Print-A-Punch

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I. Abstract

The Print-A-Punch project addresses the need for precise, symmetrical cruciform tissue samples for biaxial testing in cardiovascular research. Current manual cutting methods result in asymmetrical samples, causing inaccuracies in stress-strain analyses due to shear stress and misaligned measurements. This issue impairs research aimed at understanding tissue mechanics, especially in patient-specific models.

The solution to the asymmetry is a 3D-printed cutting jig designed for ease of use, reproducibility, and cost-effectiveness. The jig guides biopsy punches and razor blades to produce symmetrical samples with minimal setup, including stabilization mechanisms to secure tissue during cutting, and thereby reducing movement and potential asymmetry. Comparative testing between the jig and manual cutting method demonstrated significantly improved symmetry with the jig, confirmed by statistical analysis.

The jig design utilizes resin-based 3D printing for precision and transparency, enabling accurate alignment of biopsy punches. While testing yielded results supporting the efficacy of the design, testing was not made on the same decellularized tissue that is in use in the lab. Future iterations of testing should address these limitations, alongside exploring alternative stabilization techniques such as vacuum systems or adhesives.

This approach streamlines tissue preparation, enhancing the reliability of biaxial testing. By making the jig design accessible for public use, this project supports broader adoption of standardized testing methods in cardiovascular research, ultimately improving the quality and reproducibility of data for mechanical behavior analysis.

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III. Introduction

The Print-A Punch project is centered on the need for a punch that is able to cut symmetrical crucifix shape tissue samples for biaxial testing. This device must consistently cut the cardiovascular samples, be sterilizable for lab use, and available at a relatively low cost. This product is developed for the clients' Dr. Colleen Witzenburg and Mr. Daniel Pearce usage in a cardiovascular lab. However, this product is public domain to be used by any researcher or research facility. This product allows for symmetrical samples to be made, in turn allowing for more accurate testing results from tissue comparison due to having fewer discrepancies between the samples.

This device is necessary to provide more accurate samples, aiding in the research and testing of the mechanical properties of various tissue types. Although this specific device is made to be utilized in Mr. Pearce's cardiovascular lab, this model can be applied in a variety of studies. This model also boasts a low barrier to entry for use. Users need only to 3D print the jig in accordance to the correct size biopsy punches. This jig ensures that the crucifix shaped samples are symmetric, allowing more accurate testing results as the measure of skewness predicted often do not quantify the symmetry correctly [1]. While asymmetric samples can and have been used for analysis of tissue mechanics, it has been found that they do not produce adequately accurate results, as shear strain complicates assumptions in planar biaxial testing and affects stress calculations and parameter estimations in modeling. Sample shape asymmetry results in a larger, more homogeneous high shear strain region, potentially improving estimates of shear and axial coupling moduli [2].

Two current competing designs on the market are the uniaxial Print-A-Punch and the novel tissue cutting apparatus. Neither of these designs are similar or relevant enough to the scope of this project such that property infringement would be a concern. The uniaxial Print-A-Punch was created by a group of Boise State University students in 2020, utilizing 3D printed components with screws and razor blades to create a hand held punch that cuts out a dog-bone shaped tissue sample. This device bends two razor blades into the dog-bone shape required for uniaxial testing. This technology facilitates the broad adoption of standard test methods that improve the quality and reproducibility of tensile tests in soft biological tissue. Researchers can freely download a set of STL files from this study to build their own Print-A-Punch device [3]. Next, the novel tissue cutting apparatus is a design for a similar cutting process. This device cuts perpendicularly across the desired tissue samples, using 3 linear actuators, 2 load cells, and tissue holding fixture.. Blades are sterilized after each cut. The device works by utilizing 2 actuators controlling lateral movement, with the third controlling the cutting motion [4]. While this device is not directly competing with the biaxial Print-A-Punch, literature values and results may be analyzed and documented for appropriate reference.

This project aims to create a device to cut cruciform shaped samples. Current processes result in asymmetric samples, leading to inaccurate results due to aforementioned shear stresses. These forces lead to inaccurate material property measurements that affect lab data. The clients are requesting a new cutting process that will allow them to produce more uniform and accurate samples for cardiovascular tissue testing. This device should maintain these properties consistently no matter the type of cardiovascular tissue or tissue thickness. By ensuring uniformity in the cruciform samples, the system will reduce errors in material property computation, improving the accuracy of mechanical behavior estimations for soft tissues. This approach will serve as a cost-effective alternative to custom steel punches, using 3D printing to enhance accessibility and reproducibility in tissue testing procedures [5].

IV. Background

Client Daniel Pearce is a PhD Candidate currently studying the effects of heart attacks on heart tissue, working under Dr. Colleen Witzenburg, the principal investigator of the cardiovascular biomechanics laboratory. Pearce is comparing tissue samples, as seen below in Figure 1, from induced heart attacks to healthy heart samples in order to see the effects that a heart attack has on the cardiovascular tissue's mechanical properties.

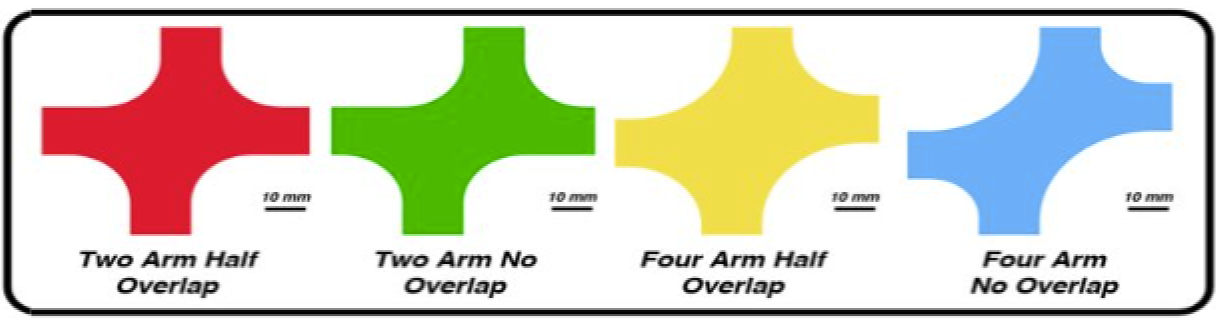


Figure 1: Computational sample shapes from FEA (adapted from [2])

The primary function of the Print-A-Punch is to generate symmetrical cuts through cardiovascular tissue; additionally, this device is to be accessible on public domain such that other labs may create their own punch for individual application. Research was conducted on the effects of impact that the tissue sample experiences during biaxial testing. This research concluded that a variety of factors may have significant influence: sample thickness, cross-sectional area, lateral contraction quantification, starting reference configuration, strain determination method, and time. These factors may impact the reconstruction of the mechanical behavior of a sample and the homogeneity of the stress field under tensile loading [6].

The main focus of the design is to achieve accuracy and precision of the punches, therefore lowering the undesirably high shear forces due to asymmetry on the cruciforms. One challenge of the cutting process is making clean, thorough cuts through the cardiovascular samples. Specific shear strains of 4-12N are required to puncture heart tissue, and a constant application of 2-4N is required for continual cutting [4]. Therefore, in creating a punch for biaxial testing, there were a few key constraints to consider for this prototype. One of these requirements is that the plastic used to create the prototype abides by ASTM: 638 [7], plastics that will not cause tissue degeneration, nor degrade under sterilization.

The biaxial tensile tests performed in a research and development manner investigate stress values at the intersection point of the specimen [8]. Biaxial testing on cardiovascular tissue uses simultaneous forces in two principal directions, allowing for analysis of strain-rate, creep, stress-relaxation, and force-controlled metrics. Strain-rate testing determines time-dependent behaviors of tissue, while creep testing shows how tissue displacement creeps to maintain peak membrane tension. Stress-relaxation testing shows tissue stress reduces from peak membrane tension. Finally, force-controlled testing reveals material's anisotropy and nonlinear stress-strain response, and has the five loading ratios: 1:1, 0.75:1, 1:0.75, 0.5:1, and 1:0.5 [9].

The main product design specifications for the project entails a focus on accuracy, ease of use, and low cost. The punch must be capable of replicating the intended punch pattern on every attempt, with the overall length and width of printed punches having an error of less than 5% [5]. For ease of use, the prototype must be able to facilitate a cut through multiple types of tissue with relative ease, without requiring excessive force to operate. The device must be simple enough to be easily replicated by other researchers for laboratory use. The device must also require minimal setup time. Print-A-Punch should be able to withstand chemical sterilization, meaning that it must not corrode when in contact with ethanol or PBS solution. For this reason, polylactic acid (PLA), resin, and nylon substrates were considered for this project, due to their chemically resistant properties [10]. Fabrication itself requires only that the punch be simple to print with 3D printers/resin casters, with a print time of under 5 hours, and a reasonable price per gram of filament/resin.

V. Preliminary Designs

Design 1: Razor 1-Step

Design one utilizes a large stamping mechanism with attached razor blades for cutting samples to the predetermined geometry. The design requires 4 bolts with wingnuts (not pictured) that screw into the frame of the punch, forcing the razors into the U-shaped geometry required for the cruciform. The razor 1-step is a singular punch, depicted with a before and after cut in Figure 2 below.

