Fall | 11

Tissue Fragment Injection System

BME 200/300
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Advisor: Dr. Randolph Ashton
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Abstract:

Currently, the most common method for implantation of the Vx-2 carcinoma model in rabbit livers is surgical (Lee K-H et al, 2009). Percutaneous methods have been explored as a less invasive alternative. Limitations to the percutaneous method include: difficulty closing the hepatic incision, tumor seeding in unwanted areas, and backflow of tumor fragments during the procedure. The goal of this project is to design a percutaneous method that eliminates these limitations and lowers the technical skill required to perform the procedure. This will allow the user to perform more procedures per day, decrease the stress on the animals, and collect accurate data more quickly.

Three design alternatives were considered. One design encapsulates the tumor cells with poly-lactic-co-glycolic acid (PLGA), which is a water soluble, biodegradable polymer, and uses a biopsy needle for implantation (Fraylich, 2009). Another design utilizes a PLGA tipped needle followed by an injection of N-isoproplyacrylamide, a thermoresponsive polymer, to plug the injection hole (Uludag, H, 2001). The third is a completely mechanical design, which uses a modified 20-gauge needle tipped with a cellular delivery mechanism to place the cells within the liver.

After evaluating each design alternative through the use of a design matrix, we concluded that the PLGA capsule design showed the most promise. After testing of biomaterials and various needles, a final design was selected. This design consists of tissue fragments encapsulated by PLGA sheets, back-loaded into a 17-gauge needle, and ejected by a blunt-tip stylet. This design effectively reduces unwanted seeding and procedure time.

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Background:

Gastrointestinal and malignant melanoma cancer tumors most commonly metastasize in the liver (Lee et al., 2009). The Vx-2 carcinoma cell line was originally induced by the Shope cottontail rabbit papillomavirus (Georges et al., 1985). This Vx-2 model was first used by Moore et al. (1959) and is the most common model for studying liver cancer growth and developing potential treatments. This is an effective model because tumors formed from the Vx-2 carcinoma cell line are fast growing and exhibit similar blood flow and genetics to a human liver tumor. **Figure 1** is a Vx-2 tumor 28 days after implantation in a rabbit (Lee et al., 2009).

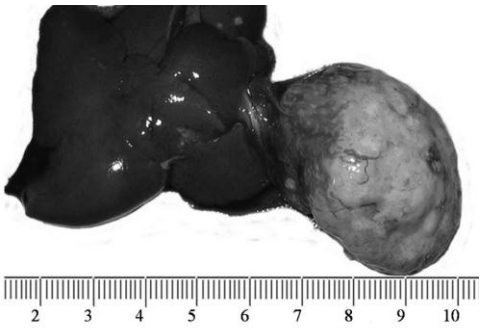


Figure 1: Vx-2 carcinoma tumor 28 days after implantation which displays extensive growth and surface blood vessels (Luo et al, 2010).

Currently, the most common method used for tumor implantation in rabbits is open laparotomy. This method allows for easy access to the site of implantation, accurate placement of cells, and minimal unwanted cell seeding in the abdominal cavity (Lee et al. 2009). Limitations to this method include long recovery time, anesthetic complications, and length of the procedure (Brace, 2011).

Percutaneous injection, using sonographic imaging methods for guidance, is a promising alternative to open surgery. This method is much less invasive than open surgery and shortens the length of the procedure. On average, the percutaneous method is five to eight minutes quicker than open surgery, which is beneficial due to a decrease in the amount of anesthesia to which the rabbits are exposed (Luo et al., 2010; Lee et al., 2009). Rabbits spend less time under anesthesia and exhibit a recovery time that is 3 times faster than the surgical method following the percutaneous procedure (Lee et al., 2009). Additionally, this method decreases the technical skill required to perform the procedure. Limitations to the percutaneous method include: increased seeding of tumors cells in unwanted areas of the abdominal cavity, difficulty closing the internal injection site, and a limited field of view. In a study conducted by Lee et al. (2009), unwanted seeding occurred in 21% of rabbits when the percutaneous method was used versus 8% of rabbits using the surgical method. This unwanted seeding arises from backflow at the injection site and seeding along the injection pathway as the needle is being inserted and removed.

