<table>
<tr><td colspan="2">Design History File

Department of
Biomedical Engineering
Wayne State University</td><td>Team: Group 1: Nerve Regeneration – Neuroma Reduction
Class: BME 4910
Semester/Year: Spring/Summer 2022</td></tr>
<tr><td colspan="3">Project: Nerve cap to reduce peripheral nerve end neuroma following a traction neurectomy.</td></tr>
<tr><td colspan="2">User Needs Summary:
- Biocompatibility
- Cytotoxicity
- Different sizes
- Durable
- Ease of implementation in the clinic
- Low-cost
- Reduce symptomatic neuroma
- Sterilization</td><td>Design Input Summary:
- Pass ISO 10993-1 defined biocompatibility testing
- Device diameters of 3, 4, 5, and 6 mm
- Integrate with soft tissue
- Storage life of at least 32 mo.
- Cost per unit < 20,000 USD
- Eliminate axon regeneration into fibrous or muscle tissue.
- Reduce pain as measured by visual analog scale</td></tr>
<tr><td colspan="2">Design Output Summary:
- Decellularized, GLU-treated bovine pericardial tissue
- Photochemical tissue bonding (PTB) to nerve end epineurium
- “Runway” length of 4-5 mm to exhaust regenerating nerve end
- Aligned nanofibers to help guide axons within cap and reduce entanglement
- Distal end anchoring tab
- Ethylene oxide sterilization</td><td>Design Verification Summary:
- Sensitization assays, and implantation tests.
- Cytotoxicity tests including MEM Elution assay, and MTT assay.
- Tensile tests
- Mechanical and biocompatible verifications reperformed on device at 32 months.
- Questionnaire for hospital staff with device.
- Theoretical sum of all costs.
- Visual analog scale (VAS).
- Sterility assessment.</td></tr>
<tr><td colspan="2">Design Interviews Summary:
- Interviewees: Dr. Harini Sundararaghavan, Dr. Mai Lam, Dr. Melissa Wrobel, Dr. Tonya Whitehead
- Feedback: Revise user needs/inputs, use more device diameters, specify surgical instructions
- Responses: Re-phrased/added user needs and quantified all design inputs, added two additional device diameters, specified surgical procedure under Design Outputs</td><td>Design Change Summary:

- Bovine pericardium in lieu of collagen/PLA composite
- Use aligned nanofibers to guide axons
- Remove partition to simplify design/manufacturing and reduce cost</td></tr>
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1. Design Input

1.1 Problem Statement

Symptomatic neuroma is a painful sequela of traumatic or oncologic nerve amputations. Our goal is to reduce/prevent neuroma by isolating transected nerve stumps within protective "caps" during surgery.

Neuromas are benign tumors of the peripheral nervous system and can manifest following nerve injury—especially amputation. A neuroma forms as an injured nerve end starts to regenerate in a disorganized way, resulting in a bulbous mass of entangled axon fibers and non-neural tissue growth. Neuroma is understood to occur from misdirected Schwann cell processes in the absence of a distal target—causing pathophysiologic axon outgrowth as the nerve cells try to reinstate continuity [1]. Aside from affecting quality of life, symptomatic neuroma can make molding a well-fitting prosthesis socket difficult, and/or prevent its successful use.

Neuroma formation phases:

1. Injured nerve end/degeneration axons
2. Axonal sprouts
3. Unorganized bundles of axons
4. Growth into muscle tissue
5. Growth into fibrous tissue (neuroma)

Current treatment for painful neuroma includes pharmacological therapy, and more commonly, surgical removal. The standard or care for surgical management of stump neuroma is traction neurectomy. During this technique, a neurolysis is performed and the nerve is exposed. Then the nerve is put under traction and transected to healthy nerve. Yet, traction neurectomy alone cannot prevent neuroma from re-forming as the nerve end regenerates. Alternatively, the nerve stump may be buried into adjacent muscle tissue or bone. While this procedure mitigates external stimulation that causes the painful sensation, it requires an additional dissection into otherwise healthy tissue, may not always be anatomically possible, and exposes the patient to further risks [2].

1.2 User Needs Statement

Design a biocompatible device or therapy to reduce neuroma re-formation in patients with symptomatic neuroma in the wrist/dorsum base of the hand who receive a traction neurectomy.

1.3 Design Specifications

A summary of the design specifications is presented in Table 1.1. This table was developed through the feedback from the initial interviews with professionals. It highlights the necessary user needs that the design will focus on to identify what the design requires to be successful. Additionally, this table provides an organization of the design inputs that are required to achieve the user needs. These inputs were identified through extensive research and analysis on existing measures and studies. Explanations for these reasons are listed within the table and highlight the results of the research that lead to the design inputs.

Table 1.1 Design inputs and user need of the design.

User Need	Input(s)	Reference	Explanation
Biocompatibility	Device needs to produce appropriate host response for the given body contact and duration conditions: - Permanent contact: implant device.	https://www.fda.gov/medical-devices/biocompatibility-assessment-resource-center/biocompatibility-evaluation-endpoints-contact-duration-periods#permanent-implant	ISO 10993-1 defines biocompatibility requirements for permanent contact, implanted devices.
Cytotoxicity	Device material(s) cannot cause significant cell damage or death as measured by a cytotoxicity assay.	https://www.fda.gov/media/85865/download	FDA requires implanted devices to undergo cytotoxicity testing at 37 deg. C for 72 hr.
Different Sizes	Use different device sizes to fit the range of nerve diameters for the digits.	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6467622/	Use cadaveric study findings on nerve diameter in the hand to determine different sizes needed.
Durable	Device cannot mechanically fail (e.g., break, tear) during and after implantation. Device is not susceptible to degradation in physiologic fluid.	https://www.sciencedirect.com/science/article/abs/pii/S016981419600056X https://www.sciencedirect.com/science/article/pii/S1369702108700890	The acceptable host response for the neuroma application is integration of the device with surrounding tissue, not degradation.
Ease of implementation in the clinic	Storage life of at least 32 mo. Does not require custom or device-specific surgical instruments for implantation.	https://pubmed.ncbi.nlm.nih.gov/9134160/	Use shelf-life study of a bio-materially similar device (bioprosthetic heart valve) to estimate reasonable storage life.
Low-cost	Cost per unit less than 20,000 USD	MKTG0074R04_axo2022CodeGuide-Avance (axogeninc.com)	Provides information on insurance reimbursement with an estimated cost of

			$23,346- 11,485 for procedure.
Ease of implementation in the clinic	Storage life of at least 32 mo. Does not require custom or device-specific surgical instruments for implantation.	https://pubmed.ncbi.nlm.nih.gov/9134160/	Use shelf-life study of a bio-materially similar device (bioprosthetic heart valve) to estimate reasonable storage life.
Low-cost	Cost per unit less than 20,000 USD	MKTG0074R04_axo 2022CodeGuide-Avance (axogeninc.com)	Provides information on insurance reimbursement with an estimated cost of $23,346- 11,485 for procedure.
Reduce Neuroma	Eliminate axonal outgrowth into fibrous tissue. Reduce pain. (100-mm VAS reading of 0 to 4 mm [no pain] or 5 to 44 mm [mild pain]).	https://pubmed.ncbi.nlm.nih.gov/14622683/ (Visual analog scale to assess pain)	Visual analog scale (VAS) is one of the most common measures of pain intensity in pain research. Used to define our input for addressing pain remediation.
Sterilization	Device must meet the appropriate Sterility Assurance Level (SAL).	https://www.fda.gov/sterilization-process-controls	FDA guidance on verification activities used to monitor and control the sterilization process.