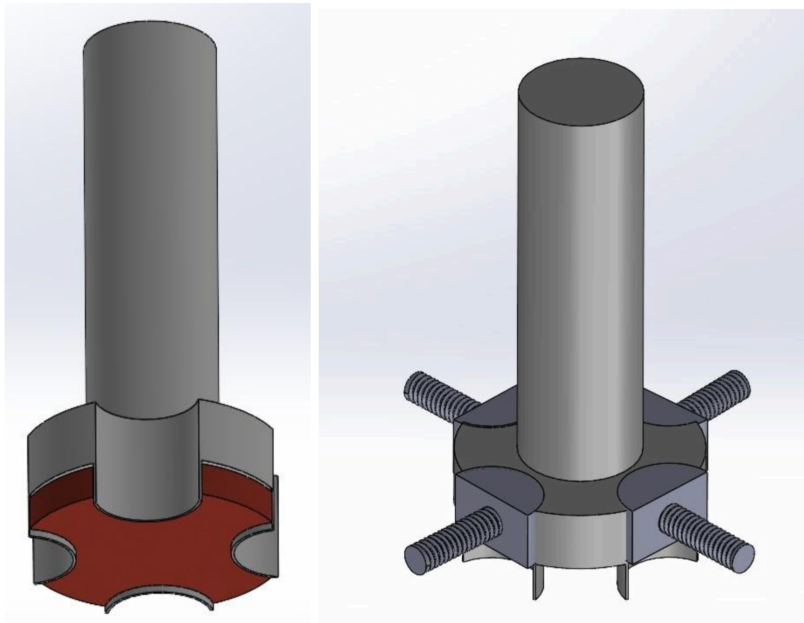


Figure 2: Razor 1-Step CAD model with and without shaping bolts

In using the razor 1-step, the user would first secure the four blades in place by tightening the wingnuts on the shaping bolts. After the wingnuts were sufficiently tightened, the user would then position the punch over the tissue sample, and plunge down in a stamping motion with approximately 4-12N of force [4], to sufficiently cut the apertures from the sample. If required, the user would then use a rolling motion of the wrist to ensure a complete cut of the tissue sample.

One of the benefits of this design consideration is ease of forming. The razor blades in this design would be much easier to shape than that of the other design considerations involving razor blades, due to the wingnuts tightening onto bolts. This element allows for better time management, safety, and overall efficiency of the design. Another benefit of the razor 1-step is a low production cost, as the 3D printed PLA handle is a cheap, versatile option that is easily accessible for most seeking to use such a device [11]. Finally, fewer razor blades would be needed for this design, as bending razor blades to less extreme angles puts less strain on the metal, reducing the factor known as metal fatigue [12].

There are also several constraints of this model to consider. Razor blades may not be the most feasible option for cutting, as successfully bending them could prove to be a challenge. Additionally, if the user desires a perfectly circular sample, this design would be enough to alone cut the tissue, and a second punch would be needed. Finally, the razor 1-step does not address the issue of the fastening the sample during the cut, as the design does not have any feature to prevent the sample from sliding or shifting on the cutting station.

Design 2: Razor 2-Step

Design two again uses a stamp-press method, where the user presses down onto the tissue sample to make a cut. As pictured below in Figure 3, the two components contain separate geometries of razor blades for making two separate cuts, as well as a framing jig as shown in the bottom of Figure 4.

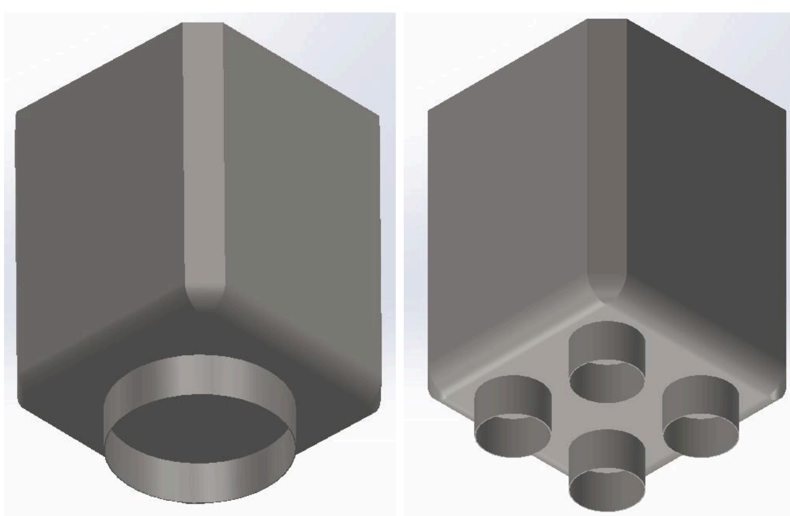


Figure 3: Razor 2-Step CAD model stamps

In using the razor 2-step, the user would first insert the pre-bent blades into the grooves of the PLA plastic handle. The user would then center the tissue sample in the framing box, and while using one hand to secure the frame, the other would stamp downwards with the circular cutter in the same fashion as the razor 1-step, cutting through the tissue sample of interest. The user would next repeat the action with the four-point punch, removing the sections from the cruciform.

There are several benefits to this design. Firstly, this design offers a high level of both precision and accuracy, as the pre-set geometry guarantees that the same cut will be made time and time again. This feature is a key component for the scope of this project, as the main goal is to ensure symmetry and consistency in the samples. Another benefit of this design is time efficiency, as only 2 swift punches are required to prepare the tissue sample for biaxial testing. Finally, the use of a framing jig allows for increased safety, as it ensures that the user's fingers will not be within the danger zone of the blades.

However, several constraints detract from the feasibility of this design option. Bending razor blades to such a precise geometry is not taken into account in this design, which could prove to be a considerable challenge without proper machinery. This in turn would increase the already proportionally high production costs, and paired with the need for several blades per cut, this design is much less viable. Finally, this model lacks a method to secure the sample to the cutting station when inside the jig, potentially resulting in misalignments and therefore asymmetries in the tissue sample. These factors were taken into consideration during design evaluations and ultimately steered the team away from this design.

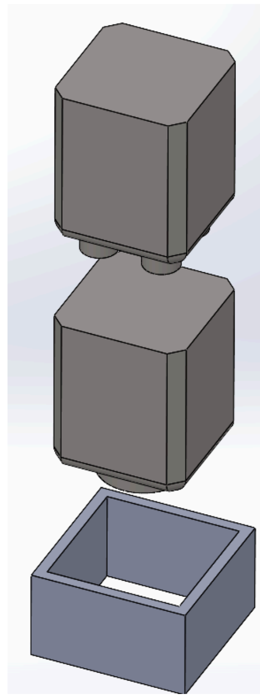
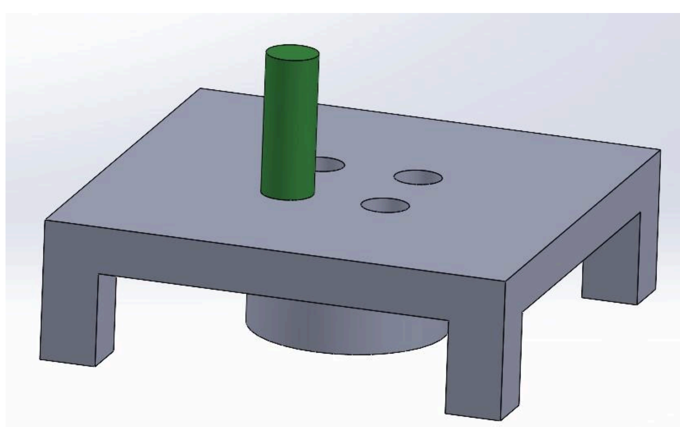


Figure 4: CAD assembly of Razor 2-Step with stamps and frame

Design 3: Biopsy Jig



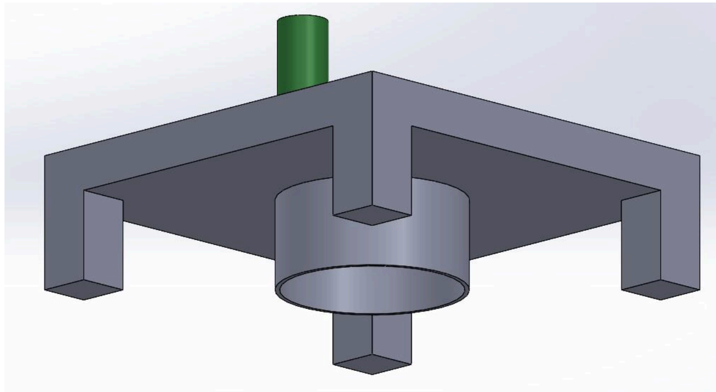


Figure 5a/5b: Biopsy Jig CAD model, demonstrating biopsy punch (green) through symmetric holes

Design three takes a new approach to the cutting of tissue samples. Rather than utilizing bent razor blades to make cuts in the sample, the biopsy jig has four symmetric holes meant for aligning a biopsy punch with the four apertures of the sample. The user would first align the tissue sample within the cylindrical holder in the middle, securing the sample to the cutting station. The user would then unpackage a biopsy punch coordinating with the diameter of the jig's holes, and press the punch vertically downwards into the sample. From that point, the user utilizes standard biopsy punch techniques (twisting, rolling, etc.) to remove the excess material from the cruciform. After all four punches have been made, the user removes the jig, where they may then use a separate razorblade to do any necessary trimming or cleanup.