Our client, Dr. Chris Brace of the UW-Madison Department of Radiology and Biomedical Engineering, has asked us to design a percutaneous tissue fragment injection system. The current procedure used in Dr. Brace's laboratory begins with the harvesting of tumor tissue from the flank of a donor White New Zealand rabbit, where the tumor was allowed to grow in vivo for approximately 2 weeks. This tissue is then cut into fragments of 2-8 cubic mm to be used in the implantation procedure. Next, a 2 cm incision is cut in the abdomen of the recipient rabbit to expose the liver.

Then, a 1-2 mm incision is cut in the liver and the cell tissue is implanted using a small forceps. After implantation, this incision is closed with 2-3 sutures after homeostasis has been met (Brace, 2011).

Problem Statement:

Certain models of cancer require surgical implantation of tissue fragments. Percutaneous injection is the preferred method for implantation over open surgery because it is less invasive. Percutaneous methods have limitations including: difficulty closing the hepatic incision, tumor seeding in unwanted areas, and backflow of tumor fragments during the procedure. Our goal is to design an improved tissue fragment injection system that effectively eliminates these complications using biocompatible materials and coaxial needles, while also lowering the technical skill required to perform the procedure.

Existing Percutaneous Injection Methods:

There are a few variations of the percutaneous method that have been tested in rabbits using the Vx-2 model. One method involves a 16-gauge needle, 14-gauge sheath, and a wire. The 14-gauge sheath is inserted first, followed by the 16-gauge needle **(A and B of Figure 2)**. Next, the wire is used to push the tumor cells out of the needle, with guidance by ultra sound imaging for accurate placement **(C of Figure 2)**. Finally, the 16-gauge needle and wire are retracted into the sheath and the sheath is removed from the animal (**D and E of Figure 2,** Lee et al., 2009). This method displays an increased level of unwanted tumor seeding in the abdominal cavity, which likely occurs during insertion or removal of the needle. Another current method, similar to the first, utilizes an 18-gauge needle with a sheath, and the injection of gelatin foam following cell insertion. This foam is used to plug the target site to prevent unwanted tumor seeding (**Figure 3,** Luo et al, 2010).

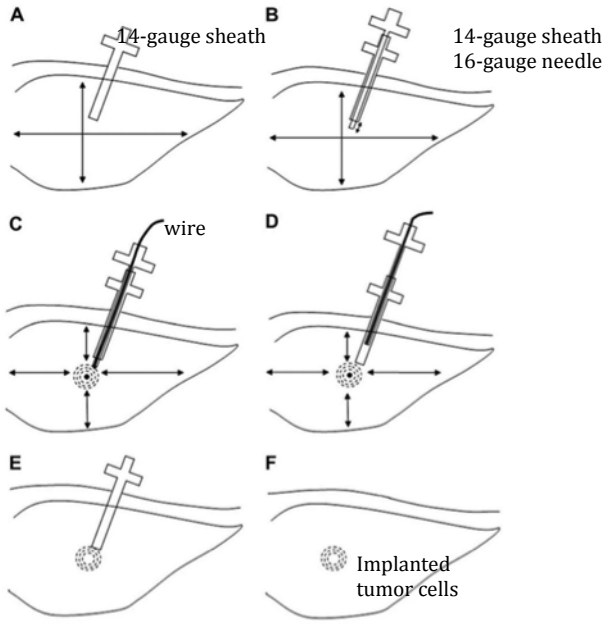


Figure 2: Existing percutaneous method schematic involving a 14-gauge sheath, 16-gauge needle, and wire. Part A: 14-gauge sheath is inserted into rabbit. Part B: 16-gauge needle, loaded with tumor cells, is inserted into sheath. Part C: Wire is inserted into 16-gauge needle and tumor cells are pushed into target area of liver. Part D: The 16-gauge needle and wire are removed. Part E: The 14-gauge sheath is removed. Part F: The tumor cells implanted in the liver of the rabbit (Lee K-H et al, 2009).

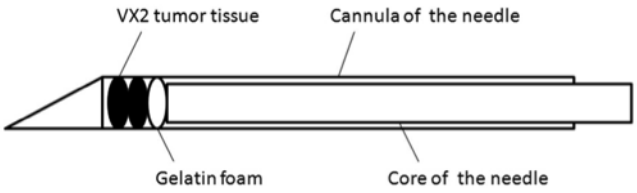


Figure 3: Needle used in percutaneous method which utilizes an 18-gauge needle, sheath, and gelatin foam (Luo et al, 2010).

