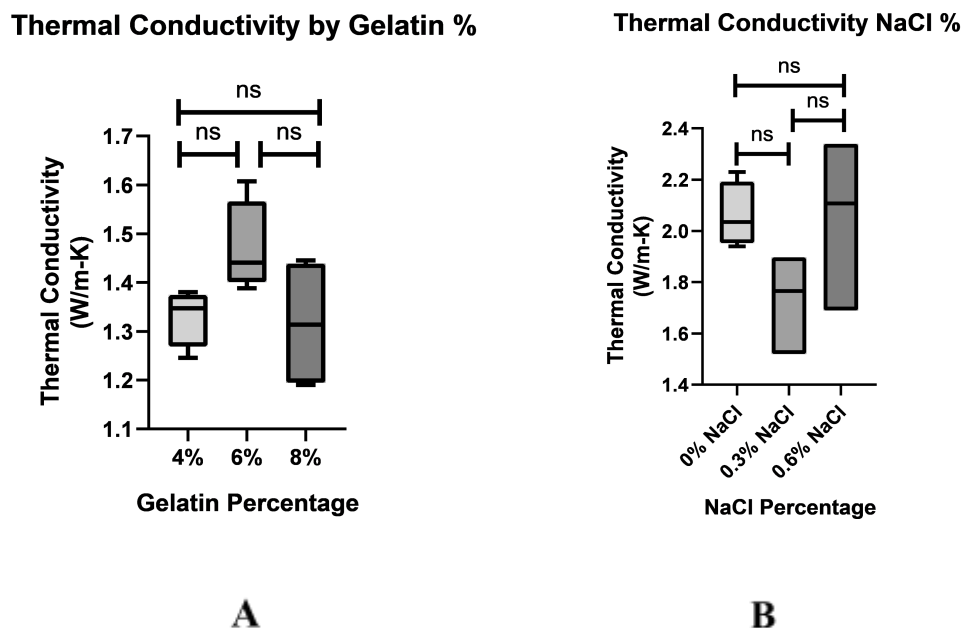


Figure 5: Thermal conductivity of gelatin hydrogels by gelatin and saline percentage. (A) Shows the thermal conductivity of gelatin samples by varying gelatin percentage between 4%, 6%, and 8%. ANOVA one-way analysis revealed no significant difference between values ($p=0.11$). (B) Shows the thermal conductivity of gelatin samples by varying saline percentage between 0%, 0.3%, and 0.6%. One-way ANOVA analysis revealed no significant difference between values ($p=0.16$).

3.2 Electrical Conductivity

To characterize the electrical properties of the chosen hydrogel, the voltage drop across samples was measured and used to find electrical conductivity (Equation II, III, and IV). In a similar fashion to thermal conductivity characterization protocols, two separate experiments were conducted, one utilizing hydrogels with 0.1% NaCl concentration that varied in gelatin percentage, the other comparing two 6% hydrogel compositions with different NaCl percentages. Varying gelatin percentage while holding saline percentage at 0.1% revealed average electrical conductivity values of 0.03, (3%), 0.04, (6%), and 0.03 (9%) S/m ($n=4$), as seen in Figure 6A. Varying saline percentages between 0.1% to 2.5% while holding gelatin constant at 6% revealed the data in Figure 6B; notably, 0.4% saline had an average electrical conductivity of 0.9 S/m and

1.2% saline had an average electrical conductivity of 0.62 S/m (n=4), values representing either ends of the reported physiological range of 0.2-0.5 S/m [14].