The biopsy jig design has several advantages over the other designs considered. Firstly, this design eliminates the need for bending any razor blades to high-stress geometries. This factor alone convinced the group of its viability. Additionally, this design allows the user to secure the sample down to the cutting station, ensuring that the sample will not slide or squish during the cutting process, leading to any asymmetries. The pre-cut holes in the jig also certify that the tissue samples will be symmetric and consistent. Finally, this design features great reproducibility, as it is a very simple 3D print job, as well as very easy to understand and assemble.

Several constraints must be considered for this design. Most notable of these is the high maintenance cost of the design. While the two other designs had a much higher initial fabrication cost, the biopsy jig's need for biopsy punches causes an increase in long-term cost [13]. This constraint was justified based on the client's current method involving approximately one punch per tissue sample. Another constraint of this design is time efficiency, as it is less efficient than other designs considered for this application, which were able to make all four necessary cuts in one to two motions.

VI. Preliminary Design Evaluations

Design Matrix

	Razor 1-Step		**Razor 2-Step**		**Biopsy Punch Jig**	
Criteria (weight)	Score	Weighted Score	Score	Weighted Score	Score	Weighted Score
Reproducibility (30)	3/5	18	4/5	24	5/5	30
Cost (20)	4/5	15	3.5/5	14	1/5	4
Ease of Use (20)	4/5	15	4.5/5	18	5/5	20
Reusability (15)	3/5	9	3/5	9	2.5/5	7.5
Ease of Fabrication (10)	4/5	8	2/5	4	5/5	10
Safety (5)	1/5	1	4/5	4	5/5	5
	Sum	66/100	**Sum**	73/100	**Sum**	76.5/100

Figure 6: Design Matrix for evaluation of preliminary design ideas

* Scores are out of 5

** Weighted Score = Weight * (Score / 5)

Design Criteria

Reproducibility: Reproducibility entails how accurate each sample will be when tested across multiple trials. Mechanism of cutting, ability to apply uniform pressure, and ease of the cut were the three factors evaluated for this criteria.

Cost: Cost refers to the price of materials needed for each design. This category considers the upfront costs of 3D printer material or resin casting, and regular maintenance cost, such as the routine purchase of fresh razor blades or biopsy punches.

Ease of use: Ease of use refers to the process of preparing and executing the cut. This criteria covers bending razors, positioning tissue samples, inserting the biopsy punch, etc. Ease of use also includes the punch itself, whether the device requires manual force, or if another tool such as a hammer is required. Devices that require the user to calibrate the press or otherwise complicate preparation would score lower on this criteria.

Reusability: Reusability evaluates the lifespan of the razor or biopsy punch. Reusability also involves the lifespan of the device before a new one must be produced. As one goal of this project was to create a device that can be used for prolonged periods, this criteria was particularly important to meet this requirement.

Ease of Fabrication: Ease of fabrication is a measure of difficulty to produce and assemble the product prior to use. Overly complicated designs also score lower in this criteria. Designs that are difficult to fabricate could lead to accuracy and safety concerns.

Safety: Safety is a measure of the general safety of both assembly and use. Safety considers the potential hazards that could arise from handling sharp objects like razors or biopsy punches, particularly if they are not properly secured or if the design exposes the user to them. Devices that minimize the risk of harm or offer protection from sources of harm will score higher on this criterion.

Design 1: Razor 1-Step

Reproducibility: The design scored a ⅗ in this category because it features only a single cutting shape, but relies on the user to make an accurate cut with little to guide them. The design does not have any system to secure the tissue for cutting, such as a jig or a frame. In addition, the force applied by the user is not guided, meaning that the user could apply force differently between different cuts.

Cost: This design scored the highest out of the designs, a ⅘, because it features inexpensive, easily accessible parts. Firstly, the body is made with a 3D printed frame, which is relatively inexpensive in comparison to other materials. Additionally, the design uses disposable razor blades to make cuts. This design scored higher than the other designs because it features the least amount of pieces to print and uses the least amount of razor blades.

Ease of use: This design received a ⅘ because it is straight-forward with few steps. Users are only required to secure the razor blades into place, tighten the screws to shape the blades, and then apply pressure to the tissue with the cutting-face. This design scored lower in this category than other designs, as it relies on the user's judgment for placement of the cut.

Reusability: This design received a ⅗ for reusability. The design scored moderately in this category because it uses disposable razor blades. Using disposable razor blades allows the frame itself to be reused indefinitely, though the razor blades themselves cannot be used for more than 3-5 cuts, meaning the user must deconstruct and replace the razor blades often, making it difficult to cut multiple samples.

Ease of Fabrication: This design received a ⅘ for ease of fabrication. The design scored highly in this category because it uses a 3D printed body. After fabrication of the body, a user only has to place the razor blades into designated slots and tighten the wingnuts. This design lost a point as it still requires the razor blades to be bent.

Safety: This design received a ⅕ for safety. This design scored lowly in safety because it relies on the user to avoid sharp razor blades. This design works with exposed blades, and an open cutting face serves as a potential hazard. The design is also relatively small, with minimal grip surface area.

Design 2: Razor 2-Step

Reproducibility: This design received a ⅘ because while it features two separate cuts, it has a frame to guide the user to align both cuts. This design secures the sample inside its frame, and then uses both cutting faces that fit into the frame to make the necessary cuts. The rigid structure of this design allows for more reproducible results. Additionally, the force applied by the user is guided in a single direction, increasing reproducibility.

Cost: This design received a 3.5/5 for cost. Like the razor 1-step design, this design is primarily made of inexpensive, disposable parts that contribute to its low cost. The body is constructed from a 3D-printed frame, which is more cost-effective compared to other materials. Additionally, it incorporates disposable razor blades for cutting, offering a lower cost alternative to traditional blades. This design scored slightly lower than the razor 1-step, as it features more parts to construct, and uses more razor blades per cut.

Ease of use: This design received a 4.5/5 because while it has more steps than the other designs, it features a frame that makes the cut easier for the user to perform. Users are only required to arrange the razor blades, shape the blades, align the first cutting-face with the frame, and then repeat for the 2nd cut. This design is higher in this category because the frame guides the user towards a well aligned cut.

Reusability: This design received a ⅗ for reusability. The design tied with the razor 1-step for the highest score in this category, because it shares similar parts. Its use of disposable razor blades allows the body to be reused for many cuts. While this allows the frame to be reused indefinitely, the blades themselves cannot be reused for many cuts. As a result, users will need to frequently disassemble and replace the blades, making it challenging to cut multiple samples efficiently.

Ease of Fabrication: This design received a ⅖ because it has multiple parts to fabricate, and requires the razor blades to be bent into a perfect circular shape. The design features three different 3D printed pieces that combine to make the body. Then, razor blades must be bent into the desired shape before cutting the sample. This design requires the blades to be formed into a perfect circular shape, which would be difficult to accomplish.

Safety: This design received a ⅘ for safety. This design scored highly in safety because it has a more secure design that protects the users. Though this design works with exposed razor blades, the cutting frame used to align the cuts provides an extra layer of safety. This design still loses a point for safety, because it still requires the user to handle blades during construction, while avoiding the multiple cutting faces.

<u>Design 3: Biopsy Punch Jig</u>

Reproducibility: This design received the highest score as it uses biopsy punches along with a jig that secures the sample in place. Securing the sample in place allows for a more controlled cut, leading to more accurate results. The biopsy punches themselves cut out perfect holes in the sample. Additionally, this design minimizes possible mistakes made by the user, requiring them to only place the biopsy punch in the slot and press down.

Cost: This design received a 1/5 for cost. This design scored the lowest out of the designs because it uses more expensive biopsy punches rather than the alternative of inexpensive razor blades. Like razor blades, the biopsy punches must be replaced after 3-5 cuts, making the price differential more stark. Though the body would be resin-cast, the price discrepancy between resin and PLA is minimal. More importantly, the recurring costs of the biopsy punches make it much more expensive to use than the alternatives that use razor blades.

Ease of use: This design also received the highest score for its ease of use. The design scored the highest out of the designs, as it has the fewest steps for assembly and simplifies the cutting process. All that is required for use is to secure the sample in the jig, then use a biopsy punch to make cuts through the 4 holes. This design scores highly because it does not rely on the user to decide where to place the cuts, and instead guides them to the correct geometry.

Reusability: This design received a 2.5/5 for reusability. The design scored moderately in this category as it uses replaceable biopsy punches for cutting. Using biopsy punches allows the frame itself to be reused indefinitely, while replacing the biopsy punch whenever it dulls. This scored slightly lower than the designs featuring disposable razor blades, as the biopsy punches are more difficult to replace.

Ease of Fabrication: This design received the highest score as it has only one part to fabricate, along with purchasable biopsy punches. This scored higher than the other designs because it does not have multiple parts and does not require the manipulation of razor blades into specific shapes. By utilizing purchased biopsy punches, the user can cut circles out of the sample without a difficult assembly process.