Design Criteria:

The requirements for this project specify that the device must consistently seed tumor cells into a specified area of a rabbit liver while reliably preventing unwanted tumor cell seeding in the abdomen. This unwanted seeding can occur due to both residual cells on the needle during retraction, as well as tumor fragments slipping out of the pocket in the liver and into the abdominal cavity. The improved procedure should also take less time than the current open surgical procedure in order to lessen the physical toll the animals experience during inoculation. Also, the redesigned procedure should lessen the technical skill needed to perform the procedure in order to yield more consistent outcomes regardless of who performed the procedure.

All materials used in the procedure must be biocompatible with the rabbits to maintain the health of the rabbits for long-term survival studies as well as to preserve the environment the tumor cells have to grow in. Additionally, the devices and materials used must either be disposable or easily sterilized to surgical standards between each procedure. The needle must be able to reach an insertion depth of 5 cm in order to implant at the thickest part of the liver, and the ideal needle size is 18-gauge.

The device should weigh less than two pounds and have few separate components allowing the user to easily handle the device during the time-sensitive procedure. There is no given maximum budget for the device, but it should be kept within reason.

Ethical Considerations:

There are several ethical considerations to keep in mind throughout the course of the design process. First and foremost, since this design will be used on animal subjects, it is critical that all designs be safe for these animals: free of any materials or mechanisms that could negatively alter the biological composition of the animal or end its life earlier than anticipated. Any materials that will remain in the liver must be FDA approved, and the device must either be disposable or easily sterilized to ensure that a clean device is used for each percutaneous injection. In

addition to the many safety considerations, originality must also be considered. There are already a few existing percutaneous injection systems used in other laboratories, thus it is necessary that our design be original, as to not infringe upon any preexisting patents or copyrights.

Design Alternatives:

PLGA Capsule:

This procedure would involve encapsulating the tumor fragments in a layer of hydro-degradable PLGA before loading the needle and injecting it into the liver. PLGA, or polylactic-co-glycolic acid, is a material that, after a certain amount of time in the body, is broken down through hydrolysis mechanisms, exposing the tumor cells to the hepatic environment (Fraylich, 2009). The time for full hydrolysis is customizable depending on the ratio of PLA:PGA used (Makadia et al, 2011). It is also a Food and Drug Administration (FDA) approved material that is biocompatible with animals, so it would not affect the behavior of the cells or overall rabbit health. When pressure molded, PLGA has a durable structure with mechanical flexibility (Makadia et al, 2011). This allows the capsule to be stiff and maintain its structure, while avoiding rigidity and incompliance, making PLGA a good candidate for our capsule material.

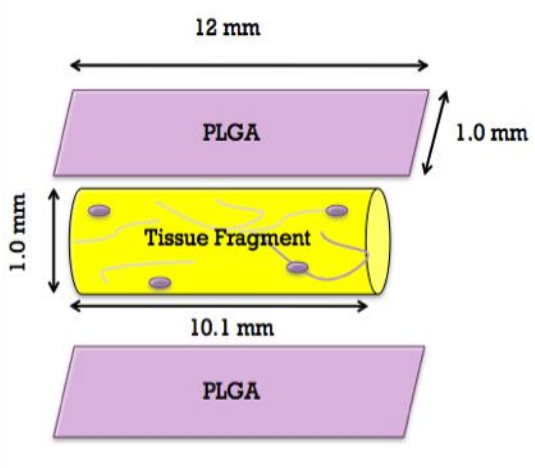


Figure 4. Dimensions and configuration of PLGA capsule. Cells and extracellular matrix can be seen in the tissue fragment.

During hydrolysis, the pH of the surrounding environment drops due to the acidity of the products of hydrolysis. Any significant change in pH could change the activity of the tumor cells and therefore the behavior of the cells. However, recent studies have shown that when Bovine Serum Albumin (BSA) proteins were encapsulated in PLGA, they maintained their activity after hydrolysis of the encapsulating PLGA microsphere (Eliaz et al., 2000). Due to the high susceptibility of proteins to pH changes, we concluded that these results could reasonably be applied to the hardy tumor cells.

In order to utilize the PLGA, the polymer would need to be die-casted into two 12mm by 1.0mm sheets, slightly larger than the dimensions of the tumor fragment. The tissue fragment would then be sandwiched in between these two sheets, effectively reducing the exposure of the tumor cells to its environment during injection (**Figure 4**). Next, the capsule would be loaded into the tissue fragment notch of an 18-gauge biopsy needle. A retractable sheath would then be released over the notch to prevent exposure during injection (**Figure 5**).