2. Design Output

2.1 Design Overview:

2.1.1 Revision 0

- Collagen/polylactic acid (PLA) composite material
- Total length of 10 mm
- Cap fits over nerve by a length of 5 mm, bonds to the epineurium via photochemical tissue bonding (PTB)

- Single partition divides axon outgrowth to reduce entanglement, provides internal architecture
- “Runway” of 4-5 mm
- Cap diameters of 3, 4, 5, and 6 mm to fit different nerve end diameters

The nerve cap is a surgical implant intended to reduce symptomatic peripheral nerve end or stump neuroma. It is one part and comprised of collagen/polylactic acid composite material. The cap clearance fits over the nerve end and the luminal surface bonds to the epineural surface via photochemical tissue bonding (PTB). PTB is an atraumatic technique which crosslinks (covalently bonds) collagen on the tissue surfaces using a photosensitizing dye and visible light [3]. A single partition within the cap divides regenerating nerve cells into two parts to reduce axon entanglement. As the regenerating nerve end exhausts itself, the cap undergoes controlled degradation.

Fig. 2.1. Rev. 0 CAD model isometric and cross-section view.

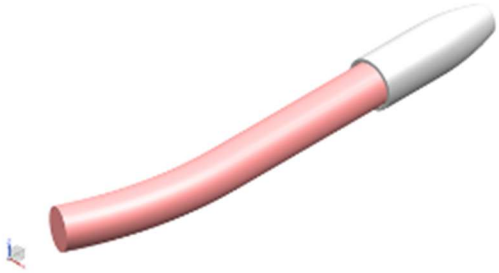


Fig. 2.2. Rev. 0 nerve cap and nerve assembly.

2.1.2 Revision 1

- Decellularized, glutaraldehyde-treated bovine pericardium
- Total length of 12 mm
- Cap thickness is 0.5±0.25 mm
- Distal end tab for anchoring of device
- Cap fits over nerve by a length of 5 mm, bonds to the epineurium via PTB
- Aligned nanofibers at the start of the lumen help guide axons
- “Runway” of 4-5 mm
- Cap diameters of 3, 4, 5, and 6 mm to fit different nerve end diameters

- Ethylene oxide sterilization

The Revision 1 nerve cap is one part and is comprised of decellularized, glutaraldehyde (GLU) treated bovine pericardium. The cap clearance fits over the nerve end and the luminal surface bonds to the epineural surface via photochemical tissue bonding. A tab on the distal end of the cap allows for anchoring to surrounding tissue. After surgical fixation of the cap, the regenerating nerve end is isolated within the cap. Aligned nanofibers at the start of the lumen help the regenerating nerve cells grow along the "runway" length, which is mm. Over time, the regenerating nerve end will exhaust itself as the cap integrates into a new protective tissue layer from mechanical stimulation and external neurotrophic signaling.

Decellularized, GLU-treated bovine pericardium is a biocompatible material commonly used in bioprosthetic heart valves or other vascular applications. The material exhibits good mechanical strength and elasticity—which is necessary for the cap to fit over and bond to the nerve. Importantly, the material vascularizes and integrates into a new, protective tissue layer, as opposed to collagen/PLA; which degrades over time.

The total cap length is 12 mm: 5 mm to fit over the nerve and bond via PTB, 4-5 mm of "runway" or gap space, and about 2 mm of anchoring tab. The 5 mm length fitting over the nerve gives the necessary PTB surface area for a sufficiently strong seal. The 4-5 mm runway is based on a study which found minimal axon infiltration past 5 mm in hollow tubes. The cap thickness is based on commonly available thickness of bovine pericardial tissue sheet or patch [4]. The given thickness is compatible with PTB [5].

Photochemical tissue bonding (PTB) is an atraumatic tissue repair technique that uses light and a photosensitizing dye to crosslink proteins on tissue surfaces. In PTB, a photoactive dye (rose bengal) is applied to the tissue surfaces, followed by illumination of the surfaces with visible light. The dye absorbs strongly at the wavelength of the illumination of the tissue interfaces, and the energy of the absorbed photons is used to drive photochemical reactions. Covalent bonds are formed between protein molecules which bind the tissue surfaces together. The overall temperature rise is negligible [1].

With the nerve cap, PTB allows for the bovine pericardial tissue to crosslink with the collagenous epineural surface of the nerve. The result is a 360° seal which fully constrains the cap and isolates the regenerating nerve end from external neurotrophic signals. The cap is sutured at the distal end in surrounding tissue.

Electrospun aligned nanofibers are placed starting at 5 mm within the cap and are intended to help guide regenerating nerve cells along the runway and reduce axon entanglement.
The cap diameters of 3, 4, 5, and 6 mm intend to fit the range of nerve diameters in the wrist/dorsum base of the hand. The interval of 1 mm between cap sizes is consistent with that of the predicate nerve cap.

Observably, the Revision 1 nerve cap uses aligned nanofibers in lieu of a partition and has a distal end tab for anchoring to soft tissue.

Fig. 2.3. Rev. 1 CAD model isometric and cross-section view.

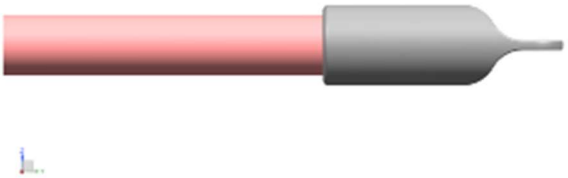


Fig. 2.4. Rev. 1 nerve cap and nerve assembly.

2.2 List of Parts/Materials

The following materials comprise the nerve cap as implanted:

Revision 0	**Revision 1**
Collagen/PLA composite	Decellularized, GLU-treated bovine pericardium
Rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein) photosensitizing dye	Rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein) photosensitizing dye
	Electrospun aligned nanofibers

2.3 Design Drawings

No formal technical drawing was made for Revision 0, as the design evolved to Revision 1 early in the design process. The technical drawing for Revision 1 is provided:

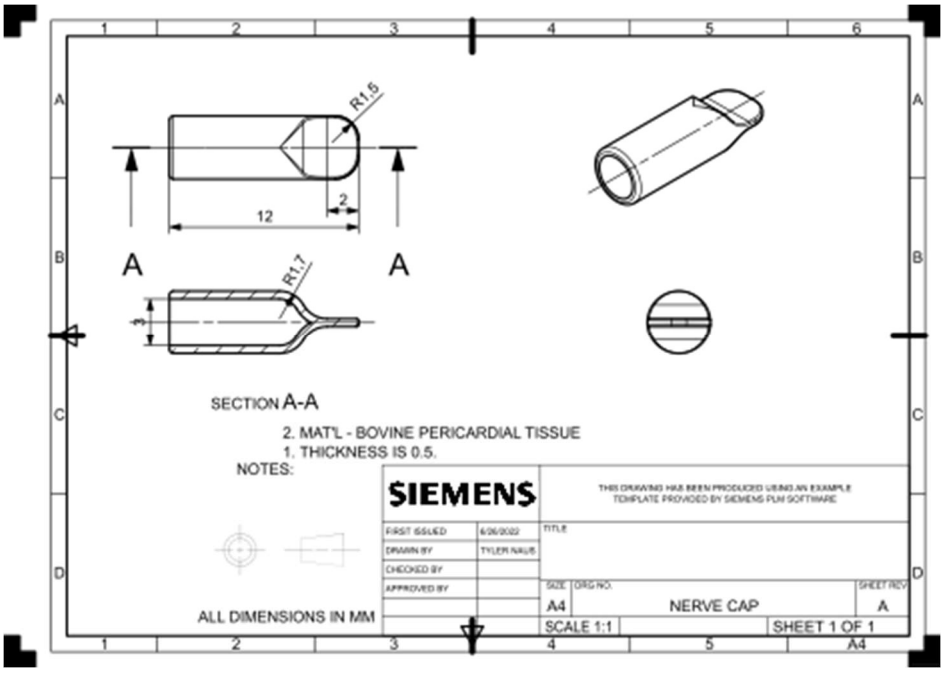


Fig. 2.5. Revision 1 nerve cap technical drawing.