Safety: This design scored the highest in safety due to the use of biopsy punches, as opposed to razors. Additionally, this design does not require the user to manipulate the biopsy punch in any way, decreasing the interaction with objects. Finally, the design features a secure rig that locks the sample into place, and protects the user from the cutting surface of the biopsy punches.

VII. Intermediary Design

Design 4: Table Jig

Design four incorporates similar mechanics to that of design three, in that this design uses preplaced holes in a jig to guide the user's manual use of a biopsy punch. However, as opposed to a cylindrical holder, the "legs" of the table are meant to be placed on the tissue surrounding the sample. By applying a slight downward force on the sample through the legs, the user can secure the sample in place during cutting.

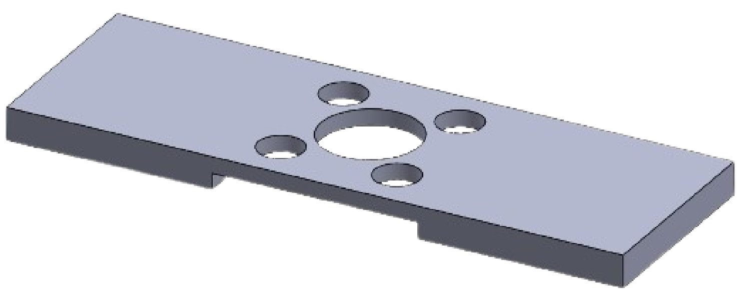


Figure 7: Table Jig CAD Model

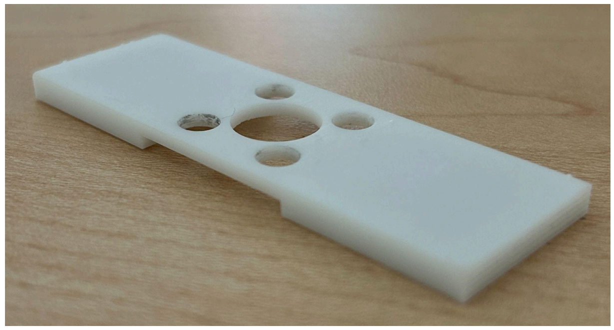


Figure 8: Table Jig (Resin Printed)

The technique for use of this design is as follows. The user would first flatten the sample onto the cutting surface and prepare the necessary biopsy punch. Placing the jig over the top of the sample, the user would align the jig in the center of the tissue sample to be tested with one hand, and apply a sufficient downward force to the device to prevent tissue motion. Using their other hand, the user would then punch through each of the four holes to cut away the apertures from the sample.

This table jig's advantages are consistent with those of the Biopsy Jig design above, eliminating the need for bending razors into high-stress geometries and securing the sample in place during cutting. One additional advantage is the alignment hole in the center of the table, which allows the user to center the jig over a particular part of the specimen, for example, a heart attack in a myocardial tissue sample. This feature allows for the table jig to be applicable in many more studies, as the overall versatility is increased.

One notable disadvantage of this design is that it does not involve a way to cut consistent arms for each sample. Although the four holes can be easily aligned and symmetrical, this device fails to address the fact that the user must then manually use a razor blade to remove the surrounding tissue to create an arm to secure into the testing machine. It is important to note that the main concern for this project was cutting the four apertures symmetrically, therefore the symmetry of the legs themselves was placed at a much lower level of import.

Design 5: Razor and Punch Jig

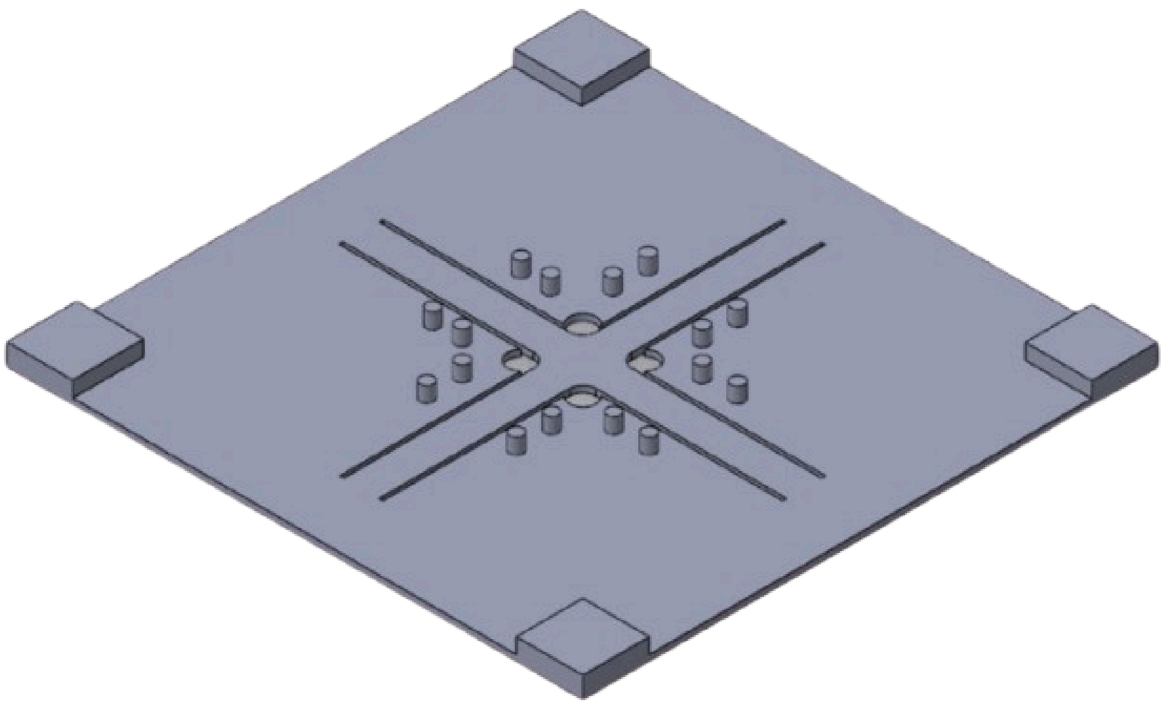
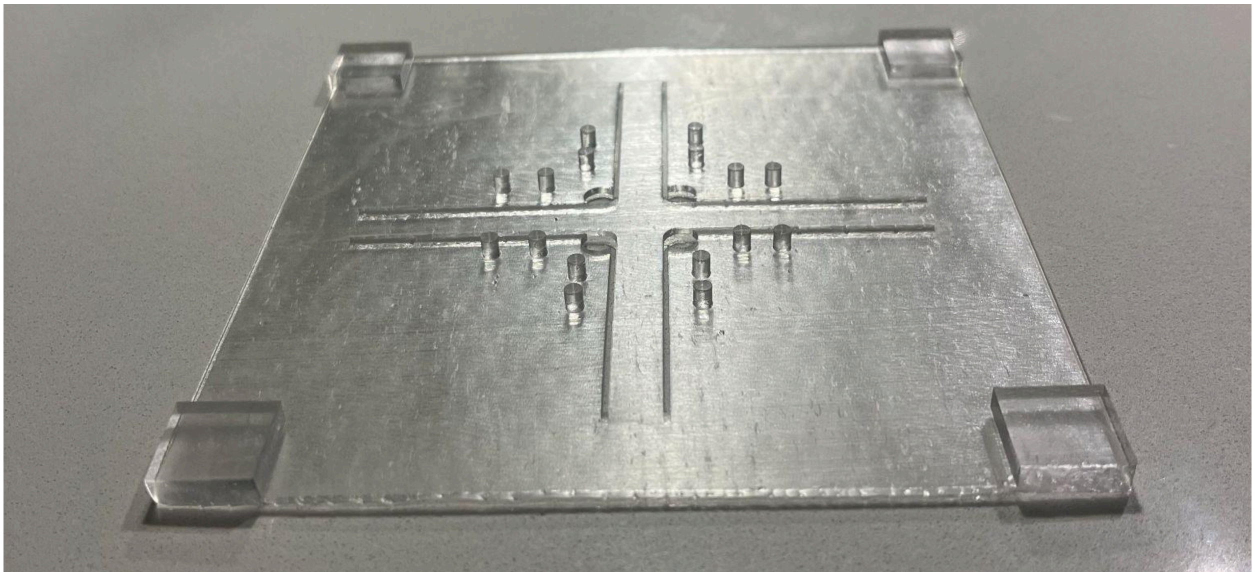
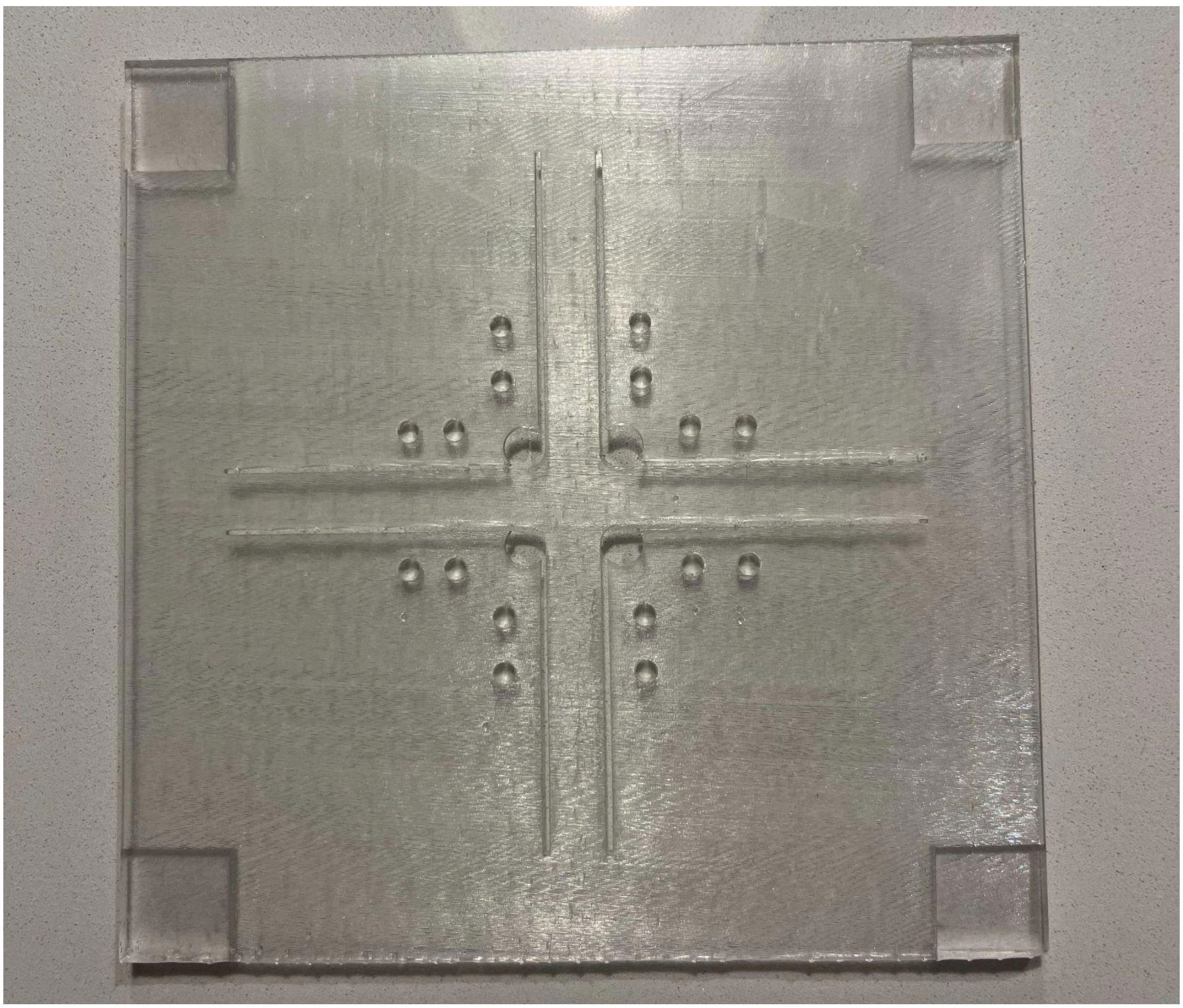