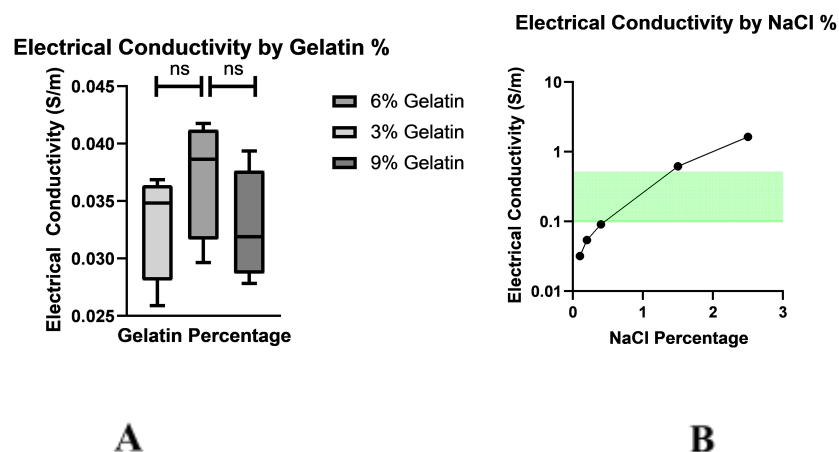
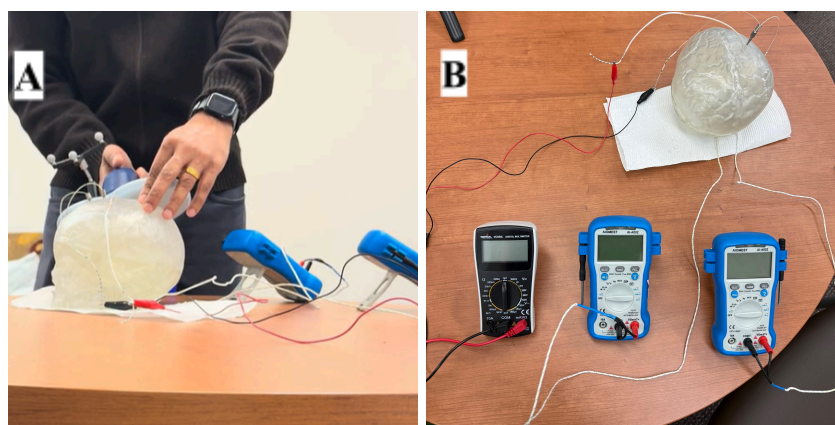


Figure 6: Electrical conductivity values by gelatin (A) and saline (B) percentage. (A) Shows electrical conductivity values as gelatin percentage varied from 3% to 9%. One way ANOVA analysis revealed no significant difference between electrical conductivity values in any condition ($p = 0.42$). (B) Shows a logarithmic curve determined by varying NaCl concentration and calculating resultant conductivity values. Highlighted in green is the target range of 0.2 to 0.5 S/m that is present in current reported literature values for brain tissue [14].

3.3 Final Phantom

After electrodes used for iEEG were inserted, single-pulse TMS (spTMS) was administered to the final phantom at increasing intensity from 80-100%. The electrodes in the phantom with hardware inserted moved vertically 0.65 ± 0.64 mm. When electrode hardware was removed, recorded vertical displacement was 0.57 ± 0.90 mm. Heating of less than 1°C was documented for electrodes with and without hardware. The induced current was 0 ± 0 uA and 0.24 ± 0.08 uA for electrodes with and without hardware, respectively. The testing setup, equipment used, and results for induced current testing on electrodes without hardware are shown in Figure 7.



C Boxplot of Measured Current Across TMS Output Intensities

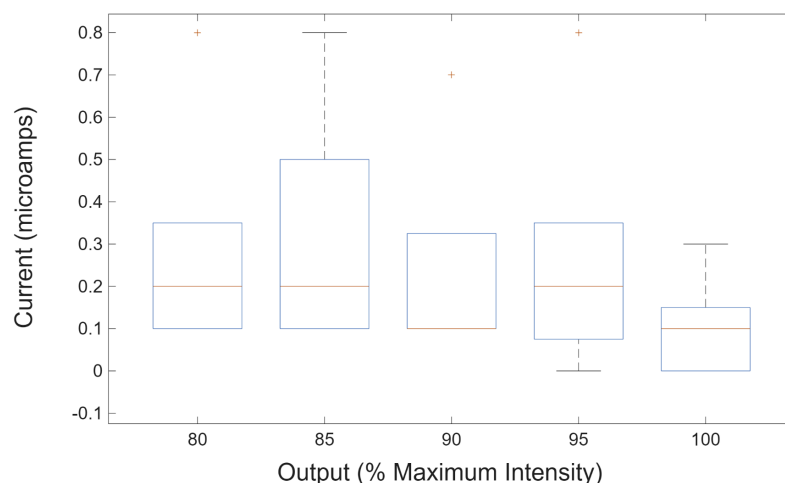


Figure 7: A) TMS operator applying a pulse to the skull phantom with electrode hardware inserted. The coil is applied approximately 5 cm from the electrode insertion. B) Three multimeters were used to measure temperature (right) and current (left) generation. Temperature was measured to a sensitivity of 1 °C with two multimeters, and current was measured to a sensitivity of 1 μ A (C) Boxplot depicting range of induced current measured during spTMS over an output intensity range of 80-100%.