Fig. 2.7 is a technical drawing of the 3 mm inner-diameter cap with 0.5 +/– 0.25 mm uniform thickness. For the other diameters (4, 5, and 6 mm), the length and thickness is the same. Rounds/fillets are used in the CAD model to mimic the smoothness of pericardial tissue and provide for an accurate geometry for finite element analysis. In manufacturing, the caps will not conform to specific radial dimensions for rounds/fillets. The critical dimensions are the cap diameter, length, and distal end tab length, and thickness. While the drawing gives macroscopic details about the product, it cannot communicate the microstructural details. Aligned nanofibers on the cap's luminal surface will start at 5 mm from the proximal end and continue for 1 mm.

2.3 Flowcharts/process description

Revision 0 was not considered long enough in the design process for the details about its fabrication to be researched and recorded. The general processing of Revision 1 nerve cap is as follows:

1. Decellularized, glutaraldehyde (GLU)-treated bovine pericardial tissue sheets are obtained from a supplier. Glutaraldehyde is a crosslinker and fixative which kills cells quickly by crosslinking their proteins and strengthens the remaining scaffold. The sheet should be within the tolerance of 0.5 +/– 0.25 mm thickness [4].
2. The sheet is cut into the appropriate length and width for it to be rolled into a hollow tube.
3. The aligned nanofiber scaffold of 1 mm width is seeded on the sheet 5 mm from the proximal end.
4. The sheet is rolled into a hollow tube and the seam is laser tissue welded.
5. The distal end of the tube is crimped to provide for a 2 mm flat tab and laser tissue welded, ensuring that the distal end is completely enclosed.
6. The cap is ethylene oxide sterilized and packaged.

Generally, the surgical procedure for Revision 0 and Revision 1 are similar, though steps unique to Revision 1 are indicated:

1. Following traction neurectomy, the nerve end is exposed, and its diameter is measured. This measurement determines which diameter cap is needed (e.g., if the nerve diameter is measured as 3.5 mm, the 4 mm cap is selected).
2. The nerve cap is taken out of its package using aseptic technique and its lumen is stained with rose bengal.
3. The nerve end is stained with rose bengal.
4. Using micro-forceps, the cap is carefully placed over the nerve to a length of 5 mm but no longer.
5. The hand-held solid-state laser (wavelength = 532 nm, fluence = 110 J/cm2) spot irradiates the nerve cap and nerve bonding site until complete photobleaching has occurred [5].
6. The surgeon inspects the bond between the cap and nerve for a complete 360-degree seal.
7. (Rev. 1) The distal end tab is used to suture the cap into soft tissue.
8. The cap should be buried sufficiently under healthy tissue so that protrusion cannot occur.
9. The incision is closed, marking completion of the surgical procedure.

3. Design Verification/Review/Testing

3.1 Design Review

3.1.1 Concept Mapping

The initiation of the design processing was with developing a concept map that detailed the possible components the design could have. These concepts where then rearranged and grouped based on common themes. The organized list can be seen in Fig 3.1. The team then selected upon different aspects from this list to use as the basis for the design.

Fig 3.1 Organized list of concept map

Materials	Cell Inhibition	Cap design	Analytical tools	Experimental approaches	Measurement Tools	Other
plastic	electrically stop growth	Spiraled design	Statistical analysis of nerve growth	computer design of shwann cell gro	Cytotoxicity assay	Cell culturing
copper	mechanically stop growth	bifurcation	Statistical analysis of toxicity (through inflammatory cell count lymp	FEA analysis of material and structu	Migration/growth assay	Minimiall invasive or robotic surgical technique
cloth	chemically stop growth	spherical design	Degradation rate analysis	CAD design of structure	Ultra sound/MRI serial imaging	Electrospin as fabrication technique
recycled materi	cut off excess nerve growth	cubical design	Histology analysis of nerve ending before and after cap	Matlab analysis of shwann cell growt	Measuring device of nerve/ area prior to surgery	
silicone	chemically kill excess growth	Pain reducing agent within conduit		Python analysis of shwann cell growth		
paper	electrically kill excess growth	Growth factors within conduit		Minitab for statistical analysis		
Polymers	conduit that degrades over time	conical design		Excel for statistical analysis		
collagen	genetically modify axon growth	tubular design		NX12 for CAD modeling		
fibrin	extend growth outside body	bioresorbable		NX12 for FEA analysis		
hyaluronic acid	photosealing	3D print cap		Electricity conductions/ simulation		
autografts	genetically modify neuron to prevent excess g	fibrous encapsulation		Technology to direct growth in pattern		
allografts	genetically modify regrowth shape	anchoring tab		Electrical component to aid in prosthetic limbs		
spider silk	chemical inhibition of growth	integration of tissue and device				
titanium						
aluminium						
brass						
steel						
Cadaver tissue						
adhesive material						
nanoparticles						
nanofibers						
Porcine Small Intestinal Submucosa						
3D scaffold						
polymer surface modification						

3.1.2 Pugh Matrix

To effectively compare the differences between the designs produced, a Pugh matrix was used to evaluate the strengths of each design. The matrix compared aspects of the design to the baseline and how well the design met the requirement. The results of the Pugh matrix are illustrated in Fig 3.2 and shows a tie between Solution 2 and Solution 3. Both solutions were evaluated as effective designs, however, the novelty of Solution 2 was appealing to the team which led to its selection as the final design. This novelty was due to the conical shape the design had.

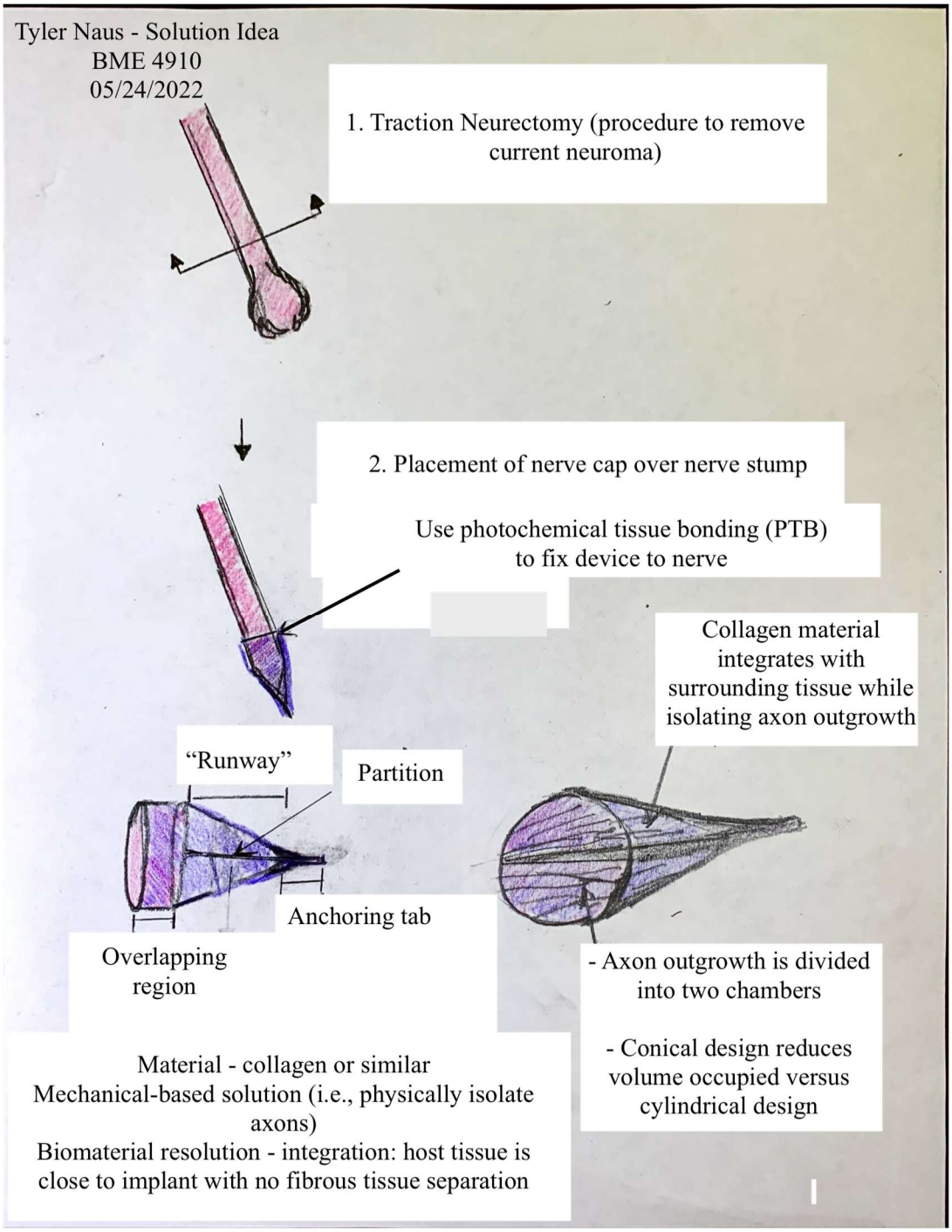
Fig 3.2 Pugh matrix of the design choices