Figure 9: Razor and Punch Jig CAD Modeling





Figures 10a/10b: Razor and Punch Jig (Resin Printed)

The final design number 5 is a design that accounts for both the punch symmetry and leg symmetry. The razor and punch jig incorporates slits in the device that allow the user to pass a razor blade down a length of the sample, after using a biopsy punch to remove the four corners from each corner. Additionally, four support pegs are placed on each quadrant of the device, which aid in stabilization and holding the sample in place during cutting.

This design also shares the same advantages as two other above-mentioned jigs, such as symmetric apertures and minimal bending of razors. However, the major advantage of this design over the other designs is that it allows the users to consistently cut uniformly wide arms for each sample. This removes uncertainty in test statistics due to the differing widths of each arm.

A disadvantage of this design is questionable stability, as the large table surface could be subject to bending or shift. The design also does not address the differing heights of samples, as an excessively thicker or thinner sample would require a new print of the device with an adjusted leg height.

VIII. Final Design

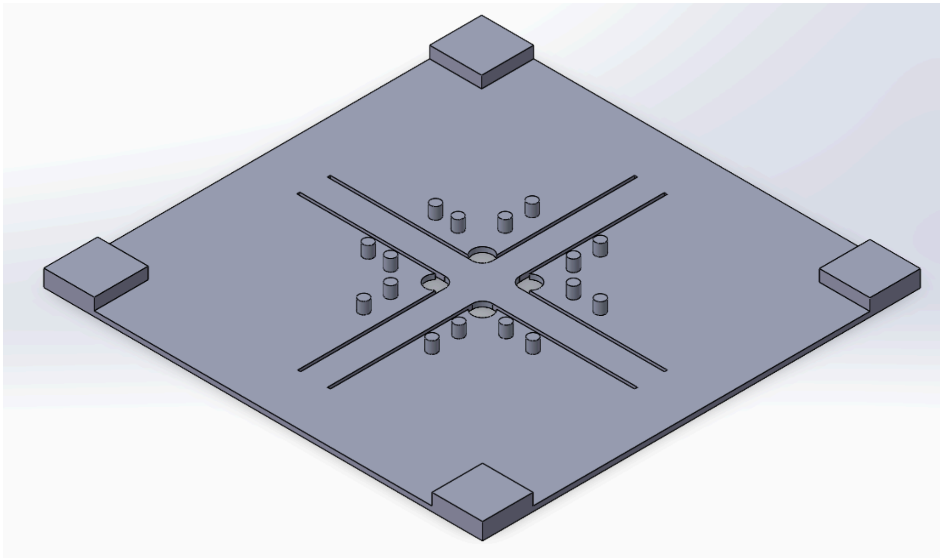


Figure 9: Razor and Punch Jig CAD Modeling

The selected final design for this project is the Razor and Punch Jig (Design 6). Although this design was not a preliminary design evaluated in the design matrix above, the team elected this design due to its significant advantages with minimal drawbacks. Deciding to take on the challenge of extending the project beyond simply streamlining the cutting process of the four apertures, this design also allows the user to cut symmetrical arms of the tissue sample. For this reason, design six is best equipped to address the criteria in the problem statement presented by the client.

IX. Fabrication

Materials

The materials required for the fabrication of the biopsy jig are easily obtainable and relatively inexpensive. The entire unit will be created in one piece using resin and/or polylactic acid plastic, as appropriate to individual usage. Resin cured printing is preferred in this instance over standard extrusion based 3D printing because resin prints can be made using completely transparent material, which is optimal to allow for the user to better center the punch guide over desired tissue sections. Along with the printed jig, the design will require the use of biopsy punches to cut the apertures from each corner of the tissue sample, as premade punches do not require time intensive set up. Resin printing prices for this project are quoted at $0.42 per gram [11]. This is comparably more expensive than PLA and other plastics but it is worth the cost to improve functionality. Biopsy punches range in cost by manufacturer and quantity, but are approximately $2.75 per punch when ordered in bulk [14].

Fabrication Plan

Fabrication for this project was conducted at design innovation space in the Wendt Commons. Resin printing, or stereolithography (SLA), was used to create the final prototype. SLA is a technique that utilizes a light source to cure resin layer by layer. This technique has an extreme high degree of precision which is necessary due to the small dimensions of the design. An STL file is prepared in the printing compatible software, and necessary supports for printing are added. After the design is printed and trimmed it will be ready to use with biopsy punches. SLA prints typically take 2-4 hours to complete, but as this project is relatively small scale, a much smaller time window is required for printing [15]. A final drawing of the razor and punch jig is referenced in the appendix.

X. Testing and Results

A two way t-test was performed to compare the results from each method of cutting, by hand and with the guide. A t-test was selected because the sample size for each method of cutting is small (n=6) and the population standard deviation was not known. The two way t-test compares the means of two independent data sets. In this instance, the data sets included symmetry values of six cruciform cuts using each method. The goal of this testing was to evaluate if there was a significant difference between the symmetry values in each cutting method. Symmetry is the focus of this testing because it was deemed the most important factor in the PDS.

Symmetry values for each sample were calculated using a python program that outputs a symmetry value about the vertical and horizontal axis. These values were then averaged to yield an overall symmetry value. Prior to importing the images into the program, the background of the images were blacked out, this allows for best processing within the program. To test the validity of the image analyzer, a perfect circle was tested. The output for each plane of symmetry was nearly 100%, giving confidence that the program works effectively. The resulting symmetry averages for by hand cutting and guide cutting were 80.97% and 86.60% respectively. The python program, raw data, calibration testing, and sample testing can all be found in the appendix.

Understanding the limitations of testing is crucial in determining what the results suggest. Sources of error in this testing protocol include human error in cutting the samples, systematic error introduced from the symmetry program, and random error. Limitations that constrain the scope of applicability include the medium tested (chicken skin) and the metric measured during testing (symmetry). Using chicken skin is a satisfactory model for representing tissue samples but only covers a narrow region of possible dimensions. There is a vast variety of thickness that can occurs. Without data from a greater variety of samples it is only possible to conclude information about this specific thickness of sample. Additionally, this model only measures symmetry, but other factors such as reproducibility and ease of use are important to consider.