The tissue fragment was modeled as a cylinder due to the cylindrical shape of the tissue fragment notch in the needle. Using 8mm^3 as the volume of the fragment and approximating the depth of the tissue notch as 0.5mm, the calculated length of the tissue fragment should be 10.1mm in order to meet the 8mm^3 volume requirement (**Figure 4**).

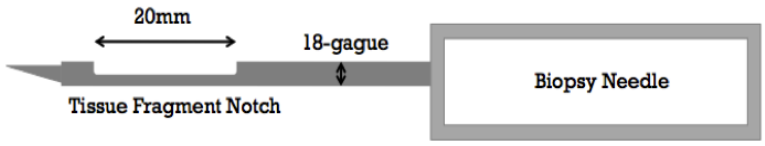


Figure 5: 18-gauge biopsy needle that will be used for injecting the PLGA capsule into the liver. There is a retractable sheath that can be released to cover the 20mm tissue fragment notch.

Cellular Delivery Mechanism (CDM):

The CDM is an entirely mechanical device that involves two different sized needles to deliver the tumor cells to the target site. First, an 18-gauge needle would be inserted into the liver to act as a guide for the second needle. A specialized 20-gauge needle would be modified with a small mechanical compartment at the tip. This mechanical compartment would open and close when a mechanism at the opposite end of the needle is triggered (**Figure 6**). The cells would be loaded into the mechanical compartment using a small forceps.

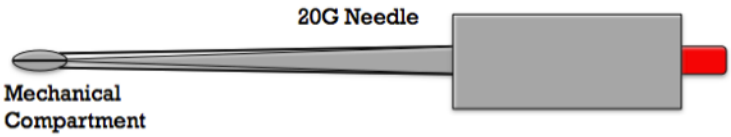


Figure 6. The CDM, image shows the mechanical compartment at the needle tip and the mechanism that that will open and close the compartment.

To begin the procedure, the specialized 20-gauge needle would be inserted into the larger 18-gauge guide needle. The opening mechanism would be triggered and then a small wire, inserted into the back of the 20-gauge needle, would force the tumor cells out of the mechanical compartment. Upon completion, all instruments would be removed and pressure would be applied to the puncture site for two to three minutes to stop any bleeding or backflow of cells.

PLGA Tip and N-IPAAm Plug:

The PLGA tip and N-isopropylacrylamide, N-IPAAm, plug design incorporates the molding ability of PLGA and the solidification properties of N-IPAAm. N-IPAAm is an FDA approved thermo-responsive polymer that changes state from a liquid to a solid as temperature increases. At a lower critical solution temperature (LCST) of 32-37 degrees Celsius, N-IPAAm will undergo a phase change and turn into a gelatin solid. Once the N-IPAAm is injected into the body, it will begin to warm up to body temperature, 37.1 degrees Celsius. After it warms past its LCST, it will change into a gelatin, sealing off the puncture hole left by the needle (Uludag et al., 2001). This will prevent back flow of cells into the abdominal cavity.

The design involves three needles. A standard 18-gauge needle would act as a guide for two 20-gauge needles. The tip of the first 20-gauge needle would be covered in PLGA, enclosing the tip to prevent unwanted cell seeding during the procedure. A tumor cell suspension would be loaded into the bare 20-gauge needle, using standard front-loading techniques, before placing the PLGA tip on the needle (**Figure 7**). Any remaining cells left on the needle would be ejected with the PLGA and left in the injection site. This PLGA tip would be die-casted prior to injection to fit the tip of the needle snugly. The second 20-gauge needle would be loaded with an empirically determined percent solution of N-IPAAm.

During the procedure, the 18-gauge needle would be inserted into the rabbit's liver in an area no less than 2 cm in thickness. The 20-gauge needle with the PLGA tip on it would then be inserted coaxially into the 18-gauge needle (**Figure 7**). The tumor cells and the PLGA tip would be pushed out of the needle into the liver using a tip release mechanism. Next, the first 20-gauge needle would be removed and replaced with the second 20-gauge needle containing N-IPAAm. The N-IPAAm would be injected, both needles removed and then pressure would be applied for two to three minutes. As the N-IPAAm warms to body temperature, its LCST is approached and it would undergo a phase change into a gelatin solid. This would block off the needle hole, securing the tumor cells in the liver. The PLGA would undergo hydrolysis and degrade in a customizable period of time, allowing the cells to interact with the hepatic cellular environment.