Requirement	Weight	Baseline	Solution 1 - Allison	Solution 2 - Tyler	Solution 3 - Yousra
Device needs to produce appropriate host response for the given body contact and duration conditions for a permanent contact ir	5	Porcine SIS	0	+	+
Device material(s) cannot cause significant cell damage or death as measured by a cytotoxicity assay.	5	Porcine SIS	0	+	+
Use different device sizes to fit the range of nerve diameters for the digits.	2	Hollow nerve conduit (tube)	+	+	+
Device cannot mechanically fail (e.g., break, tear) during and after implantation.	4	Porcine SIS	0	-	-
Device is not susceptible to degradation in physiologic fluid.	4	Porcine SIS	0	0	0
Storage life of at least 32 mo.	1	Porcine SIS	+	+	+
Does not require custom or device-specific surgical instruments for implantation.	2	Hollow nerve conduit (tube)	0	0	0
Cost per unit less than 20,000 USD	2	Hollow nerve conduit (tube)	0	0	0
		Total	3	9	9

3.1.3 Design Sketches

The final design selected from the Pugh matrix in 3.1.2 Pugh Matrix, is illustrated in Fig 3.3. This sketch details how the cap will be an effective measure in preventing neuromas. This cap is inserted during a traction neurectomy procedure and is placed over the nerve cap using photochemical tissue bonding. This bonding technique will provide a tight seal between the collagen device and the nerve. The collagen material integrates with surrounding tissue to isolate axon outgrowth. Additionally, the cap is conical in shape to reduce volume and includes two chambers for the axon to grow within. The partition within cap is intended to provide the device mechanical integrity and divide the growth to reduce entanglement. This provides a mechanical based solution as the axons are being physically isolated to mitigate their growth.

Fig 3.3 Design sketch of final design



3.3.4 Review Feedback

The feedback and questions on this design are listed below in bold with the responses to these questions listed underneath the question.

Could this possible cause smaller neuromas in those partitioned areas? (This question is about statement that the conical design with partition which reduces the area available for the neurons to grow)

No, because a neuroma is an excess growth within the tissue. If there is growth within the cap that is not a neuroma. Growth within a controlled space will not cause injury to the patient. To make this plausible we need to have design inputs that ensure that the growth does not extend the cap and the cap does not expand due to the growth.

Areas to focus on for this, is the design going to be bent or compressed if the patient moves their finger/limb?
Using collagen material, the design will be built to withstand compression or bending while protecting the nerve. The design is built in a conical design to prevent excessive bending.

I think focusing on biocompatibility is a good start. Something to think about as well, maybe beyond our scope though, is contamination of the implant during production with chemical or other contaminates.

Biocompatibility testing will be completed on the final product to determine that there is no contamination on the device, and it is sterile for input. The scaffold fabrication process during production should already ensure sterilization prior to the biocompatibility test being performed.

How easy will this be to implant? It seems like it will be pretty straight forward, but are there areas of possible failure within the implantation itself and not necessarily the design?
We intend to have this discussion with a medical professional to provide context on the risks involved with the surgery.

Will there be any type of secondary surgery needed? Will revision surgeries be likely do you think? What are the chances of a new neuroma forming?
The intention is that the design would prevent the need for an additional surgery and reduce the risk of a new neuroma formation.

Is there anything special the patient will need to do with this implant initially after surgery? E.g. immobilizing the limb, no impacts (clapping) etc.
This procedure would require the patient to limit movement within the region of surgery to prevent any excessive force or further injury to the patient as the site is healing. This is an additional discussion point that we would like to have with a specialist.

Does a nerve cap still leave room for a neuroma to form? How does the nerve cap stop neuroma formation?

It prevents regenerating nerve cells from entering muscular fibers or tissues that it was previously embedded in. In controlling the nerve growth, the development of the neuroma is prevented.

Would you better be able to direct neuronal/Schwann cell growth by using aligned nanofibers?

We will consider the use and effectiveness of nanofibers to align the growth of the nerve

What kind of injuries cause neuromas more often? Crushing, amputation, or severing?

In general, trauma to the nerve can result from all of the above. As to which injury more often leads to neuroma – I am not sure. This is a good question for a specialist.

Where does the degeneration occur in the nerve? And how does this lead to the neuroma?

Degeneration occurs in a nerve at the severed end at the distal end with intention to reattach, however in our cause we are looking at the proximal end during amputation.

Is the diagram loss of limb or neuroma formation?

Neuroma formation at the site of limb amputation in the hand.

Has the photosensitizing dye been used prior?

Yes, it has been used for tissue-to-tissue formation and tissue-to-device that is biomaterial based such as one that is high in collagen. Additionally, it has been used for reconnecting skin and cardiovascular tissue.

3.2 Risk Assessment

3.2.1 Hazard Traceability Matrix

According to ISO 14971, risk is defined as the combination of the probability of occurrence of harm and the severity of that harm. To categorize severity of the potential harm for the medical device, the appropriate descriptors in Table 3.1 are used:

Table 3.1 Severity

Rating	**Definition**	**Value**
Critical	Requires explant surgery	5
Serious	Chronic immune response/pain	4
Moderate	Mild allergic reaction/infection or moderate pain	3
Minor	Acute nerve sensitivity or pain	2
Negligible	Local, mild, transient effects	1

Categorization of probability of the potential harm is given by Table 3.2. The probabilities are given in terms of per use of the device (i.e., per surgery).

Table 3.2. Semi-Quantitative Probability Levels

Rating	**Quantitative Probability**	**Value**
Frequent	³50%	5
Probable	<50%	4
Occasional	<5%	3
Remote	<0.5%	2
Improbable	<0.05%	1

ISO 14971 requires that the manufacturer compile of list of known and foreseeable hazards associated with the product, and to consider the reasonable sequence of events that can produce hazardous situations and harm. A hazard cannot result in harm until certain circumstances lead to a hazardous situation. Table 3.3 provides a list of relationships between hazards, foreseeable sequences of events, and the resulting hazardous situations of harm that can occur for the nerve cap medical device. ISO 14971 does not define acceptable risk; this is left to the manufacturer. Table 3.3 gives a quantitative measure of risk based on the combination of severity and probability for each harm.