A two way t-test was performed in MATLab and prior to testing the alpha level and hypotheses were set. An alpha level of 0.05 was chosen and the hypotheses were as follows: H0: $\mu 1 = \mu 2$ and Ha: $\mu 1 \neq \mu 2$. The function used in MATLab was [h,p,ci,stats] = ttest2(punch,hand). The only measure of concern was the p-value which was 0.0367. This is less than the selected alpha of 0.05. The data indicates a rejection of the null hypothesis, supporting the validity of the alternative hypothesis that the mean symmetry values between cutting methods are not the same. This aligns with the expected outcome because the guide creates an outline for each cut which should increase uniformity within and between samples. Taking into consideration the sources of error and the limitations in the experiment, a conclusion can be drawn that the guide yields better symmetry results compared to the by hand method.

XI. Discussion

This study developed a device to cut cruciform-shaped samples from soft tissues in order to carry out effective biaxial testing for normal stress and strain. The final design consists of a square platform with hole cutouts for razor blades and biopsy punches. The objective of this design is to use the combination of razor blades and biopsy punches in the platform traces to cut the tissue into a cruciform shape. Additionally, there are support pins placed around the bottom of the platform to hold the tissue sample in place while cutting. This design was chosen due to its ability to not only produce specimens with symmetric biopsy punch holes but also systematically control the width of the cruciform arms.

Our results reveal an improvement in cruciform symmetry compared to the current cutting method by hand yet still could be improved upon. Due to the toughness of some tissues, while cutting the sample with the punch and razor blades, small movement and rotation of the specimen is seen. These shifts result in asymmetry of the cruciform arms along with thickness imbalances that produce inaccurate axial tensile testing results [16]. Although pin supports were introduced to reduce this movement, further work could enhance this objective through additional workshopping of the pin design, or implementing an alternative concept such as a vacuum or temporary tissue adhesive holding the sample in place.

Although the cruciform specimens are rather replicable, there are some small factors that contribute to error in the samples. The first is the use of stereolithography(resin printing) produces errors up to 0.06mm depending on the sample printed [17]. This could result in small inaccuracies of the leeway of the razor blade slits and biopsy punch hole cutouts. Another possible error includes user error. Although a standard operating procedure will be provided with the device, there is always a possibility the users do not follow the manual producing unforeseen error.

Another possible complication with this design is the need for other dimensions of cruciform shaped tissue samples. Additional CAD models must be designed to accommodate these alternate dimensions. Future work for this project could include scaling up the dimensions by using larger biopsy punches in larger guide holes and increasing the distance between razor slits for wider arms. The device can be scaled down using a similar method with smaller biopsy punches and less arm width.

XII. Conclusion

In order to accurately perform biaxial testing on soft tissues, it is necessary for tissues to be cut into symmetrical cruciform shapes. The current method of cutting tissues often leads to asymmetrical samples which skews data. Our team researched and developed new designs in order to create a more accurate and reproducible process of cutting tissues. Our chosen final design is a 3D printed cutting jig with 4 apertures, which can guide a biopsy punch to cut symmetric holes for the corners of the cruciform shape. In addition to this, it also features straight slits to be used with a razor blade or other blade, which will produce the straight arms of the cruciform. Finally, this design secures the sample with 4 supports in each quadrant, increasing the durability of the jig and keeping the sample in place.

This product was then fabricated and tested to evaluate the success of our design. Our final design was found to be a significant improvement over the current method of cutting by hand. Though this is the case, there are still some sources of error in our designed process. During the cutting of thick tissues, small movements and rotations were observed which lead to asymmetry in our results. In the future,the design could be improved by developing a better means of securing the sample, including a method to secure thicker samples. In addition, variability in thicknesses of resin printing was a small source of error in our design. This could be improved by switching to another material to print or fabricate the design. Despite these areas of error, our team was successful in developing a design to effectively cut symmetrical cruciform shapes, meeting the criteria of our PDS and satisfying the needs of our client.

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XIV. Appendix

Product Design Specifications

Print-A-Punch

Product Design Specification

Date of Last Revision: 09/19/2024

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Section 310

Function:

The Print-A-Punch device will serve as a clamping mechanism to secure razor blades in a precise configuration to cut cruciform-shaped soft tissue samples for biaxial testing. The clamping system will enable the consistent cutting of soft tissues, which addresses the challenge of asymmetry that the lab currently has with their biopsy punches. This asymmetry is mainly due to high shear strain and shear forces during testing. These forces lead to inaccurate material property measurements that affect patient-specific models. By ensuring uniformity in the cruciform samples, the system will reduce errors in material property computation, improving the accuracy of mechanical behavior estimations for soft tissues. This approach will serve as a cost-effective alternative to custom steel punches, using 3D printing to enhance accessibility and reproducibility in tissue testing procedures.

Client requirements:

- Device should cut symmetric samples of tissue
- Device should be reusable
- Device should be able to cut a consistent shape and size of soft tissue sample
- Device must be compatible with generic Stanley-brand razor blades
- Device should be fabricated from stainless steel hardware
- Device must be able to cut through various thicknesses of tissue samples
- Budget of $250

Design Requirements

1. Physical and Operational Characteristics
    a. *Performance requirements*:
        i. Prototype must be able to cut through multiple types of tissue with relative ease, without require excessive force to operate
        ii. Prototype must be able to be replicated by other researchers for laboratory use
    b. *Safety*:
        i. During assembly of punch, blades must be covered or hidden to prevent injury to user

    ii. Device usage must be easy to learn since it is universally available
    iii. Device should pose minimal risk to user during operation

c. *Accuracy and Reliability*:
    i. The punch must be capable of cutting a minimum of 3 samples before swapping blades is necessary
    ii. Body of punch must have an indefinite lifetime
    iii. Punch must be able to replicate the intended punch pattern on every attempt
    iv. Overall length and width of printed punches should have an error less than 5% [1]

d. *Life in Service*:
    i. Punch must last indefinitely with the ability to replace blades when dulled

e. *Shelf Life*:
    i. Punch must not require any unusual storage conditions
    ii. Punch must be able to withstand a large amount of external pressure from a mallet or a press

f. *Operating Environment*:
    i. Punch must be able to be used on demand with little setup time
    ii. Punch must be able to be used in a laboratory environment
    iii. Punch should be able to withstand sterilization

g. *Ergonomics*:
    i. Punch must be simple to 3D print on most 3D printers
    ii. Punch must be easy to assemble
    iii. Blades must be easy to bend during replacement
    iv. Punch should include a fast fabrication time of under 5 hours
    v. Razors should be able to be replaced safely in under 5 minutes

h. *Weight*:
    i. The device will be lightweight in order to be easily transported and shifted when working with small tissue samples

i. *Materials*:
    i. Main device structure will be 3D printed

ii. Must not corrode when in contact with tissue storage solution
iii. PETG or Nylon can be used for 3D printing due to its chemically resistant properties [4]
iv. Stainless steel hardware should be used to avoid corrosion
v. Stainless steel razor blades will be used to cut tissue samples
vi. Materials must be easy to acquire

j. *Aesthetics, Appearance, and Finish*:
   i. No color or finish preference
   ii. Punch must be appropriate for general laboratory spaces

2. Production Characteristics
   a. *Quantity*:
      i. Punch should be scalable to a variety of different size products for punching different dimension samples
      ii. CAD files and production instructions should be published in final report for public domain
   b. *Target Product Cost*:
      i. Each product should be reasonably cheap to produce (under $10 per device)
3. Miscellaneous
   a. *Standards and Specifications*:
      i. Product's plastic body should comply with ASTM D638-02a, which specifies tensile testing procedures for plastics, ensuring they meet industry requirements in laboratory use [2].
      ii. Device should comply with ASTM D412-06a, as it relates to creation of soft tissue models for biaxial testing, ensuring accurate and reproducible measurements of tensile properties [3].
   b. *Customer*:
      i. Punch is catered toward tissue testing labs and is not intended to be sold for a profit
      ii. Individual punches are to be created at owners expense
   c. *Patient-related concerns*:

    i. Not in the scope of this project

d. *Competition*:

    i. Print-A-Punch for Uniaxial Testing: Comparable design that uses a dumbbell-shaped punch intended for uniaxial tensile testing, as opposed to biaxial testing [1].

References: (CITE ALL IN IEEE)

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Material Expenses

Item	Description	Manufacturer	Mft Pt#	Vendor	Vendor Cat#	Date	QTY	Cost Each	Total	Link
3D printed material	Resin prototypes	Makerspace				10/15/2024	2	$0.76	$1.51	
3D printed material	Resin prototypes round 2	Makerspace				10/21/2024	1	$0.92	$0.92	
3D printed material	Resin prototypes round 3	Makerspace				10/25/2024	3	$5.37	$16.11	
3D printed material	Resin prototypes round 4	Makerspace				11/11/2024	2	$6.07	$12.14	
3D printed material	Resin prototypes final	Makerspace				12/4/2024	1	$11.59	$11.59	
								TOTAL:	**$42.27**	

Testing Protocol

Purpose: Determine if there is a difference between symmetry results when cutting tissue samples by hand and with the guide.