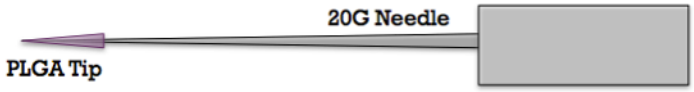


Figure 7. The first 20-gauge needle covered with the PLGA tip.

Design Matrix:

After brainstorming numerous design possibilities a design matrix was created to evaluate the designs in terms of the client's preferences (**Table 1**). The matrix was divided into six criteria: cost, ease of use, biocompatibility, ergonomics, reliability, and ease of production. Each category was assigned a weight value based on its importance in the final design, the higher the number the more important the criteria.

Reliability is our top priority and was weighted with the most points. In order to satisfy the client's problem statement by reducing the amount of unwanted cell seeding, the device must perform consistently over many procedures. If the device were to malfunction, tumors would not grow as desired, rendering the data unusable. This could result in time delays and increased expenses.

Ease of use is also a very important criterion to consider when building this device. A successful implantation of cells can vary depending on how easily the device is handled. An efficient and effective device can decrease the time required to run the operation and the technical skill of the user. This would lead to more consistent implantations, and the rabbits would benefit from a minimized time under anesthesia.

Biocompatibility was ranked equally to ease of use because most of the designs involve leaving a polymer inside the rabbit's liver as a method of delivering the cells. It is essential that these polymers do not alter the biological composition of the body. If altered, the results of the study could be influenced by such changes, and thus rendered unreliable.

Cost, ergonomics, and ease of production were ranked the lowest. The client did not specify a maximum budget, so it is not a primary concern. Due to the common application of an 18-gauge needle, it has already been ergonomically tested and is thus not a major factor in this design process. Finally, ease of production was ranked low because the client needs to find a solution that provides better results than surgical implantation. The client is willing to devote more time to preparation if the procedural outcome is more successful.

The PLGA capsule received the highest score for the ease of use category because once the tissue fragment is encapsulated, the procedure is very simple, requiring only one needle. Also, the capsule could be prepared before the recipient rabbit is anesthetized, creating a significantly shorter procedure time. The user would not be working against the clock while encapsulating and loading the needle. The PLGA capsule also scored the highest in the ease of production category because only one biomaterial needs to be prepared for the procedure.

The PLGA tip and N-IPAAm plug received the highest score in reliability because there are multiple guards against unwanted cell seeding: a removable PLGA tip that would prevent residual cells on the needle from coming into contact with the insertion pathway, and a N-IPAAm plug to prevent cells from leaking out of the hepatic pocket. However, this design received the lowest score in biocompatibility and cost because there are two biomaterials used during the procedure. Also, N-IPAAm is a non-biodegradable material that could potentially change the environment the tumor cells grow in, or encapsulate them before gelling, which would completely cut the cells off from the hepatic environment.

Finally, the CDM received the highest score in biocompatibility because no materials are left in the body, so there is little change of the procedure adversely affecting the hepatic environment. This design scored lowest in ease of production because forging a mechanical compartment, along with the releasing mechanism would be a very complicated process.

The PLGA capsule received the highest overall score, and will be the final design we pursue over the rest of the semester (**Table 1**).

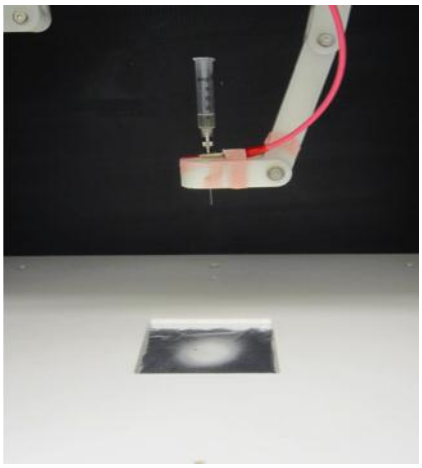
Table 1. Results of Design Matrix

Criteria	Weight Value	PLGA Capsule	PLGA tip and N-IPAAm Plug	CMD
Cost	10%	3	2.5	3.5
Ease of Use	20%	3.75	3	3
Biocompatibility	20%	2.5	2	3.75
Ergonomics	10%	3.5	3.5	3.5
Reliability	30%	3	3.75	1
Ease of Production	10%	4	3	2
Total	**100%**	**3.2**	**3.025**	**2.55**

Testing:

PLGA Optimization:

Initially, die-casting was going to be pursued as the method for forming sheets of PLGA for encapsulation, but it was determined that electrospinning was a more accessible option. Different molecular weights and concentrations of PLGA were tested in order to optimize the electrospinning process. The different molecular weights of 50:50 PLA:PGA polymers were dissolved at different concentrations into tetrahydrofuran : dimethylformamide (THF:DMF) solvent. The viscous liquid was then poured into a syringe held by an arm above an aluminum foil collection sheet, as seen in **Figure 8**. The needle is connected to a wire that delivers voltage from a voltmeter. The arm and needle were placed 16 cm above the collection sheet, also known as the working distance, and 18 kV were applied to the needle. The difference in voltage between the needle and the grounded copper plate under the aluminum foil collection sheet pulled the PLGA into fibers that were collected on the aluminum foil. Results from each trail can be seen in **Table 2**.



Figures 8. Electrospinning set-up. Using an 18-gauge needle, 18kV, and a working distance of 15cm, PLGA fibers were spun on to aluminum foil.

Each sheet was analyzed by collecting fibers for 2 minutes on a microscope slide, and then viewed under a microscope at 20X magnification. The quality of fiber formation was determined by the uniformity

of fiber direction, depletion of solvent droplets, linearity of fibers, and amount of overlap between fibers. As seen in **Figure 9a**, at the lowest molecular weight, 7,000 – 17,000 kDa, and the lowest concentration, 25 weight percent, the fibers were tortuous, oriented in many different directions, and short with no overlap. Furthermore there were many solvent droplets present, implying that the liquid was not viscous enough. At an increased concentration of 35 weight percent, as seen in **Figure 9b**, the fibers become more linear with the same orientation, however, there are still many solvent droplets and the fibers are short and not interconnected. At an increased molecular weight of 100,00 kDa and 30 weight percent concentration, as seen in **Figure 9c**, fibers are linear and highly interconnected with the same fiber orientation and nearly complete depletion of solvent droplets. These experimental conditions were considered optimal.

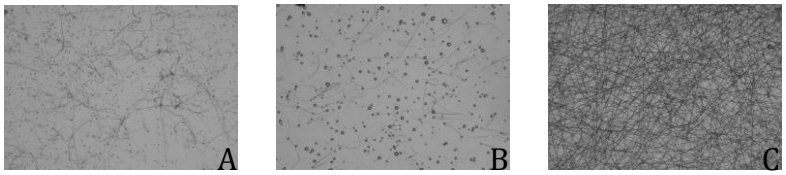


Figure 9. Images of fibers from electrospinning at 20X. **A**. 25 weight percent PLGA (Mw 7,000-17,000). **B**. 50 weight percent PLGA (Mw 7,000-17,000) **C**. 30 weight percent PLGA (Mw 100,000).

Table 2. Various concentrations of PLGA in THF:DMF solvent with a voltage of 18kV and a 16cm working distance. Two different molecular weights tested.

PLGA MW	Weight Percent	Voltage	Working Distance	Fiber alignment
7,000-17,000 kDa	25%	18kV	15cm	poor: little direction
7,000-17,000 kDa	35%	18kV	15cm	N/A (not viscious enough to stay in needle)
7,000-17,000 kDa	50%	18kV	15cm	improved: more direction
100,000 kDa	30%	18kV	15cm	optimal

Needle Testing:

To test the injection method of the tissue fragment we used liver tissue fragments to mimic the tissue sample size used in the procedure. The pieces were implanted into agar phantoms with 3 different types of needles (**Table 3**). We used a 17-gauge coaxial needle with a blunt tip stylet, an 18-gauge needle with a copper wire, and an 18-gauge automated biopsy needle. We performed these tests with unprotected liver tissue pieces as well as PLGA encapsulated tissue pieces. Without PLGA, all of the needles had similar placement results. However, the coaxial needle was the easiest to use. Limitations of unprotected tissue fragments included fragment smudging along the injection pathway during retraction and difficulty releasing the fragment without causing further damage to the phantom. With PLGA, the biopsy needle and the 18-gauge needle with copper wire proved infeasible to use. The biopsy needle was too difficult to load: requiring extremely fine motor

Table 3: Excellent (Ex) is defined as contained placement without backflow, acceptable (Acc) is accurate placement with minimal backflow, poor (P) is uncontained placement with significant backflow, while No is no placement.