Table 3.3. Hazard Traceability Matrix

ID	Hazard	Sequence of events	Hazardous Situation	Harm	Severity	Probability	Acceptable
1	Chemical (Biocompatibility)	Implantation leads to allergy/irritation	Immune response to implant material/substances	Irritation, Allergy, Delayed healing, Chronic immune response	5	1	ACC*
2	Residues/ contaminates	Product is incorrectly manufactured/sterilized/packaged	Immune response, cytotoxic, infection	Local cell/tissue damage, Infection	4	2	ACC*
3	Loss or deterioration of function	Incorrectly implanted, or device fails/degrades	Recurrence of symptomatic neuroma	Chronic pain	5	3	N ACC
4	Use error	Aseptic technique not used	Possibility of infection	Infection (bacterial, viral, or other agents)	3	2	ACC*
5	Inadequate labelling	Product is implanted in individual with known allergy to product material	Immune response to implant material/substances	Irritation, Allergy	5	1	ACC*

6	Operating instructions	Product was implanted too close to skin, not sufficiently buried in tissue	Protrusion	Wound dehiscence, Explant surgery	5	1	ACC*
7	Insufficient control of manufacturing processes	Product is not manufactured with correct material or sterilization technique	Wrong material or incompatible sterilization technique	Chronic immune response, Explant surgery	5	1	ACC*
8	Incorrect measurement	The cap's size/nerve end diameter was not accurately measured	Friction fitted cap on nerve end	Nerve compression, pain	3	2	ACC
9	Inadequate specification of design parameters	The cap is manufactured with the wrong dimensions/size	Cap is not sufficient length or thickness	Incorrect nerve repair, pain	4	1	ACC*

Table 3.3 begins by observing the chemical hazard that could be the result of an allergic response or irritation. The severity of this was evaluated at a level 5 due to the harm of delayed healing and possible risk of death. Despite this severity, the probability of this event is ranked at a level 1 because our material is collagen which has been shown to be biocompatible with the body. This makes this an acceptable risk if there is a control in place which means which should investigate efforts to minimize the severity of a chemical hazard to occur. The next hazard is the risk of residue or biological contaminates that may occur if the product is manufactured, packaged, or sterilized incorrectly. This may result in an infection and creates a cytotoxic environment resulting in local cell or tissue damage. The probability of this occurrence is at a level 2 because manufacturing issues do occur but is still not very likely. However, the severity of this evaluated at a level 4 due to the severity of the harm that may be caused by this issue. As a result, this hazard is evaluated as acceptable with a risk control in place. The third hazard is the loss or deterioration of function which is caused by an incorrect implantation or degradation of the

device. This hazard will result in chronic pain as the symptomatic neuroma returns. Due to this harm the hazard may cause, this was evaluated at a level 5 severity as it would require surgery to remove it. The probability of this occurring is placed at a level 3 due to the chance that the collagen material may degrade before nerve growth stops. This places the risk as unacceptable as the level of risk due to the severity of the harm and the higher probability of this occurrence. We will need to investigate minimizing the probability of this risk occurring.
Hazard four involves the hazard of user error that may occur is the aseptic technique is not used. Failure to follow the technique may result in the harm of infection posing a threat to the health of the safety. The severity of the hazard is a level of 3 because it poses harm, but through antibiotics and treatment the patient will be healed. The probability of this occurring is at a level 2 because it could happen but is not very likely. As a result, this risk is acceptable with a risk control in place to reduce the severity or probability of occurrence. The fifth hazard involves the inadequate use of labels to warn users of the device and its contents. This risk poses many issues, one of which is that the individual implants this device into a patient with an allergy to the material. The device would result in an immune response in the patient and severe irritation. This places this risk at a severity level of 5 but has a probability of 2 in terms of occurrence. As a result, this risk is acceptable with a risk control in place such as increasing the number of labels and their placements. The sixth hazard involves the user not following operating instructions resulting in the implant not being implanted too close to the skin or not sufficiently buried in the tissue. This may result in severe protrusion of the device and requires explant surgery to resolve. The severity of this issue is at a level 5, but the probability of this is at a level 1 due to the unlikelihood that this would occur. As a result, this hazard is acceptable with risk control in place that would reduce the severity of incorrect placement of the device.
Hazard 7 involves being insufficiently in control of the manufacturing process resulting in the product being manufactured without the correct material or sterilization technique. This hazard impacts the compatibility of the device and can result in a chronic immune response that would require explant surgery to resolve. Due to this, the hazard has a severity of 5, but the probability of this occurring is evaluated at a level 1. This hazard is then acceptable if there are risk controls in place. The eighth hazard involves the hazard of incorrect measurement of the device creating a cap diameter that does not fit well on the nerve end. This will result in friction between the nerve and the cap and may cause nerve compression and pain. Although uncomfortable, the severity of this hazard is at a level 3 and the probability of its occurrence is at a level 2. This places the risk as insignificant and acceptable. Hazard 9 results from inadequate specifications on design parameters in which the cap is manufactured with the wrong dimensions. This would produce a cap with insufficient lengths or thickness. The patient would then experience pain and the nerve would repair incorrectly. Due to this, the severity of this hazard is evaluated at a level 4 as this pain is chronic. However, the probability of this occurring is at a level 1 which indicates that this event is likely to never occur. As a result, this hazard is acceptable if there is risk control in place.

3.2.2 Risk Evaluation

The definition of risk according to ISO 14971 is the combination of the probability of occurrence of harm and the severity of harm. We have determined our risks based on our hazard traceability matrix. Also, from ISO 14971 risk analysis is described as a systematic use of available information to identify hazards and to estimate the risk. To evaluate the risk associated with each of these hazards we have created a matrix to act as a system of determining which risks are

acceptable. Hazards with a high severity of harm and probability of occurrence are considered unacceptable. Hazards with low severity and low probability are still risks but are acceptable. For the harms that are between the two extremes in severity and probability are also acceptable but are higher risks than low severity, low probability. Our system for evaluation analyzes these risks in a chart that is color coded to show the different acceptability's of risk. The area in the red is what we consider unacceptable, for our product the only risk in this section is loss or deterioration of function. The matrix showing each of our risks plotted against probability and severity are shown within Table 4, and color coded to provide how acceptable the risk is.

3.2.3 Risk Evaluation Matrix

Table 3.4. Risk Evaluation Matrix

		Severity				
		1	**2**	**3**	**4**	**5**
Probability	**1**				9	1, 5, 6, 7
	2			2, 8	4	
	3					3
	4					
	5					

Table 4 is a risk matrix where the estimated risks from Table 3.4 have been entered into the appropriate cells. The cells highlighted in green are defined as an insignificant risk. The cells in yellow are an acceptable risk with the appropriate design control(s) in place. Red is an unacceptable risk.

3.3 Technical Analysis

3.3.1 Fixation of Device

3.3.1.1. Topic of Analysis

This section details the fixation of the nerve cap to the peripheral nerve end.

Traditionally, nerve allograft, guide conduit, and cap surgeries use medical suture to fix the medical device/biologic to the nerve. For peripheral nerve applications specifically, suturing will cause trauma to the nerve as the suture is passed through it, and, depending on the suture material, may illicit tissue reactivity especially as the material is absorbed. In rare instances, suture can cause infection. It is also generally true that the smaller the structure to be repaired the more difficult the use of sutures.

In designing our nerve cap, our goal was to permit for a suture less procedure in the cap's proximal attachment to the nerve stump. Photochemical tissue bonding (PTB) is a tissue repair technique that uses light and a photosensitizing dye to crosslink proteins on tissue surfaces. In PTB, a photoactive dye is applied to the tissue surfaces, followed by illumination of the surfaces with visible light. The dye absorbs strongly at the wavelength of the illumination of the tissue interfaces, and the energy of the absorbed photons is used to drive photochemical reactions. Covalent bonds are formed between protein molecules which bind the tissue surfaces together;

analogous to welding. In the PTB technique, the photosensitizer used stains the tissue such that effects are limited to the surface. The overall temperature rise is negligible. This technology is generally applicable with collagenous tissues [10].

With our nerve cap, PTB allows for the bovine/porcine pericardium cap material to crosslink with the collagenous epineural surface of the nerve. The result is a 360° seal which fully constrains the cap and isolates the regenerating nerve end from external neurotrophic signals. The cap is sutured at the distal end in surrounding tissue [11].

The purpose of this technical briefing is to verify that PTB can be implemented with our device; particularly in its mechanical strength and compatibility with being used when the surgical site is deep within tissue.