4. Discussion

4.1 Hydrogel Testing

ANOVA analyses on initial thermal conductivity results for gelatin hydrogels did not show statistically significant differences while varying both gelatin percentage and saline

percentage. As such, the group determined that neither of these variables would greatly affect the thermal conductivity of the hydrogel brain moving forward; aiming to keep close to literature values of hydrogel brain phantoms in order to best match mechanical properties, 6% gelatin was chosen as the final gelatin concentration [15]. While the thermal conductivity values found for 6% gelatin far exceed those of pediatric brain tissue at 1.47 W/m-K and 0.543 W/m-K for our phantom and literature values, respectively, this did not exclude the composition from further usage for two reasons [12]. First, a thermal conductivity value higher than physiologically accurate does not impede the phantom's ability to effectively determine the potential of TMS to induce temperature change in brain tissue, and in fact it provides a threefold factor of safety for evaluating this parameter. Secondly, the mechanical integrity of the brain phantom needed to be taken into consideration. Attempts to fabricate significantly lower gel percentage hydrogels revealed extremely poor mechanical properties that were not conducive to creating an anthropomorphic brain phantom. The team therefore decided to vary gel percentage only within a small range close to 6%, effectively our working range, within which there was insignificant variability of thermal conductivity. Determination of final saline concentration was left until the conclusion of electrical conductivity testing.

Following analysis of thermal conductivity testing, it was revealed that thermal conductivity of gelatin hydrogels was not dependent on gelatin percentage nor saline percentage. We then sought to evaluate whether electrical conductivity followed the same trend. After varying gelatin and saline percentage, ANOVA analyses revealed that, while gelatin percentage between 3% and 9% did not have a significant effect on electrical conductivity, varying saline percentage did. We then set up a concentration series of saline-tuned gelatin hydrogels, from which we created a linear regression to determine a saline percentage that corresponded to physiological values. Of the compositions evaluated, 6% gelatin with 1.2% saline most closely matched the electrical conductivity of pediatric brain tissue, at 0.62 S/m (our phantom) as compared to 0.2-0.5 S/m (brain tissue) [14]. The team selected this composition as our final brain tissue material, which we used to fabricate our final prototype and test under TMS with implanted iEEG electrodes. Despite the hydrogel composition having an electrical conductivity at the upper range of native brain tissue, the team selected 1.2% saline in our composition to ensure that our phantom did not artificially repress current conduction by not accommodating for the full range of potential values.

4.2 Phantom Testing

The pediatric brain phantom underwent testing using TMS and iEEG in tandem to assess electrode displacement, heating, and electric field generation. There were several challenges noted during this process.

Primarily, due to the specific equipment required for pulse administration – that is, a MagStim² TMS system and corresponding coil – it was necessary that the final testing process occur at the PNL. This led to limited options for additional testing equipment that was both portable and highly accurate. With diminished accuracy for this preliminary testing round, therefore, it is important that the next step be to determine a more robust measurement system that can guarantee electrode heating, displacement, and field generation results to a higher degree of confidence.

Additionally, while the phantom maintained general physical integrity, the hydrogel brain continued to slowly, gradually increase in temperature unrelated to TMS application. While this was unavoidable, it may be necessary to institute a time limit for future testing endeavors, should sessions run longer, to avoid degradation of hydrogel quality.