	Trial 1		Trial 2		Trial 3		Trial 4	
Needle Type	+ PLGA	- PLGA	+ PLGA	- PLGA	+ PLGA	- PLGA	+ PLGA	- PLGA
Coaxial 17G Needle	Acc	P	Acc	Acc	Ex	Ex	N/A	Acc
18G Needle with Copper Wire	No	Ex	No	No	No	No	No	N/A
Biopsy Needle	Acc	Acc	N/A	Acc	N/A	Acc	N/A	Acc

skills. The18-gauge needle was rejected because the copper wire pierced through the capsule before it could be properly loaded into the needle. Due to its larger size, the 17-gauge coaxial needle allowed for greater variability in capsule dimensions, making it more consistent in loading and placement.

Final Design: PLGA Capsule:

Using PLGA of a PLA:PGA ratio of 50:50 and a molecular weight of 100,000 kDa, a 30 weight-percent solution will be made in 1:1 ratio of tetrahydrofuran:dimethylformamide solvent. Once this solution is homogenous, it will be loaded into the electrospinning apparatus and spun into a sheet using a voltage of 18 kV and a 16 cm working distance. Prior to loading the cells into the needle they will be wrapped in PLGA. The individual pieces of PLGA will measure 5mm by 3mm so that the 8 cubic mm tissue fragment can be completely encapsulated (**Figure 10**). The capsule will then be back-loaded into a 17-gauge coaxial needle. A blunt tipped stylet will be used to push the tissue fragment roughly 2 cm from the tip of the needle. The needle will then be inserted into the rabbit's liver to a depth of approximately 5 cm. This will be visualized through ultra sound guidance. Once the

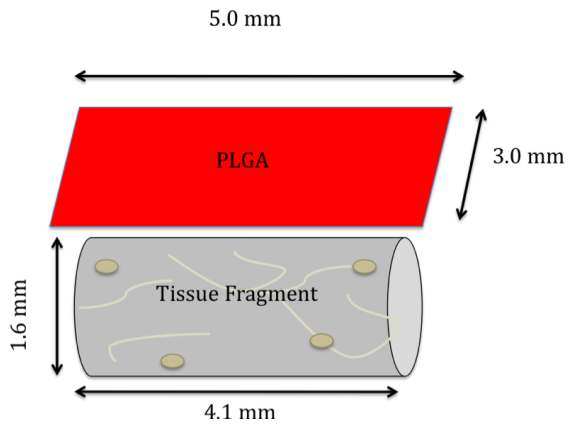


Figure 10. Dimensions and configuration of PLGA capsule. Cells and extracellular matrix can be seen in the tissue fragment.

target site has been reached, the stylet will then be pushed to the end of the needle, expelling the capsule. The stylet will then be retracted about 2 cm into the needle sheath to prevent contamination during removal. Finally, both elements of the needle will be removed, leaving the encapsulated tissue fragment implanted in the liver.

All elements of this design must be sterilized before contact with and injection into the rabbit liver. Sterilization of PLGA will be done using gamma radiation. The coaxial needles will be sterilized before packaging and delivery and disposed of after each procedure.

Future Work:

With our final design selected, there are a few necessary steps we would like to take before implementing this design into Dr. Brace's lab. Some of these steps involve improvements to elements of our existing design, and the final step involves adding an additional element to the design.

First, we would like to determine and customize an optimal degradation time for the PLGA capsule. To do this, it would be necessary to simulate the environment of a rabbit liver. Considerations would include temperature, pH, and other important characteristics. Next, we plan to improve the efficiency of the encapsulation processes. Developing a systematic protocol for the encapsulation process would help to even further reduce the technical skill and time required for this procedure. Additionally, it is crucial that a proper method of storing sheets of PLGA be determined. One large sheet of PLGA will last for about 75-100 procedures. This means that electrospinning will only need to be performed once every 75-100 procedures. While this provides a major advantage of reducing procedure time, it requires that the sheets be appropriately stored in order to ensure they maintain their desirable properties.

Preliminary testing has showed that N-IPAAm would likely be a very effective way of closing the injection site after the tissue fragment has been implanted in the liver. However, before a N-IPAAm plug can confidently be implemented into our design, it is necessary that additional testing of injection methods and temperature sensitivity be performed. If added to the final design, N-IPAAm would be loaded into a second needle, and injected through the sheath after the tissue fragment has been implanted and the blunt-tip stylet has been removed from the sheath.

Once these steps have been completed and total confidence in the device and method of our final design has been achieved, it would be ready to be implemented into Dr. Brace's lab to be tested on rabbit subjects.