3.3.1.2. Methods and Materials

To test for mechanical strength of the bond, an experiment was devised using PTB with chitosan adhesive films and calf intestine. Chitosan is a natural material derived from the chitin shells of crustaceans. PTB was achieved by applying a solution of rose bengal (a stain) between the tissue edges of the chitosan film and calf intestine, which are irradiated by a laser to facilitate the cross-linking of collagen fibers [5].

The solid-state laser used had a wavelength, l, of 532 nm, Fluence of about 110 J/cm2, and spot size of 0.5 cm. A single column tensiometer tested the bonding strength. Thermocouples recorded the temperature at the bonding surface during the application of the laser. Human fibroblasts were seeded on the adhesion and cultured for 48 hours to assess cell growth [5]. Deacetylated chitosan from crab shells dissolved at a concentration of 1.7% wt./vol. in deionized water containing 2% vol./vol. acetic acid and rose bengal was used. The chitosan solution was stirred for two weeks at 25°C in a vial shielded from light to avoid photo-bleaching of rose bengal. The chitosan solution was spread over a sterile and dry perspex plate and allowed to dry for two weeks at 25°C. The resulting chitosan film was detached from the plate. A digital micrometer measured the film's thickness. All films were cut into rectangular strips, placed between sterile glass slides to preserve their flat shape and wrapped in aluminum foil for light shielding. A UV-Visible spectrophotometer measured the laser attenuation at 532 nm within the chitosan film. The wavelength 532 nm is strongly absorbed by rose bengal in phosphate buffer solution (PBS) and corresponds to the laser wavelength used in tissue repair. The adhesion of the Rose bengal adhesive was activated by a diode-pumped solid-state laser that was coupled to a multimode optical fiber. The fiber was inserted in a hand-held probe to provide precise beam delivery by the operator. Because the laser is not eye safe, safety googles were worn during use of the laser. The adhesive strength was tested *in vitro* on calf intestine, which was harvested immediately after euthanasia and stored at -80°C. Prior to use, the tissue was immersed in deionized water for 15 minutes to defrost and hydrate at 25°C. Intestine sections (2 x 1 cm) were cut using a #10 blade under a microscope. The intestine was kept moist using deionized water; excess water was absorbed with cotton tips prior to tissue repair. A chitosan adhesive was positioned across the intestine section on the serosa layer with microforceps. The laser operator spot-irradiated for about 5 seconds, verifying the beam scanned the whole surface area. After PTB, tissue was maintained in wet gauze before tensile testing to mimic *in vivo* conditions. A

sample was clamped to the tensiometer using mechanical grips, which moved at a rate of 22 mm/min. until the two tissue stumps separated. Strips of chitosan adhesive without laser irradiation were tensile tested to serve as the control [5].

The temperature increase at the bonding site was measured in a separate experiment. The thermocouple was positioned between the calf intestine and chitosan adhesive. The thermocouple was inserted through a hole punched in the intestine with a 10 gauge needle. The operator ensured full contact between the adhesive and thermocouple using a microscope. The thermocouple was calibrated and connected to a digital multimeter to record and store data every 5 seconds. The beam was applied at a 1 mm offset for 30 seconds of recording [5].
To assess adhesive cytotoxicity, human fibroblasts were seeded on the adhesive samples after the samples were sterilized with 100% ethanol and washed in PBS [5].

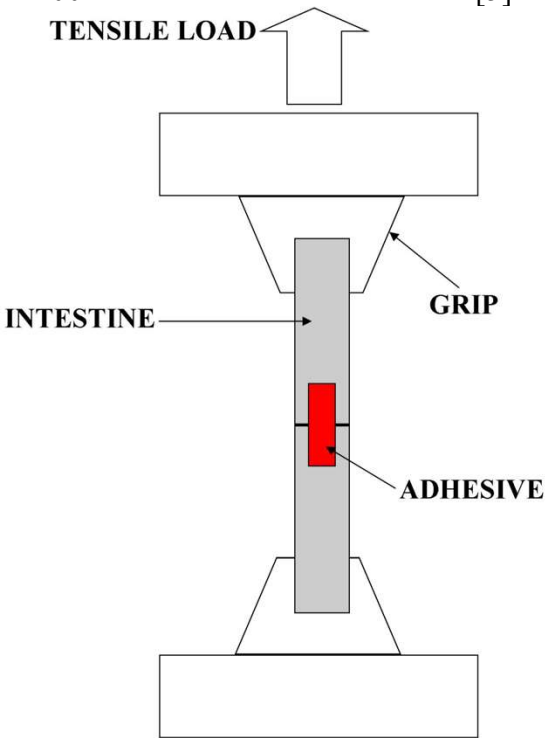


Fig. 3.3.1. Experimental tensile test setup.

3.3.1.3 Analysis Results and Discussion

Tensile test results:
The chitosan film bonded to the intestine upon laser irradiation achieved a maximum load at failure of 0.91 ± 0.07 N. The adhesive strength was estimated as the maximum load divided by the adhesive surface area, which was calculated as 15.1 ± 1.2 kPa [3].

Temperature results:
The average temperature of the chitosan adhesive during irradiation was 32°C. The temperature increased by approximately 6°C during the 30 seconds of laser activation [3].

Adhesive cytotoxicity results:

After 48 hours of incubation, fibroblasts grew confluent on the chitosan adhesive. No morphologic changes were observed under the microscope in the cells attached to the adhesive compared to the cells in the control culture [3].

Discussion:
Given the adhesive strength was 15.1 kPa, and our nerve cap design calls for 5 mm of length over the nerve for PTB, the surface area for the 3 mm cap (smallest cap diameter) is determined as:

Using the surface area for right cylinder:
Nerve cap PTB surface area = (2)(p)(3 mm)(5 mm) + 2(p)(3 mm)^2 = 150.796 mm2

The relation for stress is: F = σ A
Solving for force (and with 15.1 kPa = 0.0151 N/mm2):
= (0.0151 N/mm2)(150.796 mm2) = 2.27 N (or 0.510 lb)

Findings for temperature verify that PTB is safe for surgical use. PTB performed well in cytotoxicity experimentation, with no finding of toxicity to cells. Rose bengal dye had no significant toxic effect on human fibroblasts. Rose bengal should not have significant toxic effects in the body as tissue is more resilient than cells to photochemical damage [5]. Previous studies have confirmed *in vivo* no thermal injury in tissue where PTB was applied. It should be noted that the thickness of the chitosan film was about 0.2 mm. Our design calls for thickness of 0.375 ± 0.125 mm. It is theorized that thicker material will cause more tissue heating in the PTB technique. We anticipate that our design specifications will not cause excessive heating, given that the maximum temperature observed in this study was only 32°C (body temperature is 37°C).

Last, PTB has been successfully implemented in repair models of similar invasiveness/depth as the surgery for peripheral nerve; PTB was implemented in vascular and peripheral nerve (guide conduit and cap) applications alike. [5]. The procedure for nerve capping in a rat model using PTB involved applying rose Bengal solution to the material for one minute, then fitting the cap over the nerve to allow for an approximately 3 mm circumferential interface with the epineurium. Microforceps were used to place the cap, ensuring it was in full 360° contact with the epineurium. The site was irradiated in two increments of 180° around the nerve stump for 60 seconds each. Post illumination photobleaching of the stain indicated bonding of the tissue surfaces [11]. In conclusion, PTB is biocompatible and durable. We are confident it can be employed successfully with our nerve cap.