4.3 Assumptions and Simplifications

While creating this brain phantom model, we greatly simplified the physiology and anatomy of the pediatric human brain and skull. While we prioritized matching the thermal conductivity, electrical conductivity, and mechanical property values of pediatric brain and skull tissue, there are a wide variety of tissue types in vivo that present very different properties. Each part of the brain, including the cerebrum, cerebellum, brainstem, and their associated cell types, has its own unique thermal, electrical, and mechanical properties which are difficult to recapitulate in our simplified model [16]. By assuming the brain is a uniform structure and targeting electrical conductivity values of 0.2-0.5 S/m and a thermal conductivity of 0.536 W/m-K, we must acknowledge that this model cannot fully represent the complexity of human brain anatomy [12].

4.4 Limitations

There were several factors across multiple aspects of the project that impacted the successful creation of the pediatric brain phantom. First, inconsistencies in the equipment that

were used introduced variability in both fabrication and testing of the hydrogels. Specifically, the hot plates used to heat and stir the gels did not maintain stable or accurate temperatures, as their displayed values often differed from those measured with an infrared thermometer. This inconsistency made it difficult to reproducibly prepare gels with consistent properties. To compensate for this, the temperature of the hot plate had to be continuously monitored with the infrared thermometer and manually adjusted, likely leading to inconsistencies. Additionally, when inserting the thermocouple into the gels during thermal testing, the associated Arduino software often malfunctioned and displayed inaccurate temperature readings, leading to potential inconsistencies.

Second, current literature regarding the fabrication of a pediatric brain phantom is limited. The most closely related study focuses on testing the safety of iEEG and TMS together specifically in adult patients, not pediatric. While some pediatric brain phantoms have been developed, they are typically designed for vastly different applications and do not address the combined use of TMS and iEEG.

Third, time constraints further limited the overall success of the brain phantom. The phantom was designed, fabricated, and tested within approximately seven months, with a significant portion of this time dedicated to iterating a model that best matches the properties of native pediatric brain and skull tissue. Persistent issues with materials and testing delayed progress and reduced the time that was available for testing TMS and iEEG together.

5. Conclusions

Completion of this phantom will allow for validation of the use of TMS in conjunction with iEEG for pediatric patients. While previous studies have addressed concerns with using TMS and iEEG in conjunction in adult patients, they fail to consider more stringent safety standards and physiological differences present in pediatric patients [4]. Epilepsy is the fourth most common neurological disease, often manifesting in pediatric patients before the age of one year [1]. iEEG is routinely used in surgical planning for epilepsy in adult and pediatric patients, and TMS may provide complimentary information for mapping critical regions of the brain that should be avoided during surgery. However, there are still many safety concerns around the use of TMS in patients with iEEG electrodes actively implanted. Our aim was to develop a phantom for validation of use of TMS in pediatric patients with implanted cortical electrodes.

Based on the current state of the model and testing, definitive conclusions about the safety of combined use of TMS and iEEG cannot be made at this time. For future testing, improvements to the resolution of equipment must be made to effectively sense changes in temperature, any potential current generation, and displacement that may occur. While the current thermal conductivity of the hydrogel brain introduces a factor of safety, further improvements could be made to the composition to bring that number closer to the thermal conductivity of 0.536 W/m-K target. Additionally, verification of the rheological properties should be completed on the final hydrogel composition to ensure accuracy of mechanical properties. After improving model fidelity in this way as well as improving the testing set up, more comprehensive TMS testing will be conducted to investigate the safety of combined TMS and iEEG. Testing done on the final skull and brain phantom assembly met the assigned safety guidelines; however, the quality of equipment used was below preferred standards, and additional testing which provides higher accuracy must be done in order to confidently claim TMS and iEEG are safe to use in tandem for pediatric patients. Repeat fabrication and testing should also take place using the described methods for a range of ages, as brain tissue characteristics as well as brain size vary greatly during childhood development. Following these intensive testing procedures, a claim about the safety of joint TMS and iEEG use will be made based on electrode displacement, electrode heating, and induced current generation.

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Supplementary Information

Protocol for Processing a CT Scan in 3D Slicer

Part 1: Import STL and prepare for editing in 3D Slicer

1. Open 3D Slicer.
2. Import the STL file using drag and drop or Add Data.
3. Go to the Segmentations module.
4. Select Import model to convert the STL into a segmentation.
5. Click Specify geometry and set the geometry to fit the imported segment.
6. Set voxel size to approximately 0.5 to 1 mm to preserve detail.