Protocol:
A two sample t test for significance will be performed on collected results. An alpha of 0.05 will be chosen in this instance and each sample will have 6 data points. Data is collected using image analysis software created in python.

Hypothesis:
H0: $\mu_1 = \mu_2$
Ha: $\mu_1 \neq \mu_2$
μ_1 = by hand method
μ_2 = guide method

Assumptions:
- Independent events
- Equal variances

- Normality

Data Collection:
Perform 6 sample cuts using each method
- To reduce error cut will be performed by the sample individual using the same process each time

Data Analysis:
A two sample t test will be performed using Matlab to determine significance level
[h, p, ci, stats] = ttest2(x , y , Name,Value)

P-value less than alpha = 0.05 results in the rejection of the null hypothesis in favor or the alternate hypothesis

Results:
The calculated p-value = 0.0367 which is less than the selected alpha of 0.05. Thus, we fail to accept the null hypothesis and suggest that the alternate hypothesis is true. The mean symmetry values between cutting methods are not the same.
Code for symmetry analysis:

```python
from PIL import Image
import numpy as np
import matplotlib.pyplot as plt

# Load and preprocess the image
image_path = "C:\\Users\\cbail\\OneDrive\\Documents\\School\\24-25\\BME 300\\circlebandw.jpg"

image = Image.open(image_path).convert('L')  # Convert to grayscale
image_array = np.array(image)

# Binary thresholding (white shape on black background)
threshold = 128
binary_image = (image_array > threshold).astype(int)

# Crop the binary image to focus only on the shape
def crop_to_shape(binary_image):
    rows = np.any(binary_image, axis=1)
    cols = np.any(binary_image, axis=0)
    top, bottom = np.where(rows)[0][[0, -1]]
    left, right = np.where(cols)[0][[0, -1]]
    return binary_image[top:bottom+1, left:right+1], (top, bottom, left, right)

cropped_binary_image, bounds = crop_to_shape(binary_image)

# Calculate the centroid of the binary shape
def calculate_centroid(binary_image):
    y_coords, x_coords = np.nonzero(binary_image)
    centroid_y = int(np.mean(y_coords))
    centroid_x = int(np.mean(x_coords))
    return centroid_y, centroid_x

centroid_y, centroid_x = calculate_centroid(cropped_binary_image)

# Function to calculate symmetry by comparing flipped shapes
def calculate_shape_symmetry(binary_image, centroid_y, centroid_x):
    height, width = binary_image.shape

    # Split the shape into halves
    top_half = binary_image[:centroid_y, :]
    bottom_half = np.flipud(binary_image[centroid_y + 1:, :])  # Flip bottom half

    left_half = binary_image[:, :centroid_x]
    right_half = np.fliplr(binary_image[:, centroid_x + 1:])  # Flip right half

    # Resize halves to match dimensions (if needed)
    min_rows = min(top_half.shape[0], bottom_half.shape[0])
    min_cols = min(left_half.shape[1], right_half.shape[1])
    top_half = top_half[:min_rows, :]
    bottom_half = bottom_half[:min_rows, :]
    left_half = left_half[:, :min_cols]
    right_half = right_half[:, :min_cols]

    # Compare halves for symmetry
    horizontal_overlap = np.sum(top_half == bottom_half) / top_half.size
    vertical_overlap = np.sum(left_half == right_half) / left_half.size

    return horizontal_overlap, vertical_overlap
```

```python
# Calculate symmetry
horizontal_symmetry, vertical_symmetry = calculate_shape_symmetry(cropped_binary_image, centroid_y, centroid_x)

# Visualization
plt.figure(figsize=(12, 8))

# Original binary image
plt.subplot(2, 2, 1)
plt.title("Original Binary Image")
plt.imshow(binary_image, cmap="gray")
plt.axis("off")

# Cropped binary image
plt.subplot(2, 2, 2)
plt.title("Cropped Binary Image")
plt.imshow(cropped_binary_image, cmap="gray")
plt.axhline(centroid_y, color='red', linestyle='--', label="Horizontal Symmetry Line")
plt.axvline(centroid_x, color='blue', linestyle='--', label="Vertical Symmetry Line")
plt.legend()
plt.axis("off")

# Top and bottom halves comparison
plt.subplot(2, 2, 3)
plt.title("Top vs Bottom Halves")
plt.imshow(np.vstack([cropped_binary_image[:centroid_y, :],
                      np.flipud(cropped_binary_image[centroid_y + 1:, :])]), cmap="gray")
plt.axhline(centroid_y, color='red', linestyle='--')
plt.axis("off")

# Left and right halves comparison
plt.subplot(2, 2, 4)
plt.title("Left vs Right Halves")
plt.imshow(np.hstack([cropped_binary_image[:, :centroid_x],
                      np.fliplr(cropped_binary_image[:, centroid_x + 1:])]), cmap="gray")
plt.axvline(centroid_x, color='blue', linestyle='--')
plt.axis("off")

plt.tight_layout()
plt.show()

# Output the symmetry results
print(f"Horizontal Symmetry: {horizontal_symmetry * 100:.2f}%")
print(f"Vertical Symmetry: {vertical_symmetry * 100:.2f}%")
```

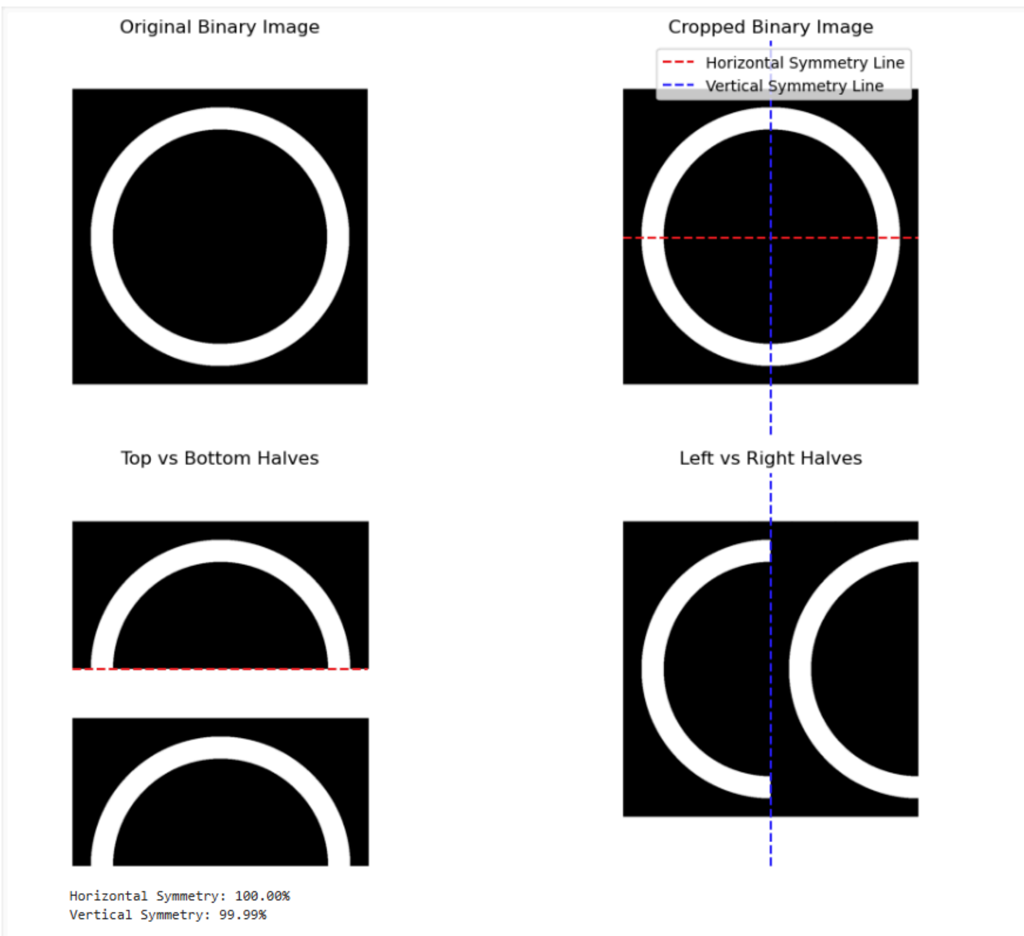
Initial symmetry output of samples cut on 21 Nov 2024 - using IsolatedSym program:

Guide samples	Horizontal Sym	Vertical Sym	Avg
Sample 1	88.84	91.73	90.285
Sample 2	82.92	82.03	82.475
Sample 3	83.57	79.16	81.365
Sample 4	82.70	80.38	81.54
Sample 5	92.37	91.83	92.1
Sample 6	88.53	95.09	91.81