3.3.2 Isolation and Guidance of Regenerating Nerve Cells

3.3.2.1 Topic of Analysis
This section covers the importance of mechanical testing in the guidance and regeneration of nerve cells in our nerve cap. For the device to reduce neuroma with the design created it needs to be able to hold its structure under various forces. These forces include any compression or tension on the hand through everyday activities, and the use of a prosthetic hand. We also want the material to be able to hold its shape without breaking if the axons begin to grow over each

other in layers within the “runway” portion of the design. Tensile testing is a mechanical testing used to determine a material’s ultimate tensile strength, yield strength, elongation, and Poisson’s ratio. The purpose of a tensile test is to provide information on the force a material can undergo when being stretched before it breaks. The output of yield strength provides the force that a material can undergo and remain elastic. Once the material surpasses the yield strength it enters the plastic deformation area. For the purpose of our device, we are focused on the ultimate tensile test to determine if it is considered brittle to the material that is on the market. The goal for our material is to be less brittle in comparison. We are also looking at performing compression tests on the conduit to determine that the device can withstand the same everyday activities and prosthetic use based on compression forces. It has been stated in a study on peripheral nerves that nerves under compression can cause symptoms including numbness and pain. If the device is under compression forces that then act on the nerve endings this can cause the patient discomfort that we are trying to limit with the insertion of the device [12] . If the device undergoes tensile forces, it can create a transverse contraction, which can be viewed in the figure below causing compression on the nerve, and again pain to the user.

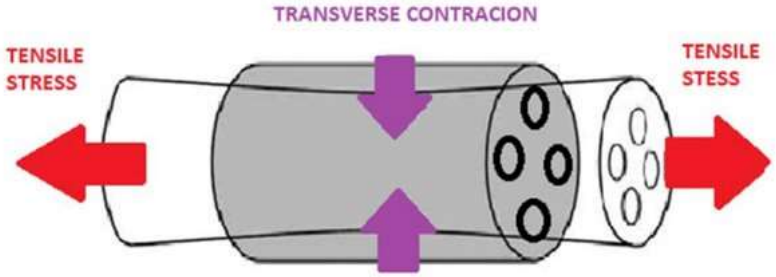


Figure 3.3.2 1 Transverse Contraction

Compression testing outputs information on modulus of elasticity, proportional limit, compressive yield point, compressive yield strength, and compressive strength. Once again, we are looking at the ductility of the material to determine if it is durable for our implementation.

3.3.2.2 Methods and Materials

For the scale of the device a micro tester is needed. For the purpose of our theoretical approach this testing is backed through the explanation of being able to test with a micro tester such as Admet eXpert 4000. Compression testing is done on the final design of a product, and therefore we will use research on the materials and previous mechanical testing of devices to determine the outcome of our device [5]. For our device we will represent this technical testing on an upscaled version using a universal testing machine.

Materials:

-Porcine small intestine submucosa

- Decellularized, glutaraldehyde-treated bovine pericardium

-Universal testing machine

To begin we will take the porcine small intestine submucosa sheet and clamp either end into the universal testing machine. From there we will turn on the universal testing machine on. The machine will pull apart the material for the tensile test a few inches or until it rips. The force is

measured up to 1000 times per second during the test and the average of all the force readings are reported. The data is then outputted to create a graph such as Figure 3.3.2 2:

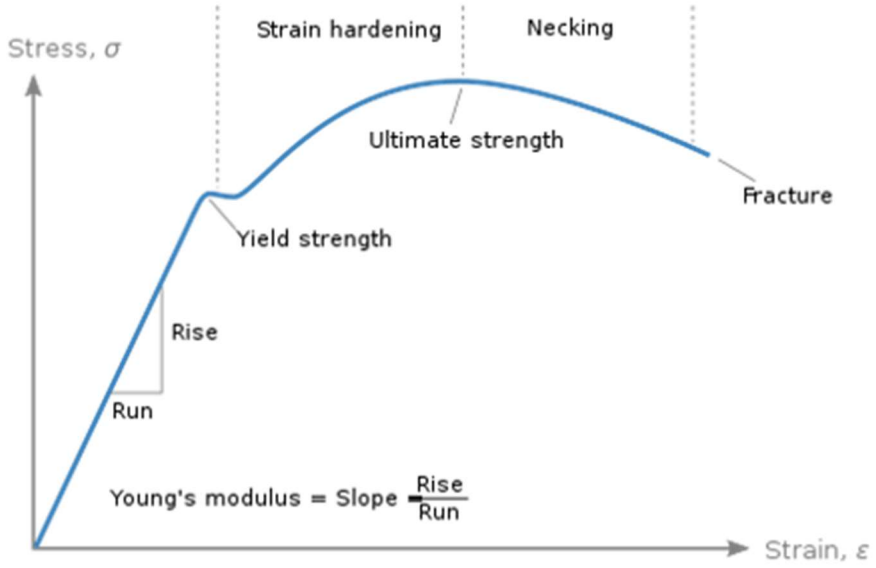


Figure 3.3.2 2 Stress, Strain Curve

The graph is then read using the labels in the figure to determine the yield strength, ultimate strength and young's modulus. We will then repeat this procedure for the decellularized, glutaraldehyde-treated bovine pericardium, and compare the results.

3.3.2.3 Executive Summary

An indepth literature review will be necessary to determine the forces that act on a hand and its peripheral nerves. This type of data has yet to be found in our previous data collections, and it is unknown if there is a comprehensive review of such forces. With this being the case, we may investigate retrieving this data on our own through a series of educated guesses from readings and understanding of forces. For the upscale version the tensile test will output information that we can use to analyze if the material is ductile in comparison to the material on the market. After our understanding of this information, we can confirm our results with results produced on the material in literature.

3.3.3 Biocompatibility of Pericardium Material

3.3.3.1 Topic of Analysis

In the development of the nerve cap, the cytotoxicity of the pericardium material is evaluated to determine the safety of applying this material. This material's cytotoxicity is analyzed through an agar diffusion test which are intended for implant devices with elastomeric closures [6].

3.3.3.2 Methods and Materials

For this analysis, the following materials will be required:

- sample of pericardium
- 0.9% sodium chloride
- zinc dithiocarbonate (ZDBC)
- L-29 mammalian fibroblast cells

- Minimum essential medium (MEM)
- Polyurethane film
- Microscope
- Humidified incubator
- 60 mm diameter plate
- 5% Carbon dioxide

To begin, squares of less than 100 square millimeters (mm) of pericardium are prepared with 0.9% sodium chloride. This samples are what will be used for the agar diffusion assay. Polyurethane film with ZDMC will be used as a positive control. From there, L-29 mammalian fibroblasts are grown in MEM to a confluence greater than 80%. The culture is added to each 60 mm diameter plate and is the agar layer of the assessment. This layer must be thin enough to allow leached chemicals from the samples and is set aside to solidify. The sample of prepared pericardium and negative control are added to the top of the agar surface. This can be repeated to create duplicates with no more than three specimens per prepared plate. These cultures are incubated at 37 +/- 1ºC in a humidified container with 5% carbon dioxide. The cultures are then evaluated under a microscope to determine the biological reactivity of the samples.

3.3.3.3 Executive Summary

This method of analysis provides an evaluation of the pericardium material that will be used in our cap design. Additionally, our cap design is shaped as an enclosure and is elastic. The agar diffusion test is intended for implanted devices with elastomeric closures. Results of the test are evaluated on a scale of 1 to 4 as seen is Table 1 which was adopted from Ethide Laboratories [8]. Requirements of the agar diffusion tests are met if the biological responses are not greater than a level 2. The results of the test are valid if the negative controls are at a level 0 and the positive controls are at least grade 3. Our design is expected to follow this requirement and meet the necessary validity this test requires.

Grade	Reactivity	Description of Reactivity Zone
0	None	No detectable zone around or under the specimen
1	Slight	Some malformed or degenerated cells under the specimen
2	Mild	Zone limited to an area under specimen and less than 0.45 cm beyond specimen
3	Moderate	The zone extends 0.45–1.0 cm beyond the specimen
4	Severe	The zone extends greater than 1.0 cm beyond the specimen.