Part 2: Initial cleaning in 3D Slicer

1. Open Segment Editor and select the created segmentation.
2. Ensure geometry is defined; source volume can remain None.
3. Apply Smoothing with the Closing method.
4. Start with a kernel size of 1 mm and increase gradually up to about 2 mm if needed to fill holes.
5. Inspect the model in 3D view to confirm small holes are filled.
6. If larger holes remain, install the SurfaceWrapSolidify extension.
7. Use Wrap Solidify with outer surface mode and smallest detail set to approximately 1 to 2 mm.
8. Remove remaining artifacts using the Scissors tool in 3D mode or Islands to remove small disconnected regions.

Part 3: Export cleaned model from 3D Slicer

1. Return to the Segmentations module.
2. Select Export to models.
3. Save the output as an STL file.

Part 4: Refinement in Meshmixer

1. Open Meshmixer and import the cleaned STL.
2. Go to Analysis and select Inspector.
3. Run Auto Repair All to detect and fill remaining holes.
4. For more control, select individual holes and apply Smooth Fill.
5. Inspect the mesh for remaining holes and surface irregularities.
6. Apply light smoothing if necessary, avoiding excessive detail loss.

7. Perform a plane cut to separate into the top half (delete bottom). Repeat for the bottom so that two separate models of the two halves of the skull are achieved.
8. Remove remaining vasculature inside the brain using the shrink/smoothing tools.
9. Export the final cleaned STL.

Part 5: Import into Fusion 360

1. Open Fusion 360.
2. Upload the STL file.
3. Insert the mesh into the workspace.
4. Switch to the Mesh workspace if needed.
5. Use Repair and Close Holes to fix any remaining minor defects.
6. Optionally re-mesh or convert to BRep if further CAD operations are required.

File Processing from STL to Workable CAD

Part 1: Import Structural MRI Data into 3D Slicer and Verification of Image Quality and Orientation

1. Open 3D Slicer
2. Select “DICOM” from the main toolbar
3. Import the MRI dataset into the DICOM database
4. Load the T1-weighted scan into the viewer
5. Examine the scan in axial, sagittal, and coronal views
6. Confirm that the entire head is included in the scan
7. Verify that no significant motion artifacts are present
8. Confirm that voxel resolution and orientation are appropriate
9. If necessary, reorient or resample the volume using the Volume module

Part 2: Export of MRI Volume

1. Select the loaded volume in the Data module
2. Open the Save dialog
3. Export the volume as a NIfTI file (.nii or .nii.gz)

Part 3: SimNIBS Segmentation Pipeline

1. Open the SimNIBS command prompt
2. Run the segmentation command:
charm stealth T1.nii.gz

3. Allow the pipeline to complete automatic processing steps, including skull stripping, bias-field correction, tissue segmentation, cortical surface generation, and head mesh creation

Part 4: Generation of Subject Model Directory and Outputs

1. Locate the generated directory (e.g., m2m_stealth)
2. Navigate to m2m_stealth/surfaces/ for cortical surface files
3. Access additional segmentation outputs and mesh files within the main directory
4. Identify relevant surface files:
 - lh.central.gii
 - rh.central.gii
 - lh.pial.gii
 - rh.pial.gii

Part 5: Refinement of Surface Models in Autodesk Meshmixer

1. Open Meshmixer
2. Import the surface model (converted to STL if necessary)
3. Go to Analysis and select Inspector
4. Run “Auto Repair All” to detect and fill mesh defects
5. For manual refinement, select individual defects and apply Smooth Fill
6. Inspect the surface for irregularities and apply light smoothing if needed
7. Remove unwanted internal structures using smoothing or sculpting tools
8. Export the refined surface model as an STL file

Material Testing and Sample Fabrication Protocols

Part 1: Sample Fabrication

1. Create samples by dissolving 6 % w/v in MilliQ water.
2. Begin by spinning at 400 rpm on a hot plate kept at room temperature, approximately 20°C.
3. After the gelatin has soaked for approximately 5 minutes, set the hot plate to 80°C and monitor the solution temperature until it reaches 65°C.
4. Add NaCl powder at 1.2% w/v and spin on the hot plate for a further 20 minutes. Pour into silicone molds using a serological pipette.
5. Allow to firm overnight or at least 4 hours in a 4°C fridge.