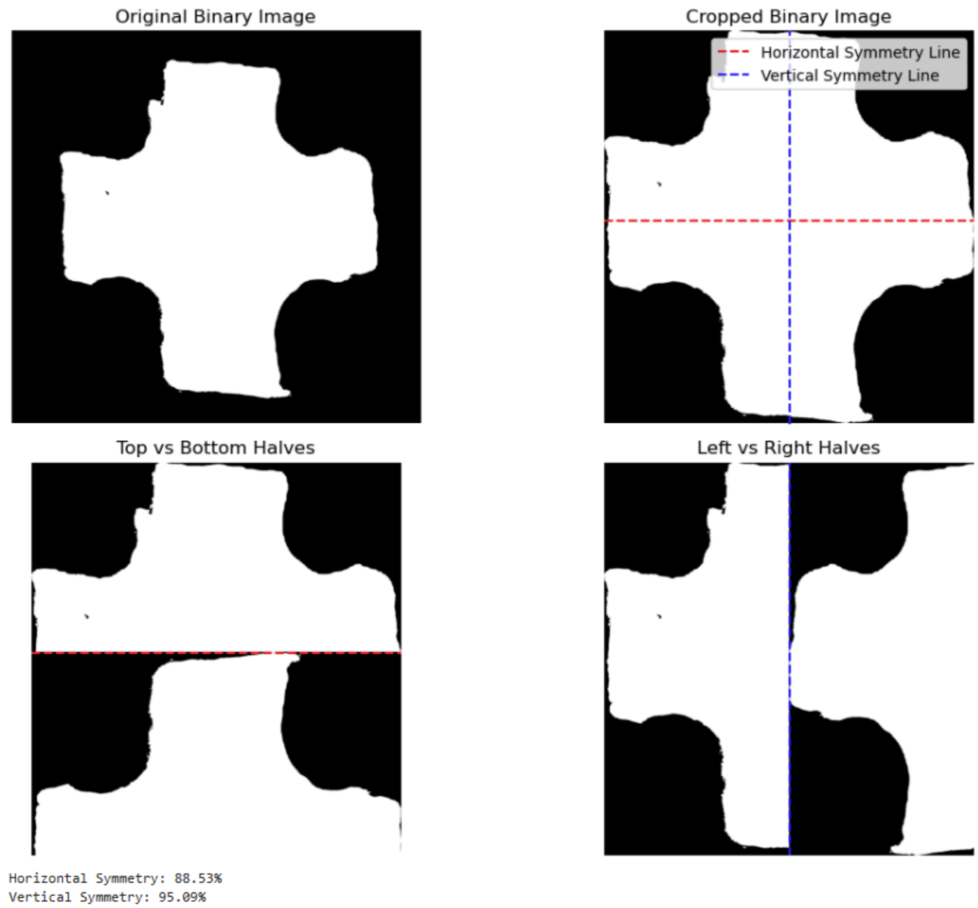
Hand Sample	Horizontal Sym	Vertical Sym	Avg

Sample 1	77.93	83.89	80.91
Sample 2	74.04	83.57	78.805
Sample 3	83.32	77.76	80.54
Sample 4	83.83	85.05	84.44
Sample 5	81.03	83.37	82.2
Sample 6	78.48	79.35	78.915

Calibration testing:

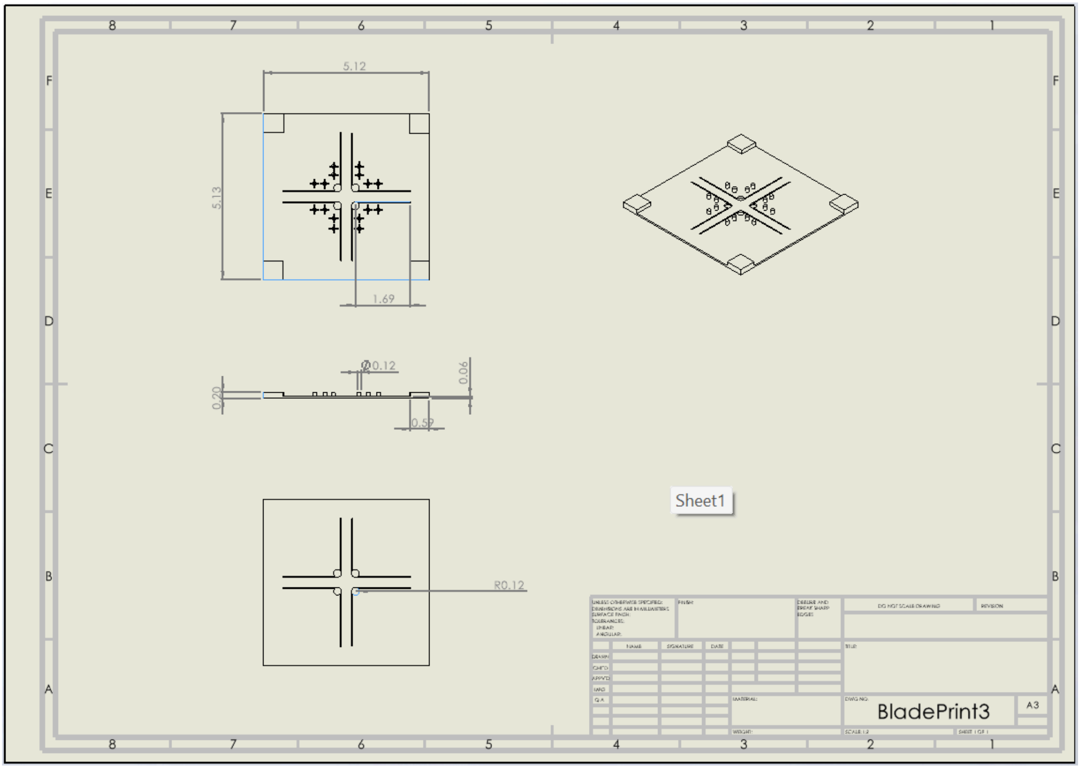


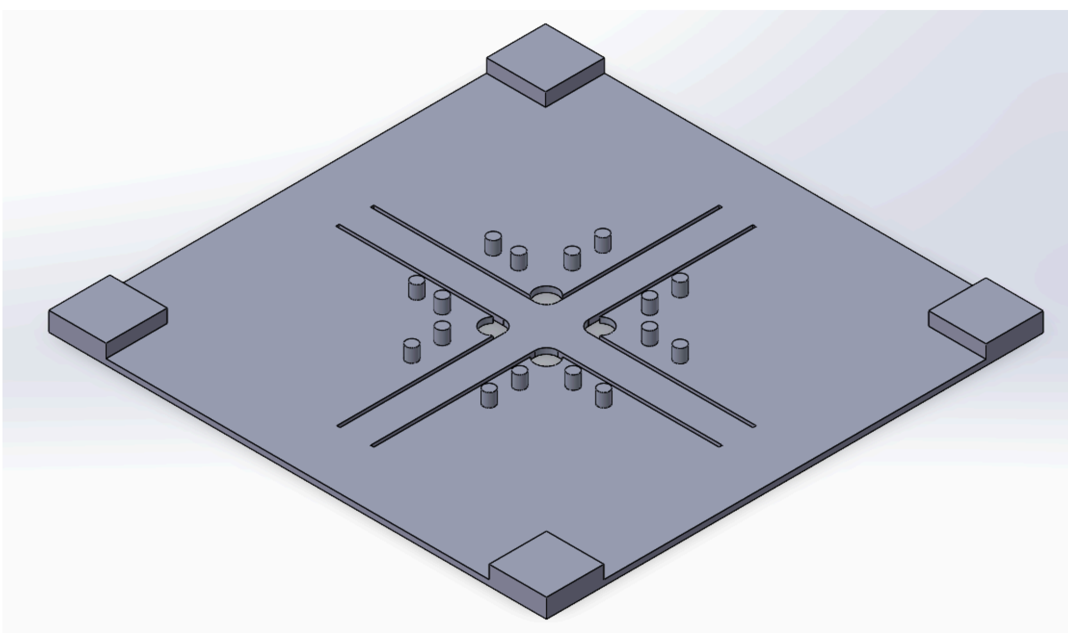
Figure[11] - Celebration testing for symmetry program



Figure[12] - Output example from symmetry program

Final Design File





Standard Operating Procedure(SOP)

Purpose
The purpose of this SOP is to provide a method of producing cruciform shaped tissue samples from irregularly shaped bit. The primary purpose of having cruciform shaped samples is for biaxial tensile strength testing but can be used for other research purposes where necessary.

Application
This document includes the procedure/steps to ensure the most symmetric specimen is produced along with a CAD .stl file with 8mm arms for users printing purposes. Also attached are optimal biopsy punches are razor blades for making the cuts.

Procedure steps
1. Gather an approximately 15-20 mm wide tissue sample of thickness less that 2mm
2. Place the tissue sample on a rough cutting board thickness evenly distributed and level
3. Center the four hole cutouts around the tissue area of interest with pins pressing down on the side tissue
4. Use 5mm biopsy punch(attached below) through one of the four holes pressing down hard then twisting 180° clockwise then back counterclockwise
5. Continue this procedure for the other three holes starting with the diagonal punch
6. Next, use a single edge razor blade(attached below) in the jigs slits starting with both sides of a single arm then the opposite arm and last the two remaining arms
7. Remove the jig from above the able and discard any scraps that are cut off
8. If any scraps are still attached, use a razor blade by hand to cut them off where necessary

Resources
Razor Blades
Biopsy Punches