Table 1. Biological Reactivity Grades for Agar Diffusion Test

3.4 Design Specifications

Refer to Revision 1 of the Design Output section for the product specifications. The following acceptance criteria apply to the device and are based on the design inputs:

1. Biocompatibility (as defined by ISO 10993-1): Initial Evaluation Tests for Consideration for an Implant device with permanent tissue/bone contact: Sterilization, Irritation, Systemic toxicity, Sub-chronic toxicity, Genotoxicity, and Implantation.

Acceptance criterion: Pass all initial evaluation tests listed according to ISO 10993-1.

2. Cytotoxicity: In addition to ISO 10993-1, FDA requires implant devices undergo *in vitro* cytotoxicity testing for 72 hours at 37 C.

Acceptance criterion: Pass *in vitro* cytotoxicity test for final finished device.

3. Different sizes.

Acceptance criterion: The specified nerve cap inner diameter needs to be accurate within 0.1 mm.

4. Low-cost:

Acceptance criterion: Estimated total cost < 20000 USD.

5. Reduce symptomatic neuroma:

Acceptance criterion: Reduction in pain as measured by a visual analog scale and reading of 44 or below (mild to no pain) post-surgery.

6. Sterilization:

Acceptance criterion: Final finished form of the device is sterile (no contaminants or residues).

Table 3.1 below details the verification activities corresponding to the design outputs listed in Table 3.1. Design verification is defined in 21 CFR 820 with its purpose to ensure that specified requirements of the device have been fulfilled.

Number	Verification
1	The verification for this section will be completed on the end product with a series of sensitization assays, and implantation tests. See section 3.5.1 for our approach on this verification.
2	The verification for this section will be completed on the end product through cytotoxicity tests including MEM Elution assay, and MTT assay. *in vitro* cytotoxicity testing for 72 hours at 37 C. See section 3.5.2 for our approach on this verification.
3	Tensile tests will be performed on the material as well as the material with adherence from the PTB in in vivo studies. See section 3.5.3 for our approach on this verification.
4	Mechanical and biocompatible verifications reperformed on device at 32 months outputting results with no significant difference. Questionnaire for hospital staff with device insuring not extra equipment was needed during their procedure See section 3.5.4 for our approach on this verification.
5	This will be verified as a theoretical sum of all costs based on literature searches, and See section 3.5.5 for our approach on this verification.
6	Visual analog scale (VAS) Test will be completed by patient one month post operation.

	See section 3.5.6 for our approach on this verification.
7	The final finished form of device will undergo a sterility assessment. See section 3.5.7 for our approach on this verification.

3.5 Verification

For the device specifications found in the previous section our team has created a verification matrix for the device considering it will eventually be implemented in a clinical setting. The verification techniques mimic those that we would require our product to see if on the market. Due to our goal deliverable being a prototype of this design backed by a theoretical approach using literature searches only a few of these verification techniques will be performed throughout the following semester. We currently have no data to verify our design specifications, however the following table provides the plan if an end device was an applicable goal of ours:

Table 3.5 Verification Matrix

Design Specification	**Acceptance Criteria**	**Design Output**	**Verification**
Biocompatible	Passes ISO 10993-1 which categorizes the appropriate host response for permanent contact of implant device.	Decellularized, glutaraldehyde-treated bovine pericardium	The verification for this section will be completed on the end product with a series of sensitization assays, and implantation tests. See section 3.5.1 for our approach on this verification.
Non-cytotoxic	Passes ISO 10993-1 which categorizes the appropriate host response for permanent contact of implant device.	Decellularized, glutaraldehyde-treated bovine pericardium	The verification for this section will be completed on the end product through cytotoxicity tests including MEM Elution assay, and MTT assay. *in vitro* cytotoxicity testing for 72 hours at 37 C. See section 3.5.2 for our approach on this verification.
Durable	Can withstand x forces.	Decellularized, glutaraldehyde-	Tensile tests will be performed on the material as well as

	This force has yet to be determined within our research we are looking to continue our data search as well as speak to experts to understand the input for this. Photochemical bonding adhesion does not break.	treated bovine pericardium, PTB	the material with adherence from the PTB in in vivo studies. See section 3.5.3 for our approach on this verification.
Ease of implementation in the clinic	Storage life > 32 months No device specific surgical instruments for implantation	Decellularized, glutaraldehyde-treated bovine pericardium, PTB, Ethylene oxide sterilization	Mechanical and biocompatible verifications reperformed on device at 32 months outputting results with no significant difference. Questionnaire for hospital staff with device insuring not extra equipment was needed during their procedure See section 3.5.4 for our approach on this verification.
Low-cost	< $20,000 per unit	Decellularized, glutaraldehyde-treated bovine pericardium, PTB, 3D printing, packaging	This will be verified as a theoretical sum of all costs based on literature searches, and See section 3.5.5 for our approach on this verification.
Reduces Symptomatic Neuroma	Reduce pain. (100-mm VAS reading of 0 to 4 mm [no pain] or 5 to 44 mm [mild pain]).	Aligned nanofibers at the start of the lumen help guide axons, 12 mm length, 4-5mm	Visual analog scale (VAS) Test will be completed by patient one month post operation.

		"runway", 0.5±0.25 mm thickness	See section 3.5.6 for our approach on this verification.
Sterile	Meet appropriate Sterility Assurance Level (SAL) which is 10^-6 for implant devices	Ethylene oxide sterilization	The final finished form of device will undergo a sterility assessment. See section 3.5.7 for our approach on this verification.

3.5.1 Biocompatibility Verification

Since our team does not have access or the skill set to perform biocompatibility testing in a wet lab we will conduct a literature review on decellularized, glutaraldehyde-treated bovine pericardium when used in in vivo testing. We will compare and collect data found on the biocompatibility of this material done within other labs, and the outcome of its use in implantable devices. Specifically we want to look for data on tests such as sensitization assays, and implantation tests to confirm that this device will be biocompatible.

The FDA's guidance document titled "Use of International Standard ISO 10993-1, 'Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process" gives the evaluation endpoints for consideration for an implant device with permanent contact with tissue/bone. The biological effects to be assessed are: cytotoxicity, sensitization, irritation, acute systemic toxicity, material-mediated pyrogenicity, subacute/subchronic toxicity, genotoxicity, implantation, chronic toxicity, and carcinogenicity.

While the proper method is to follow the FDA guidelines for each endpoint and submit complete test reports for each endpoint, given our limited resources, our approach will be to conduct literature searches to verify to our best ability each of the above endpoints while following FDA documentation guidelines within the guidance document.

3.5.2 Cytotoxicity Verification

For our team to verify that the device will not cause cytotoxicity we will again conduct a literature review of the material being used, decellularized, glutaraldehyde-treated bovine pericardium. We will look specifically at cytotoxicity assays in this search.

3.5.3 Durability Verification

We will be performing a series of mechanical testing to complete this verification for our theoretical approach. Refer to technical report section 3.3.2 of this document for the tests that will be performed. A literature review of the mechanical strength of nerves and nerve conduits will need to be performed to confirm the quantifiable input for the device.

3.5.4 Ease of implementation in the clinic

Due to this verification being done on the final product over a long period of time we will not be testing this component of our device. We will continue to back this design specification with research on the material, as well as interviews with surgeons if applicable.

3.5.5 Low Cost

For this verification we will be doing a sum of the data found of prices for the material, design creation, packaging, shipping, and surgery. Our design input or acceptance criteria for this user need has been determined based off an initial understanding of prices through the Axogen Nerve Cap. To confirm this criteria we will compare the price of that nerve cap to NEUROCAP® – Polyganics, and compare the price with our summed amount from our literature search.

3.5.6 Reduces Symptomatic Neuroma

For our theoretical approach we intend to further explain the verification that would be done in vivo before the produce is on the market. We will produce a report of the basis of animal testing and reviewing histology to confirm that the device reduces neuroma. Our format for this prediction will align with the data provided by Axogen and Polyganics.

3.5.7 Sterile

For this section we are going to conduct a literature search on Ethylene oxide sterilization to make sure it is compatible with our material, and design parameters including mechanical properties, and biocompatibility.

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