Part 2: Thermal Conductivity Testing

1. Cut $n > 3$ samples from the hydrogel mold. For consistency, they should all be approximately the same size. Make these as close to rectangular as possible.
2. Weigh samples and take their dimensions, noting which face of the sample will be in contact with the hot plate. This will be the surface area (A) dimension.
3. Measure the length of the thermocouple to be inserted into the sample. Mark this value, and subtract it from the height (measured as the dimension orthogonal to the face in contact with the hot plate) to calculate the ΔX value.
4. Set the hot plate to $35\text{ }^{\circ}\text{C}$ and allow it to come to temperature.
5. Wrap the sample in an insulating material and insert a thermocouple. Measure and record starting temperature.
6. Allow the sample to sit on the hot plate for 10 minutes. Each minute, take a temperature measurement and record it. After 10 minutes, the sample at the location of the thermocouple will not be $35\text{ }^{\circ}\text{C}$, but it should be increased by a number of degrees C.
7. Take the sample of the hot plate and calculate thermal conductivity from the given equation.

Thermal Conductivity Thermocouple Testing Code

```
int sensorPin = A0; // select the input pin for the potentiometer
int sensorValue = 0; // variable to store the value coming from the sensor
float tempSum = 0.0;
int count = 0;

void setup() {
  // declare the ledPin as an OUTPUT:
  Serial.begin(9600);
  pinMode(ledPin, OUTPUT);
}

void loop() {
  // read the value from the sensor:

  sensorValue = analogRead(sensorPin);
  float voltage = (sensorValue * 0.0049);
  float temp = (voltage + 0.267) / 0.0143;
```

```

//Serial.print("Temp: ");
//Serial.println(temp);

count++;
tempSum = tempSum + temp;
float temp_ave;
if (count == 100) {
  temp_ave = (tempSum/100);
  //Serial.println(tempSum);
  Serial.println(temp_ave);
  tempSum = 0;
  count = 0;
}
delay(10);

}

```

Electrical Conductivity Testing Protocol

Materials:

- Keysight 33210A Function/Arbitrary Waveform Generator 10MHz
- Keysight 34450A Digital Multimeter
- Astro AI M4KOR Digital Multimeter
- Two copper electrodes
 - Copper wire stripped approximately 2.5 cm at each end
- Additional connecting wires
 - Two sets of multimeter probes and BNC coaxial cable
- Infrared thermometer
- 4x 3D-printed PLA box of dimensions 3.474cm x 3.007 cm x 8.554 cm
- Fabricated hydrogels: 6% gelatin, 0.1% NaCl
 - Left to sit for 24 hours, minimum, refrigerated at 4°C

Methods:

1. Collect the starting volumetric and mass measurements of gels to be tested:
 - a. Mass, depth of gel within housing
 - b. Take the temperature of the gel that is being tested

2. Assemble the testing set-up, focusing on one gel at a time:
 - a. Insert the end of a copper electrode at each end of the gel's length, measuring depth of insertion
 - b. Utilizing the two digital multimeters, complete assembly so that one is connected in series, and the other in parallel
 - i. The digital multimeter in series should be set to record current, while the multimeter in parallel should be set to record AC voltage
3. Before turning power on, ensure that all devices are running and working properly.
4. Generate a waveform on the generator with the following specifications:
 - a. Sine wave
 - b. $10 V_{pp}$
 - c. Frequency ranging from 100-500Hz (measurements will be noted at 100, 200, 300, 400, and 500 Hz, respectively)
5. Upon pressing "output" and generating the waveform, observe and record the reported induced voltage and current between the electrode components
6. Repeat at each frequency point in the frequency range desired above.
7. To solve for resistance of each gel, utilize Ohm's Law and the reported induced voltage values corresponding with each signal

Final Prototype Fabrication Protocol

Skull:

1. Upload the workable STL file from the skull CT scan into Formlabs Preform.
2. Print with tree supports out of Formlabs Clear V5 resin on a Formlabs SLA printer.
3. After the print is complete, carefully remove tree supports.
4. Using a 1/8 inch center drill and a size 39 drill bit, carefully mark and drill three holes to accommodate the iEEG hardware.