Conclusion:

The design and implementation of a percutaneous tissue injection system will both simplify the procedure and generate more accurate results in the study of

Vx-2 carcinoma in the liver of New Zealand white rabbits. By encapsulating the cells in PLGA during delivery, any unwanted seeding will be successfully avoided. Through the consideration of biocompatibility, reliability, ease of use, and other important factors, a design consisting of cells encapsulated in PLGA delivered via coaxial needle has been selected as the best solution to the client's request.

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Appendix A:

Project Design Specifications
#25 – Tissue Fragment Injection System

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Function:

Certain models of cancer require surgical implantation of tissue fragments. Percutaneous injection is the preferred method for implantation over open surgery because it is less invasive. Percutaneous methods have limitations including: difficulty suturing the site of incision, tumor seeding in unwanted areas, and backflow of tumor fragments during procedure. Our goal is to design an improved tissue fragment injection system that effectively eliminates these complications using biocompatible materials and coaxial needles, while also lowering the technical skill required to perform the procedure.

Client Requirements:

- Cost effective
- Efficient
- Ease of use

Design Requirements:

1. Physical and Operational Characteristics:
    a. *Performance Requirements:*
        i. Decrease inoculation time in order to lessen the stress experienced by the rabbits, therefore increasing the number of experiments that can be fit in one day.
        ii. Should be disposable or easily sterilized to surgical standards.
        iii. Needle must be able to reach a 5 cm insertion depth in order to reach the thickest part of the liver.
        iv. Must prevent unwanted cell seeding in the abdominal cavity.
    b. *Safety:*
        i. The device must be biocompatible in order to preserve long-term health and to preserve the environment the tumor cells grow in.
        ii. The device should be non-toxic to the user.
        iii. Needle must be able to fit into a biohazard needle disposal compartment.
        iv. The device must be sterile.
    c. *Accuracy and Reliability:*
        i. Must consistently seed cells in the target area of the liver, which must be at least 2cm thick, with the assistance of sonographic imaging.

ii. Must reliably prevent unwanted tumor cell seeding in the abdomen due to both residual cells on the injection needle, and tumor fragments slipping out of the liver into the abdominal cavity.
iii. Needle must effectively enclose tumor fragments from the pathway during injection.
iv. Device must prevent residual cells from seeding in the pathway during retraction.

d. *Life in Service:*
   i. Needle and other biomaterials will be used one time.
   ii. Device will be in use for years.

e. *Shelf Life:*
   i. Should be stored vertically to maintain calibrations if needed.
   ii. Should be stored at room temperature and off the floor.
      1. PLGA stored at -20 degrees Celsius for an extended period of time.
   iii. Needles are sealed and can be stored according to manufacturer's specifications.

f. *Operating Environment:*
   i. Device will be used at laboratory and operating room temperatures.
   ii. Device will be used in a sterile environment.
   iii. Device will be used in vivo, thus should be biocompatible.

g. *Ergonomics:*
   i. Materials must not react negatively with animal tissues.
   ii. Device must have a comfortable grip to allow for extended and repeated use.

h. *Size:*
   i. Device should be portable.
   ii. Device must be easily held by user for the duration of the procedure.
   iii. Needle should not exceed 18-gauge.

i. *Weight:*
   i. Device should not weigh more than 2 pounds so it can reasonably be used throughout the multiple procedures performed in one day.

j. *Materials:*
   i. Injected biomaterials must not be detrimental to the subject. ie. change in pH and enzyme activity.
   ii. Materials must be sterile.

k. *Aesthetics:*
   i. No specified aesthetic requirements.

2. **Production Characteristics**
   a. *Quantity:*
      i. One device.
   b. *Target Product Cost:*

        i. Client gave a flexible budget.

3. **Miscellaneous:**
    a. *Standards and Specifications:*
        i. Must be up to RARC standards.
    b. *Customer:*
        i. Client is interested in thermally expanding materials as a method to close the point of insertion.
        ii. Client is interested in a dissolvable material.
        iii. Client prefers a thin-walled needle, no larger than 18-gauge diameter.
    c. *Patient-Related Concerns:*
        i. Reduce the amount of anesthesia and stress on the animal, therefore increasing recovery time.
        ii. Must be sterilized or replaced in between uses.
    d. *Competition:*
        i. Existing percutaneous surgical methods.
        ii. Open surgery procedures.