Brain:

1. Upload the workable STL file from the brain MRI scan into Bambu Studio slicing software.
2. Slice the file with tree supports and print in ABS.
3. Once the print is complete, vapor smooth the print by placing it in a glass container with vaporized 100% acetone until the surface texture has smoothed.
4. Dry the mold completely. In a container that can completely accommodate the size of the print, mix equal parts of Part A and Part B of your silicone mixture

5. Place the brain carefully in the silicone mixture. Pop any bubbles by tapping the container on the table.
6. Leave the mold to set overnight.
7. Remove the silicone mold from its container. Cut the mold in half carefully and remove the ABS brain
8. Mix the gelatin composition as described above. The final mixture used for the brain phantom had a volume of 1100 mL.
9. Pour the gelatin mixture into the negative mold and allow to set overnight at 4 °C

Transport and Assembly:

1. Should the phantom need to be transported, utilize an insulatory carrier to preserve the hydrogel's temperature prior to testing.
2. Upon assembly of the full model, carefully separate the top and bottom skull halves and remove the gelatin brain from its mold.
3. Fit the brain portion snugly within the skull receptacle and begin testing promptly.

Brain Phantom Testing Protocol: Transcranial Magnetic Stimulation

Materials:

- Assembled phantom:
 - Skull receptacle
 - Holes for the electrode hardware are to be drilled ahead of time (one for each electrode/bolt pair)
 - Hydrogel brain
- Depth electrodes
 - One of each of the following depth electrode conformations:
 - 6-point
 - 8-point
 - 10-point
 - Associated hardware: securement bolts for insertion within the skull
- Magstim 200², Magstim, United Kingdom

- Transcranial magnetic stimulation (TMS) pulse generator used in tandem with a Magstim D70² figure-eight coil
- Tripods (2) and iPhone cameras (2)
- Multimeter (3)
 - Additional connective hardware: alligator clips and temperature probes
- Caliper
- Tape measure

Testing:

1. During set-up, place the phantom on a flat, stable surface that is accessible for the TMS operator. The coil will need to be as proximal as possible to the phantom for proper pulse administration. Place the tripod and filming device in a location that offers an unobstructed view of the phantom's surface with the depth electrodes inserted. Provide a reference object in the camera's view and measure it for later calibration.
2. To assemble the electrodes and bolts, first remove the cap from the bolt by unscrewing it. Insert the electrode into the bolt with each half still separate, such that the position of the bolt can be easily adjusted. With a partner lifting up the top half of the skull, insert the electrode through the pre-drilled hole in the skull and into the brain gel until all copper points are completely inserted. Carefully place the top half of the skull back on the phantom. Screw the bottom half of the bolt into the hole by twisting five times. The thread is tapered, and will become harder to turn. Finally, secure the cap back onto the bottom of the bolt to prevent the electrode and bolt from sliding relative to one another.
3. While the TMS operator turns on and calibrates the Magstim machine, take a temperature reading of the brain phantom and record the observed result.
4. The coil will then be used to apply pulses across a stimulation intensity range of 50-100% machine output delivered at 10–40 Hz, at two different positionings: approximately 5cm away from the collection of insertion points, and directly adjacent to the collection of insertion points.
 - a. 30 pulses will be administered, 5 at 80% intensity, 5 at 85% intensity, and so on until 100% intensity is reached.
 - b. The number of pulses delivered should be recorded.

- c. The following should be recorded:
 - i. Length of electrode inserted (once, prior to pulse administration).
 - ii. Temperature reported at electrode (after every pulse).
 - iii. Current reading at electrode (after every pulse).
 - iv. Length of electrode AFTER pulse administration (at the end of the 30 pulse interval).
- d. Between the two trials – corresponding to the two positionings of the coil – record any noted change in temperature over a 5 minute period to account for the possibility of the gelatin hydrogel slowly coming to room temperature.
- e. Start a new video for each trial to allow for recalibration after set up.
5. After all pulses have been delivered, make any visual observations of the integrity of the phantom.
6. After testing has concluded, consult the video recordings to mark any other visual displacement throughout the process.